FURTHER STUDIES IN EXPERIMENTAL ATHEROSCLEROSIS.

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PLATES 40 TO 43.

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Several years ago, the writer[1] reported some experiments on dogs as a contribution to the study of experimental atherosclerosis. Since that preliminary report, the experiments have been continued and though much of these very elusive problems is still far from being clear, it is thought best to report some of the results of further study. It may be well to summarize briefly the contents of the article just referred to. Infectious disease has frequently been insisted upon as an important etiological factor in atherosclerosis. In order to test this, dogs were infected with pure cultures of various pathogenic bacteria and also with bacterial toxins, by means of injections into the jugular vein. The animals were kept in a state of mild chronic sepsis for periods ranging from 4 to 7 months. The results, as far as the blood vessels were concerned, were entirely negative. Lead and nicotine also gave negative results. General overexertion maintained for long periods by means of a treadmill, both with and without the hypertension produced by the injection of large doses of adrenalin, also proved without effect. The parenteral methods were then abandoned and feeding experiments were taken up. Some attempts were made with cottonseed-oil, which, however, proved too toxic. Then two dogs were fed daily with pure cholesterol in half-gram doses enclosed in capsules. These two dogs died after 1½ and 2½ months respectively. In the former publication positive results are claimed for these two experiments, because a number of whitish yellowish streaks, very slightly raised above the level of the intima, was found in the aortas. In the light of later experience these can no longer be considered as positive results. The doses of cholesterol were too small, dogs eliminate cholesterol more rapidly and completely than rabbits, and under these conditions 1½ and 2½ months would appear altogether too short a time for the development of so insidious and chronic a lesion as atherosclerosis. Moreover, experience has shown that these insignificant ridges and patches are found fairly regularly in the aorta of young

[1] Adler, I., Studies in experimental atherosclerosis. A preliminary report, *J. Exp. Med.*, 1914, xx, 93.

and healthy individuals both canine and human. There will be occasion to speak of this more in detail presently. It is not necessary to recount here all the varieties of experimentation that were attempted. It is important however to mention two dogs that received, besides their usual food of bread and meat, dilute hydrochloric acid up to sixty drops daily for 2 months and 3 months respectively. Both these dogs showed very definite and unmistakable arteriosclerotic patches in various portions of the aorta and in the one case, especially well marked in the lowest portion of the abdominal aorta. There were no traces of ulceration or calcification. Microscopically these patches showed the typical picture of arteriosclerosis, the details of which need not be gone into, except to mention the fact that cholesterol, in its free state, was abundantly present. That these findings could not be accepted unhesitatingly as proving hydrochloric acid to be an important factor in the etiology of arteriosclerosis is obvious. Two experiments are altogether insufficient to clinch so important a conclusion. The time during which the hydrochloric acid feeding was continued was too short, and more important than everything else, the age of the animals could not be ascertained and both dogs were in all probability rather old. It is easily possible, therefore, that the lesions found were not due to experimentation but were spontaneous, for it can no longer be doubted, though it is perhaps not generally recognized, that arteriosclerosis, closely analogous to the human, occurs spontaneously in old and debilitated dogs. It was necessary therefore to continue these acid experiments on a little broader basis.

The writer is still of the opinion that if it be possible to produce atherosclerosis, analogous to, or identical with the human type, experimentally and with reasonable certainty, the methods employed should give positive results not only in rabbits, but with a fair constancy in all animals that are subject to spontaneous or typical senile atherosclerosis. Unfortunately animal pathology has been more or less neglected in this respect, and it is possible that when more is known of vascular disease in the animal kingdom, more favorable subjects for the experimental study of atherosclerosis will be found. Until then, however, dogs would seem sufficiently well adapted for this class of experiments. They are omnivorous as the human; the structure of their blood vessels, especially of the aorta, differs not so very greatly from the structure of human arteries; and while they are subject to pronounced senile atherosclerosis, the lesions are not obscured and rendered uncertain by such forms of median necrosis as occur spontaneously in so large a percentage of rabbits. It is the writer's firm conviction, therefore, that the following conditions

should be fulfilled before it can be justly claimed that a genuine atherosclerosis has been artificially produced by experiment. (1) The gross lesions must be closely analogous, if not quite identical in every microscopic detail with the human arteriosclerosis. (2) These positive results must be so constant and regular that it is reasonably to be expected that every experiment will give a positive result, with perhaps only an occasional failure under exceptional conditions. Definite conclusions from apparently positive findings in only one or two animals are not permissible. The number of positive results must be sufficiently large, though an exception here and there may occur. In man also there occur instances, from the celebrated case of Harvey's down to modern times, where persons can live even to extreme old age, apparently immune to arteriosclerosis, their arteries remaining soft and elastic up to their death. (3) In order to avoid the probability of the lesions being due to spontaneous degeneration, the experiments should all be done on young animals. It might be objected to this that the youthful organism is perhaps possessed of powers of resistance and repair which are materially diminished or lost in older individuals and that therefore experiments on very young animals would not be likely to give positive results by the method in question even if they fulfilled all conditions on older individuals. In answer to this it may be said that since it has of late years been positively demonstrated that very young human individuals, even babies, are subject to unmistakable arteriosclerotic lesions under certain conditions, it seems more than probable that the same is true of dogs.

It was now necessary to test the action of acids to better advantage. The acids employed were: (1) hydrochloric acid, to verify if possible the apparently positive result that had been obtained in the two former experiments; (2) lactic acid, because O. Loeb[2] had claimed positive results with this acid and had developed a plausible theory concerning the mechanism of its action; (3) acetic acid, selected because of its occasional occurrence in certain types of digestive disturbance. Other acids, as for instance butyric, might logi-

[2] Loeb, O., Ueber Arteriosklerose. II. Ueber experimentelle Artherienveränderungen mit besonderer Berücksichtigung der Wirkung der Milchsaüre auf Grund eigener Versuche, *Deutsch. med. Woch.*, 1913, xxxix, 1819.

cally and profitably have also been tested, but for various reasons this could not very conveniently be done.

Hydrochloric Acid.

The series includes three dogs. All of them are young. Two were males, one female. In every case the ordinary chemically pure hydrochloric acid (specific weight not determined) was used. The initial dose was ten drops mixed with the food of the animal and increased by five drops every 3rd day. In every instance it took some time until the animals became accustomed to this unusual admixture to their diet. They would leave portions of their food, and at times would not eat at all; sometimes the dose of acid would have to be diminished; at other times the administration of the acid would have to be stopped altogether for a few days. But after a longer or shorter period the animals became thoroughly habituated to the acid and ate with great appetite, while the doses of acid were steadily increased. This is true, it should be said, not only of the hydrochloric acid series but of the two other acids as well. In the hydrochloric set there was one experiment (No. 1) that was disappointing.

Experiment 1.—Brown dog, male; weight 8,500 gm. Dec. 5, 1914. Commenced with 10 drops increased by 5 drops every 3rd day. Eats well until Dec. 28 by which time the dose reached 65 drops. Now begins to leave some food and to lose weight. Dec. 30. Weight, 7,600 gm. After a few days of 70 drops, dog is kept at 60 from Jan. 1, 1915, to Feb. 10. During all this time, eats well but loses weight steadily. 70 drops up to Feb. 16. Refuses food entirely for 2 days. Feb. 18. Died. Weight at death 5,500 gm. Experiment had lasted only about 2½ months during which time the dog took about 250 cc. of hydrochloric acid.

Besides the usual small punched out erosions in the stomach and the upper intestine and a great number of round worms, there are no definite lesions apparent, except general atrophy and marasmus.

Experiment 2.—Young brindle bull, female; weight 9,500 gm. Dec. 1, 1914. Commenced with 10 drops as usual. After a few days, weight begins to decrease. Acid has to be stopped, then again resumed; sometimes the dog eats well, at other times leaves great portions of food. Weight increases slightly, then is again diminished, the lowest weight being 6,800 gm. on Mar. 22, 1915. Appetite, however, is excellent and 80 drops of acid are given daily. From now on the dose of acid is increased by 10 drops about every 2 weeks. Weight increases steadily;

the animal is in excellent condition. Aug. 25. The dog gives birth to six healthy pups. From July 6 to Oct. 19, when the animal was killed, 140 drops were taken daily. Duration of the experiment 10 months, 19 days; total amount of hydrochloric acid consumed somewhat over 2,000 cc.

The autopsy shows a very slight hyperemia in scattered areas through the gastrointestinal tract. Otherwise there is nothing abnormal.

Experiment 3.—Black and white dog, male; weight 8,700 gm. Feb. 19, 1915. Dog started at once at 40 drops but though it does not eat well and is losing weight, the dose is steadily increased; weight varies. Though there are times when it absolutely refuses to eat, the dose is still increased and the dog begins to gain steadily in weight. Oct. 15. Killed; weight 9,700 gm. From July 30 to Oct. 15 the dog had taken 140 drops daily, ate all the food, and seemed in excellent health. The experiment lasted about 8 months, during which time the dog had consumed a little more than 1,600 cc.

The autopsy shows besides the usual intestinal parasites, fairly normal conditions. There is not even any very extensive hyperemia in the stomach and intestines.

Lactic Acid.

On the whole it seems that the animals did not take to lactic acid quite so easily as they did to hydrochloric.

Experiment 4.—Young fox-terrier, female; weight 6,000 gm. Dec. 1, 1914. Commenced with 10 drops daily; after a week 20 drops were given, and animal refused food, and weight decreased very rapidly. Dec. 10. The lactic acid is stopped altogether. Feb. 16, 1915. Weight is about 4,800 gm. The animal is kept on normal food without any acid until June 4. On that day having been eating well and being in good condition, lactic acid is given again with an initial dose of 90 drops. From now on appetite is excellent and increase in weight steady. The dosage of lactic acid is rapidly increased and from Sept. 19 until Oct. 14, when the dog is killed, 140 drops are taken daily. Weight at death 11,606 gm. The dog was under observation for 10½ months, but the lactic acid experiment, counting from June 4, 1915, lasted a little over 4 months in which somewhat more than 1,000 cc. were administered.

The autopsy shows some patchy hyperemia in the gastrointestinal tract, otherwise no organic lesions. No definite macroscopic signs of arteriosclerosis, although the yellowish ridges and patches in the arch and in the descending aorta are a little more pronounced than usual.

Experiment 5.—Bull-terrier, male; weight 13,000 gm. Dec. 1, 1915. Similar history. Eats well for several weeks, though weight decreases. Leaves large portion of food for about a week then begins to eat well again. Weight and ppetite vary. Sometimes the dose of lactic acid had to be diminished; some ames stopped altogether for a day or two. The dose had been increased to ti

175 drops, then it had to be diminished again to 80. Feb. 24, 1916. Lowest weight reached 10,300 gm. From now on dog increases steadily in weight and eats everything. Oct. 8. Killed. From June 14 to Oct. 8, 200 drops of lactic acid taken daily. The experiment lasted about 10 months, the total amount of lactic acid being approximately 3,000 cc.

At the autopsy no gross lesions of any kind are found.

Experiment 6.—Brown and white cur, female; weight 10,700 gm. Dec. 29, 1914. History similar to the foregoing. It took until about Feb. 24, 1915, for the animal to become accustomed to the lactic acid. On that day the minimum weight of 6,500 gm. was reached, but from now on the dog showed excellent appetite, ate everything it could get, steadily increasing in weight. Oct. 13. Killed. From June 4 to Oct. 13, 160 drops were taken daily, and the weight at death was 12,700 gm. The experiment lasted about 9½ months. Total amount of lactic acid consumed somewhat over 2,000 cc. The dog had given birth to a litter of pups 2 days before death.

The autopsy shows no gross lesions.

Acetic Acid.

Again the history is about the same as in the foregoing two series. 50 per cent glacial acetic acid, chemically pure, was used.

Experiment 7.—Brindle bull, male; weight 8,100 gm. Dec. 29, 1914. There are the usual variations in weight up to a certain lower limit. The dosage of acid is increased or diminished according to the amount of food the animal takes. It takes till about Mar. 9, 1915, for the dog to become fully accustomed to the acid. From now on its appetite is excellent, health good, and weight steadily increasing. Oct. 7. Killed. From June 14 to Oct. 7 the daily dose was 150 drops. Weight on day of death 12,000 gm. Experiment lasted 9 months and 10 days. Total quantity of glacial acetic acid consumed approximately 2,000 cc.

The autopsy shows no gross lesions.

Experiment 8.—Coach dog, male; weight 9,700 gm. Dec. 1, 1914. There are similar variations in weight and in dosage. It takes till Feb. 23, 1915, for the dog to become accustomed to the acid. From then on, dose of acid is steadily increased, and the weight increases though there are occasional bloody stools. June 1. Killed in a fight. Weight at death 10,200 gm. From Apr. 25 to date of death 150 drops of acid were taken daily with excellent appetite. Experiment lasted 6 months. Total amount of glacial acetic acid approximately somewhat over 1,000 cc.

The autopsy shows no gross lesions.

It is not necessary to give any details of the microscopic examinations. No lesion was discovered which could be interpreted as anal-

ogous to arteriosclerosis. The positive findings in the two dogs reported by the writer several years ago were, therefore, evidently not due to the hydrochloric acid, but were spontaneous and probably of the senile type. Loeb's claims of positive results with lactic acid are also not convincing. The one dog 8 years old, Loeb himself disregards on account of his age. In the other dog, only $1\frac{1}{2}$ years old, he regards as arteriosclerotic various small ridges and patches in the intima of the aorta. He gives no microscopic details and it is therefore not at all unlikely that these patches and ridges were no more significant than those found so frequently in the aorta of normal dogs, both young and old. It can therefore be safely asserted that the feeding of dogs with large quantities of various acids for a comparatively long period, though the cause of weeks of severe indigestion and marasmus, has not resulted in any lesions comparable to arteriosclerosis.

As cholesterol still appears to occupy the most prominent place in studies on arteriosclerosis, it was resolved to revert once more to experiments with that substance. Meanwhile a paper by Klotz[3] had appeared reporting the effects of injections into the ear vein of rabbits of cholesterol dissolved in pure oils or as a soap emulsion. It was decided therefore to try somewhat similar experiments on dogs.

Some attempts were made to develop a technique by means of which cholesterol was to be injected directly into the heart or into the aorta. The only result of these tentative experiments was the production of some cases of hemorrhagic and exudative pericarditis. It was then decided to inject cholesterol through the jugular veins. This series includes four dogs. All four were young animals, probably barely a year old. The cholesterol was obtained from gall stones and thoroughly purified; it was dissolved in sesame oil which was cheap and practically non-toxic. Its phytosterol content, being as in all similar oils less than 1 per cent, could, in the small quantities used in each injection, be considered negligible. It was found that 0.2 cholesterol in about 8 cc. of sesame oil at body temperature gave a perfectly clear solution. This solution was employed exclu-

[3] Klotz, O., Vascular changes following intravenous injections of fat and cholesterin, *J. Med. Research*, 1915–16, xxxiii, 157.

sively. Any attempt to increase the quantity of oil or use a higher concentration of cholesterol gave rise to more or less grave disturbances, as for instance, acute pulmonary edema and rapid death of the animal. For the first few injections, ether was given and a very small incision into the skin was made and the jugular laid bare. It soon became evident, however, that the incision was not necessary and that the vein could be reached with certainty, after a little practice, through the intact skin. Two injections a week were given regularly and without any exception for a period of between 8 and 9 months. The total amount of cholesterol consumed by each animal during that period was about 11 gm. No deleterious effect of the injection was noticed at any time. To observe whether a permanent rise in blood pressure had any effect upon the result of the injections, in two of the dogs, before any injections were made, the abdominal aorta was tied by the Halsted[4] method. A flap of the fascia lata was taken, with which the aorta was tied somewhat below the right renal artery. The ligature was tightened so that pulsation in the femoral artery could just be felt. In both cases the operations were uneventful and the wounds healed without any difficulty.[5] As the autopsies later on showed, permanent stricture of the aorta was accomplished by this operation. This must necessarily have affected not only the pressure in the aortic system, but also, indirectly at least, in the pulmonary circulation into which the cholesterol was primarily discharged.

The further history of these four dogs is uneventful. During the very hot and humid summer of 1916, the animals suffered from the mange, but with the coming of cool weather and with the necessary dermatological attention, they recovered fairly well, and were in good condition when they were killed. One dog did not recover its health entirely after the mange was cured, but continued losing weight. The injections were, however, continued regularly. On November 16 the dog was killed. At the autopsy it was found that the entire intestinal tract, including stomach and colon, showed hemorrhagic

[4] Halsted, W. S., Partial occlusions of the thoracic and abdominal aortas by bands of fresh aorta and of fascia lata, *Proc. Soc. Exp. Biol. and Med.*, 1912–13, x, 113.

[5] The operations were performed by Dr. M. J. Sittenfield, to whom I am indebted for his valuable assistance.

swellings with partial destruction of the muscularis. The contents of the intestines were bloody and the mucosa itself was in many places hemorrhagic. There were numerous ulcers, some of them extending down to the serosa. Great numbers of *Ascaris mystax* were present. In all four dogs the lungs at autopsy were found much firmer than normal and not completely collapsed. There were also present small wedge-shaped hemorrhagic infarctions, and patches of brown pigmentation evidently due to old hemorrhages. The liver, kidneys, and pancreas appeared normal. The adrenals were not enlarged and showed no gross lesions. The spleen in nearly every case was somewhat enlarged and showed very large prominent follicles. In one case there was some softening of the pulp, probably due to hemorrhage. In one of the dogs, in which the aorta had been tied, a definite, fairly close stricture of the vessel was seen surrounded by an area of thickening, but there was no noticeable dilatation proximal to the obstruction. In the other operated dog, there was also an area of thickening about the place of ligature, but the aorta appeared of practically uniform caliber, possibly very slightly constricted at the place where the ligature had been applied. No gross lesion could be recognized in any of the jugulars and the microscopic examination also showed perfectly normal conditions, with only here and there a small droplet of fat in the adventitia. Neither the frequent passage of oil and cholesterol through the vein nor its puncture several hundred times by a rather large needle seems to have had an injurious effect upon the delicate vessel. No trace of inflammation or degeneration could be seen. The heart muscle in every instance was flabby and was found in diastolic dilatation. There was no hypertrophy of the right ventricle. The animals were killed by the injection of chloroform into the jugular vein, and to this the flabby and dilated condition of the heart muscle may be ascribed. The aortas showed some ridges and patches and wrinklings here and there, more frequently around the orifices of branching vessels. These irregularities in the intima of the aorta are usually scarcely visible to the naked eye, especially in the fresh object, unless one has become familiar with them and searches for them carefully. After the artery has been kept in formalin for some time, they become a little more distinct. Besides these, however, more extensive sclerotic thickenings were plainly visible. This was

especially the case in one aorta (Fig. 1) in which, in the sinus Valsalvæ behind the semilunar valves, these sclerotic areas protruded more distinctly. In the bulb of this aorta also and near the orifices of the branching intercostal arteries a number of ridges was noticed which tended to confirm the impression of incipient sclerosis.

The oil and cholesterol injected into the jugular is of course carried through the upper cava and the right auricle into the right ventricle, and from there through the pulmonary artery into the lungs. It might be confidently expected, therefore, that if any changes in the arteries are to result from this procedure, they would show primarily in the pulmonalis, rather than in the aorta, for the first powerful impact of the blood laden with cholesterol is directed against the former. This expectation was completely verified. In all four dogs very definite changes, producing the distinct impression of arteriosclerotic thickening and loss of elasticity, appeared in the pulmonalis (Fig. 2). There were no signs of ulceration or calcification nor was there any appreciable dilatation. These departures from the normal were principally of the nodular type, protruding more or less prominently into the lumen of the vessel. They commenced immediately behind the valvular cusps, showed the faint whitish yellowish tint common to all sclerotic lesions of the intima, and extended, more or less, further up into the vessel. These nodulations were most pronounced in the two dogs in which the aorta had been tied. In the other two dogs the lesions, though quite definite and plain, were much less in extent and prominence. It is not possible to say whether this is merely coincidental, or whether the increased blood pressure is responsible. The most marked sclerotic changes were found in the dog whose aorta showed comparatively slight obstruction. While the gross lesions of the main trunk of the pulmonary artery closely resembled ordinary intimal arteriosclerosis before the development of atheroma has begun, the microscopic examination revealed a condition entirely unexpected, and in many ways rather surprising. Hypertrophy of the intima with proliferation of the endothelium, new formation of connective tissue, and the splitting of the elastica interna as described by Jores was distinctly in evidence. This hypertrophy of the intima however was comparatively limited and not commensurate with the amount of thickening. In many places there was hardly any prolif-

eration of the intima or it was entirely absent. The most pronounced and constant and evidently primary lesions were found in the media, and especially in its innermost portions adjoining the elastica interna. Within this region there had taken place much damage to the elastic tissue. Everywhere the breaking up of elastic fibers into larger and smaller pieces and into granules (*Körnchenzerfall*) could be seen. Very often it appeared as if the elastic fibers, especially the larger ones, had suddenly snapped and the ends could be seen some distance apart coiled like the lash of a whip. Another lesion, evidently secondary to this forcible disruption of the elastic tissue, was the separation of the muscular elements into bundles of varying dimensions and at varying angles, which were so pushed aside as to leave wider or narrower gaps between them. There seems to be no doubt that this disorganization of the elements of the media was largely responsible for the prominent areas of apparent thickening, and that the proliferation of the intima took only a minor and secondary part in the process (Fig. 3). These comparatively wide gaps between the dislocated groups of muscle cells usually contained remnants of broken up elastic fibers, but otherwise appeared empty (Fig. 4). That they were not empty could be surmised from the compactness and solidity of the nodules. Edema could be positively excluded; staining for fibrin or mucin gave no results, but with certain stains, as for example alum-carmine, the faintest possible tinge could be obtained, sometimes showing slight inequalities and suggesting some very delicate coagulated material. It is probable that these wide areas contain, as Ribbert[6] suggested, coagulated blood plasma. He finds these gaps, however, only in the intima, where he believes they are the effect of excessively high blood pressure. He attributes the deposit of cholesterol to this also. No sign of necrosis could be detected and there was nothing to suggest the median necrosis as seen in rabbits or in human arteries. The muscle cells, although detached from their normal position were in perfect condition, and the elastic fibers though shattered and disorganized retained their normal staining properties. There were no inflammatory

[6] Ribbert, H., Über die Genese der arteriosklerotischen Veränderungen der Intima, *Verhandl. deutsch. path. Ges.*, 1905, viii, 168.

symptoms and the vasa vasorum were normal. It seemed clear that the entire process was purely mechanical. The sclerotic areas in the aorta showed the same median disorganization even more strictly confined to the media, for the intima was mostly not affected, but normal.

The lungs presented a very constant and characteristic microscopic picture. Even a superficial examination showed that the bulk of the oil and its cholesterol content was filtered out through these organs. The sudanophil masses were deposited almost exclusively in the interalveolar septi, and there mainly in the capillaries. The latter were much distended and filled to bursting with oil. Very frequently the distended capillaries were surrounded by an infiltration of leukocytes, both processes together causing a considerable thickening of the septi with very marked encroachment upon the alveolar air spaces. The alveoli themselves were practically free from oil. Here and there an overdistended capillary would burst and a few drops of oil be left in the alveoli. Dispersed throughout the lung there were clear indications of hemorrhages. Numerous red blood corpuscles were found in the air spaces and in the septi, and hemorrhagic infarctions were also irregularly scattered throughout the lungs, especially near the margins. Notwithstanding all this the pulmonary apparatus seemed quite adequate to combat the enormous overburdening caused by the oil injections. Clinically there were no symptoms of respiratory disturbance and this could be accounted for by the fact that though the pulmonary circulation was heavily overtaxed, nevertheless capillaries and alveoli always remained unaffected in sufficient numbers to ensure the necessary aeration of the blood. There is, moreover, good reason to believe that the oil was very rapidly discharged from the lung. If the dog was killed within 24 hours of an oil injection the lungs were found overcrowded with sudanophil masses, while if 2 or 3 days had elapsed between the injection and the killing, comparatively little sudanophil material remained, and the lungs presented an appearance more nearly approaching the normal. It appears that there is a marked difference between the reaction to cholesterol in oil as shown in the lungs of the dog and that described by Klotz[3] in the lungs of the rabbit. A very careful study of a large number of sec-

tions of the lungs of all the dogs and from all parts of the lung failed to show any crystals or any doubly refracting substances. Phagocytes with granular fatty inclusions were scattered throughout the lungs, mostly in the septi, occasionally in the alveoli. Extensive areas of the lung however appeared free from phagocytes. On the other hand, it is the writer's impression that in certain localities, often near the margins, or where the pulmonary tissues had become more or less indurated by hemorrhagic infarctions and the subsequent reactive processes, and where accordingly the circulation was materially interfered with and the blood vessels were presumably no longer able to carry off the fatty material with sufficient dispatch, the phagocytes accumulated in considerable numbers, presumably to replace or rather materially to aid the work of the blood vessels. In such spots large phagocytic cells filled with granular sudanophil material are heaped in close proximity. Frequently several of these cells merge into one large giant cell, the several nuclei being distributed irregularly, some in the center and others pushed aside towards the circumference. Many large cells filled with granules of brown or blackish pigment and resembling very closely the *Herzfehlerzellen* are also found in these indurated areas.

The blood vessels in the dog's lung also showed somewhat different reactions from those described by Klotz for the rabbit. The pulmonary veins as well as the bronchial arteries appeared to be normal. The capillaries also, notwithstanding the tremendous strain to which they were subjected by the overcrowding with oil, did not show structural lesions. The smaller and smallest branches of the pulmonary artery were also without exception normal. It was only in the right and left main branches of the pulmonary trunk and their subdivisions of the second and third order that definite structural changes in the vascular walls were found. It was impossible to decide whether there was any dilatation of these larger vessels as there were no normal measurements available that might have served as a standard, but the general impression was that if any dilatation had actually taken place, it could not be very serious. Several small globular nodules were found in the two main branches, though they were not so large or so extensive as those described in the main trunk. The microscope showed that these little globules were the

result of a very marked and peculiar hyperplasia of the media. The elastic lamellæ and the muscle fibers were not arranged in the usual symmetrical layers but formed more or less regular concentric circles or in other places abutted upon each other at various angles, thus producing upon the whole the impression of a whorl of tissue. Within these peculiar nodulations some of the elastic fibers had also given way and snapped asunder and here also spaces apparently filled with plasma and containing remnants of broken elastic tissue occurred. The dislocation and forcing apart of the elements of the media, and the destruction of elastic tissue are, however, minimal when compared with the same processes as seen in the main trunk. Another peculiarity of this strange form of nodulation is, that it is practically impossible to decide where the media ends or the intima begins. No elastica interna is visible, and the innermost layers of the vessel consist of fibrous and muscular tissue all included in a fine network of delicate elastic fibers forming irregular meshes, and the whole is lined with moderately proliferating endothelium. Rather small phagocytes are occasionally, but on the whole rarely to be found, as also very minute sudanophil droplets between the cells. Very definite and typical hyperplasia of the intima, independent of those hypertrophic median nodules, is also found in the two main branches, but especially in some of the larger arteries well within the lungs (Fig. 5). Here there is comparatively little change in the condition of the media although very small spaces filled with plasma and containing fragments of elastic fibers are sometimes seen just below the elastica interna. These thickenings of the intima, though they distort the lumen of the vessel but slightly, agree accurately with the description given by Jores.[7] There is the splitting of the elastica interna, the new formation of fibrous and muscle cells, and some proliferation of the endothelium, but there is this difference, that there is no pronounced fat phanerosis or fatty degeneration. It is true that some fat phagocytes and minute fatty droplets are found in the intima and even sometimes in the inner layers of the media, but they are few in number and not constant in occurrence or regular in distribution.

[7] Jores, L., Wesen und Entwickelung der Arteriosklerose, Wiesbaden, 1903.

It is obvious, therefore, that though the pulmonary circulation was for many months overcrowded with cholesterol in oil; though there must have existed a certain amount of hypercholesteremia, as shown by the excess of sudanophil bodies appearing throughout the heart muscle, the liver, spleen, kidneys, and adrenals; and though the pressure within the pulmonary, and also, though perhaps to a lesser degree in the aortic system, must have been very great; the cholesterol evidently took no essential part in the processes that led to such profound changes in the structure of the arterial walls.

What relations if any, do these findings bear to human atherosclerosis? It is now very generally believed that the latter is an affection primarily of the intima, essentially degenerative in character, and that cholesterol and its esters are very important factors in this process. The results of the present experiments on the other hand are apparently in direct opposition to this theory. Though to the naked eye the nodules protruding into the lumen of the vessels, and also the occasional diffuse thickening of the vascular walls, closely resemble arteriosclerosis at a stage in which ulceration and calcification have not as yet begun, the real localization of the lesion is found to be mainly in the media. The structural disruptions and dislocations appear to be the result of the action of mechanical forces rather than of chemical degenerative reactions. The hypertrophy or hyperplasia of the intima is obviously merely secondary to the lesions in the media and lastly it would appear that cholesterol does not count as a factor in the process. Notwithstanding these seemingly important differences, the present writer is nevertheless of the opinion that a very close analogy, if not identity, exists between human atherosclerosis of the pulmonary blood vessels and the lesions produced experimentally in our dogs. Our knowledge of human atherosclerosis is as yet far from complete. The controversy between those who assume a mechanical disturbance as primarily the cause of arteriosclerosis, those on the other hand who are more inclined to make toxic and other chemical influences responsible, and those who, pursuing a middle course, seek to explain the sclerotic phenomena by combining in various ways the extreme views of both sides, is still unsettled. Aside from the problems of atherosclerosis in general, however, what is known of sclerosis of the pulmonary system must

be taken specially into account. It is not many years ago that it was supposed that sclerosis of the pulmonary artery, if it occurred at all, was extremely rare. Bichat[8] seeks to show that vessels carrying venous blood seldom if ever show traces of sclerosis and hence, according to him, the pulmonary artery, in which only venous blood circulates, is practically exempt. During the last 12 to 15 years the subject has been more intensely studied. A very complete review of the literature on this subject has been given by Posselt,[9] and more recently by Ljungdahl.[10]

It is fairly certain now that pulmonary sclerosis is found quite frequently if it is only carefully looked for. Genuine senile sclerosis of the pulmonary artery seems to be quite rare, but according to Ljungdahl, contrary to the view of many authors, it does occur, but mainly in association with special complications, pulmonary emphysema for instance, that tend to raise the pressure within the artery considerably. Fischer[11] found in several cases of advanced atherosclerosis of the aorta and peripheral arteries, that the pulmonary arteries were healthy, but in these cases the lungs were free from emphysema or other chronic lesions and there was no hypertrophy of the right heart. Posselt finds that sclerosis of the pulmonary artery seems to affect youthful persons more readily than is the rule in sclerosis of the aortic vessels. According to this author 97 out of 187 cases were found in individuals under 40, 125 in subjects under 50, and only 60 in persons above 50 years of age. All authors, however, seem agreed that hypertension within the pulmonary circulation is a main factor in the etiology of sclerosis of the pulmonary artery, which is generally looked upon as an *Abnutzungskrankheit* as defined by Marchand.[12] The fact frequently observed that the artery remains intact, though the blood pressure is steadily much above normal, strongly suggests that hypertension is not the only factor, but that other etiological elements, such as local or functional variations, toxic or other influences as yet unknown, are also involved. For the pulmonary artery too, the dogma that the intima is the primary seat of the pathological changes is very generally accepted.

[8] Bichat, quoted by Brüning, H., Untersuchungen über das Vorkommen der Angiosklerose im Lungenkreislauf, *Beitr. path. Anat. u. allg. Path.*, 1901, xxx, 457.

[9] Posselt, A., Die Erkrankungen der Lungenschlagader, *Ergebn. allg. Path. u. path. Anat., Ite Abt.*, 1909, xiii, 298.

[10] Ljungdahl, M., Untersuchungen über die Arteriosklerose des kleinen Kreislaufs, Wiesbaden, 1915.

[11] Fischer, W., Über die Sklerose der Lungenarterien und ihre Entstehung, *Deutsch. Arch. klin. Med.*, 1909, xcvii, 230.

[12] Marchand, *Verhandl. Kong. inn. Med.*, 1904, xxi, 23.

There are, however, some dissenting voices. Ehlers[13] finds that the human pulmonary artery differs from the aorta in that its media is made up of two distinct layers and according to him the innermost layer assumes a very active part in pathological processes. Durante[14] reports a case of congenital atheroma in a premature infant. He finds the vasa vasorum normal, the intima almost normal. The main process is in the inner third of the media where there is some necrosis and calcification. He also describes broad, almost unstainable gaps in the media. There is some secondary hypertrophy of the intima but only over points of most advanced degeneration of the media. The aorta appears macroscopically normal, but under the microscope it is seen that in its media there are similar processes developing as in the pulmonary. Except for the median necrosis and calcification, the conditions in this case seem to bear a striking resemblance to those obtained experimentally in our dogs.

These few brief notes from the literature will suffice to show that the results of the experiments here described present some obvious analogies to the sclerosis of the human pulmonary artery, but at the same time distinct differences. The proliferation of the intima wherever it is found is the exact counterpart of what is considered typical for human atherosclerosis, and in the section taken from a branch within the lung in which the media is barely affected (Fig. 5) the picture is identical with any that can be taken from an arteriosclerotic artery that has not as yet undergone an appreciable amount of degeneration. One of the chief points of difference is that in our dogs it is not the intima but the media which is without doubt the primary and principal localization of the lesion. That this is not necessarily peculiar to the dog but can be found also in human pulmonary arteries is sufficiently proved by the above quotations from Ehlers and Durante. Perhaps greater experience and improved methods may bring forward a greater number of similar human cases. Our experiments, moreover, have shown the process at a much earlier stage than is usually seen in the human. That the peculiar structure of the pulmonary artery as described by Ehlers is not to be held responsible for this localization in the media is obvious because identical lesions may be found at the same time in the aorta.

[13] Ehlers, W. E., Zur Histologie der Arteriosklerose der Pulmonalarterie, *Virchows Arch. path. Anat.*, 1904, clxxviii, 427.

[14] Durante, G., Athérome congénital de l'aorte et de l'artère pulmonaire, *Bull. Soc. anat. Paris*, 1899, lxxiv, 97.

One is apt, therefore, to recur to the original theory of Thoma[15] who assumed some defect in structure or in function of the media as the initial stage in the development of atherosclerosis. The almost uncomplicated hyperplasia of the intima as seen in some of the larger arteries within the lung itself might also be readily accounted for by the early theories of Thoma. These arteries are without doubt dilating under pressure, the current is probably considerably slower than normal, and hence the compensatory thickening of the intima. It might be assumed that the effect upon the media was comparatively slight because in arteries of that order the muscular apparatus is not sufficiently developed to exert any great resistance to the pressure within the artery. The disorganization in varying degree of elastic lamellæ and fibers is admitted by all observers but is generally conceived as taking place principally in the intima, and only to a very limited extent in the media. It is taken to be a direct consequence of fatty degeneration. According to Torhorst[16] degeneration does not commence before the completion of the hyperplastic process in the intima, and he as well as most other investigators, especially Aschoff,[17] see the cause of the breaking up of the elastic elements in the fatty degeneration of the cement (*Kittsubstanz*) that serves to hold the fibers together.

In the present set of experiments, no trace of degeneration, fatty or otherwise, could be found. The violent destruction of the elastic elements, the forcible displacement of the muscle cells, the broad gaps filled with plasma, all point to a purely mechanical force, in all probability the intense straining of the musculature of the arteries to overcome the resistance offered by the extensive blocking of the pulmonary circulation in the lung. It will be of great interest to observe whether the cholesterol injections, possibly with some modifications, and continued during a much longer period, will eventually lead to fatty degeneration and calcification, though Ljungdahl seems to believe that 7 to 8 months of increased resistance on the part of

[15] Thoma, R., Über die Histomechanik des Gefässsystems und die Pathogenese der Angiosklerose, *Virchows Arch. path. Anat.*, 1911, cciv, 1.

[16] Torhorst, H., Die histologischen Veränderungen bei der Sklerose der Pulmonalarterie, *Beitr. path. Anat. u. allg. Path.*, 1904, xxxvi, 210.

[17] Aschoff, L., Arteriosklerose, *Beiheft med. Klin.*, 1914, x, 1.

the lungs and increased pressure from the right heart is a sufficiently long time to permit degenerative changes to develop in human pulmonary arteries.

One would think that if cholesterol or its esters participate actively in causing sclerotic lesions, such activity would be plainly demonstrated by the present experiments. That cholesterol is practically inert, that no esters, at least no doubly refracting bodies, have been met with, appears to be one of the striking differences between the arterial lesions of our dogs and human atherosclerosis. This discrepancy is, however, not so great as it would seem. The modern trend of opinion, especially as represented by Aschoff, seems to be that deposits of cholesterol esters are secondary to the mechanical conditions by means of which the intima, and perhaps also the media, undergoes serious structural alterations. Plasma is forced into the intima and the amount of lipoid deposit that takes place depends largely on the cholesterol ester content of that plasma. With this precipitation of lipoids, degeneration begins, the *Kittsubstanz* is demolished, and those processes are initiated that lead to atheroma.

This conception is, however, not as yet a well grounded fact, but rather an hypothesis. It is true that cholesterol and its esters are invariably found in every case of human atheroma and that the extent of the degeneration is approximately proportionate to the quantity of lipoids accumulated within the vascular tissues, but up to date nothing definite is as yet known about the exact conditions that induce the lipoid deposits or of the specific functions of the latter. Perhaps there may be some truth in the theories propounded by Lemoine and Gèrard,[18] according to which the cholesterol acts as an antitoxin and is heaped up in the degenerating vascular tissues in order to neutralize and to assist in transporting the toxic material injected into the blood vessels by the various processes and diseases that are supposed to lead to arteriosclerosis. In this connection it is significant that Klotz[8] finds that the hemolytic properties of sodium oleate are neutralized by an adequate admixture of cholesterol. Some added interest as regards the function of cholesterol in arterio-

[18] Lemoine and Gèrard, in Bertrand, Nature de l'athérome et rôle de la cholestèrine dans sa formation, *Rev. mod. mêd. et Chir.*, 1913, xi, 380.

sclerosis may also be found in recent studies of sclerotic lesions as found in very young persons and babies, especially by Stumpf[19] and Saltykow.[20] The present writer has also examined a relatively large number of infantile aortas, of several fetuses, and also of dogs. He hopes to publish his results in the near future, but it may be stated now that in the majority of the baby aortas, no matter what the cause of death, no matter how old or how young the child, minute ridges and patches slightly elevated above the level of the intima, and of a faint yellowish color, could be found. They were most frequently found in the sinus Valsalvæ, the bulb or the arch, but often in the descending and abdominal aorta and usually in the neighborhood of the orifices of branching vessels. Under the microscope there could regularly be seen a very distinct and typical hyperplasia of the intima, a practically intact media, very minute sudanophil droplets scattered in the interstices between the elements of the intima, and also fairly large phagocytes filled with sudanophil granules. The same phenomena could with great constancy be found also in the aorta of young dogs. In these cases there were no signs of degeneration, nor did there appear to be any tendency towards atheroma, and it must be a subject for further study to ascertain the functions of the cholesterol, and for what reason and by what mechanism a baby 1 day old or a 7 months fetus had small areas of hyperplastic intima and sudanophil phagocytes in the aorta. It seems probable that the distribution and the presumptive action of cholesterol in these cases and that found in the pulmonary arteries of our dogs are similar.

As a result of this brief discussion it is believed that the arterial lesions experimentally obtained in our dogs are essentially identical with those found in the human pulmonary artery, and that for the first time atherosclerosis, very closely analogous to the human, has with certainty been produced experimentally in dogs.

[19] Stumpf, R., Über die Entartungsvorgänge in der Aorta des Kindes und ihre Beziehungen zur Atherosklerose, *Beitr. path. Anat. u. allg. Path.*, 1914, lix, 390.

[20] Saltykow, S., Jungendliche und beginnende Atherosklerose, *Cor.-Bl. Schweiz. Aerzte*, 1915, xlv, 1057, 1089.

SUMMARY.

Cholesterol dissolved in sesame oil and injected regularly for a period of from 7 to 8 months into the jugular vein of four young dogs has caused in each animal larger and smaller nodules protruding to some degree into the lumen of the pulmonary artery and also here and there some diffuse thickenings, the whole closely resembling human arteriosclerosis. These changes commence at the origin of the pulmonary artery immediately behind the semilunar valves. They seem a little more pronounced in those dogs that had the aorta tied. Microscopically the lesions are seen to be primary in the media. They consist of a more or less violent disorganization of the elastic elements and displacement of the muscular tissue. As a consequence wide gaps, apparently filled with plasma, are formed in the media. The entire process is localized principally in the inner third of the media. There is no evidence of fatty degeneration either in the elastic elements or the muscle. Typical sclerotic hyperplasia of the intima is found over some of the prominent median nodulations while over others the intima is normal. Hyperplastic sclerosis of the intima is also found in places where the media is intact or only slightly damaged. Some of the larger branches of the pulmonary artery well within the lung tissue are somewhat dilated and show extensive hyperplasia of the intima over almost normal media. Peculiar small nodules are seen in the media of the two main branches of the pulmonary artery which are found to consist of frank hyperplasia of the elastic and muscular elements arranged in more or less regular concentric circles. The capillaries and smaller and smallest arteries within the lungs are not affected. Cholesterol in phagocytes and in droplets between the tissue elements is present in very small quantities and does not take any active part in the process. The entire process represents in all probability an early stage of arteriosclerosis, a result of purely mechanical stress brought about by the very extensive but more or less intermittent blocking of the pulmonary circulation by the oil. The musculature of the artery appears to be the main force applied to overcome the resistance in the circulation. In no case has hypertrophy of the right heart been found.

The lesions in the pulmonary arteries of the dogs produced experimentally are closely analogous to atherosclerosis of the human pulmonary artery.

EXPLANATION OF PLATES.

PLATE 40.

FIG. 1. Left heart and aorta of a dog whose aorta had been tied, showing sclerotic nodules behind the cusps and ridges in the further course of the vessel.

FIG 2. Extensive sclerosis of the pulmonary artery, possibly some dilatation of the right ventricle.

PLATE 41.

FIG. 3. The widespread disorganization of the media, the large gaps containing fragments of elastic fibers, and faintly stained coagulated material are shown. Muscle cells normal, extensive splitting of elastica interna. No change in the intima. Hematoxylin and Sudan III; Zeiss oc. 4, apochromatic 3 mm.

PLATE 42.

FIG. 4. Frozen section. The disruption of the inner portions of the media and moderate hyperplasia of the intima are shown. The elastica interna is only partially intact. Hematoxylin and Sudan III; Zeiss oc. 4, apochromatic 8 mm.

PLATE 43.

FIG. 5. Paraffin section of a branch of the pulmonary artery within the lung. Typical sclerotic hyperplasia of the intima, some disorganization of the inner portions of the media. To the right of the picture the intima with its endothelium is seen to be perfectly normal. Hematoxylin and eosin; Zeiss oc. 4, apochromatic 16 mm.

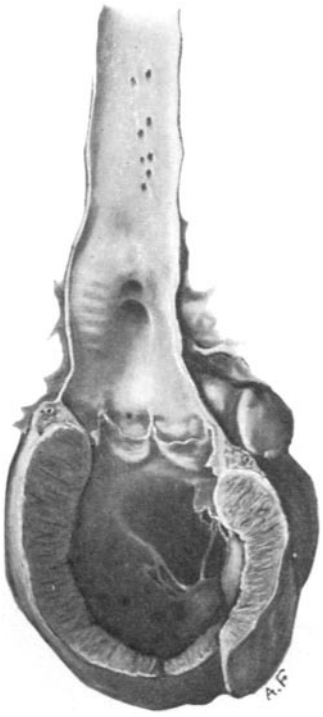

FIG. 1.

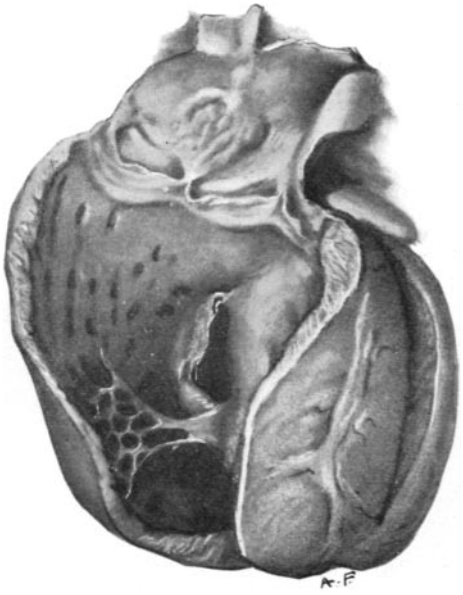

FIG. 2.

(Adler: Experimental atherosclerosis.)

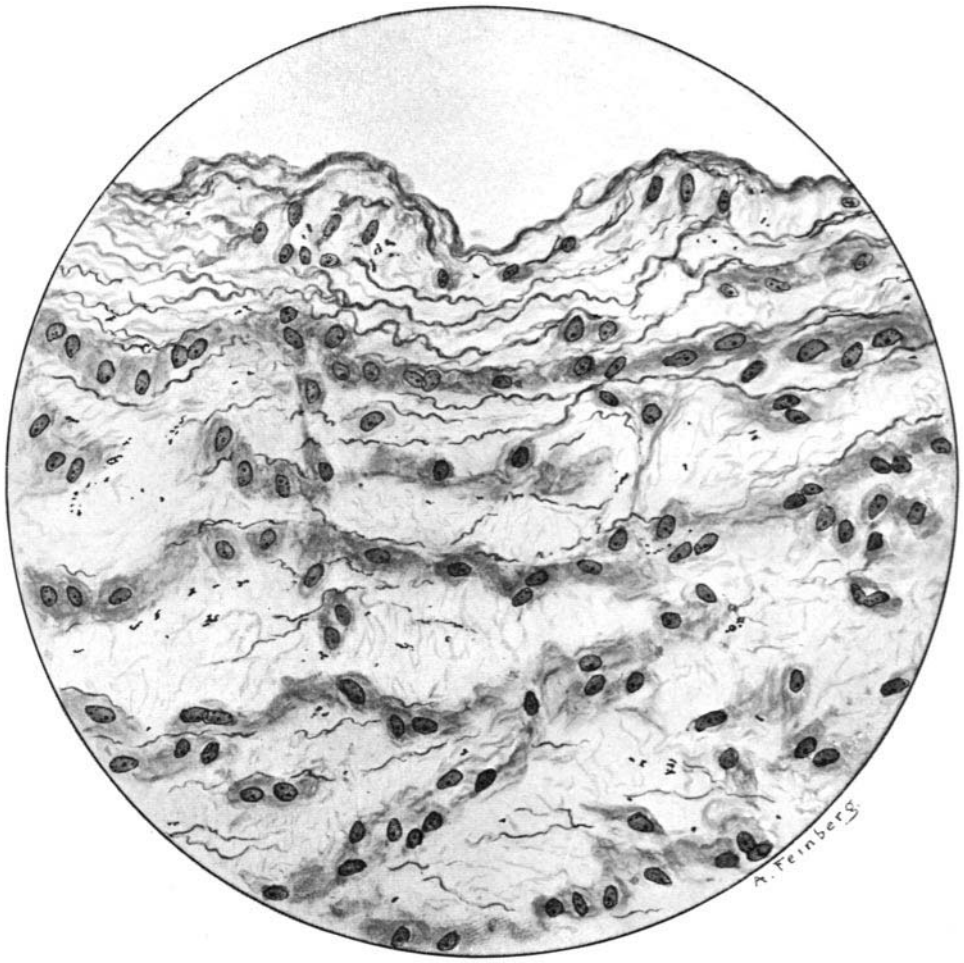

FIG. 3.

(Adler: Experimental atherosclerosis.)

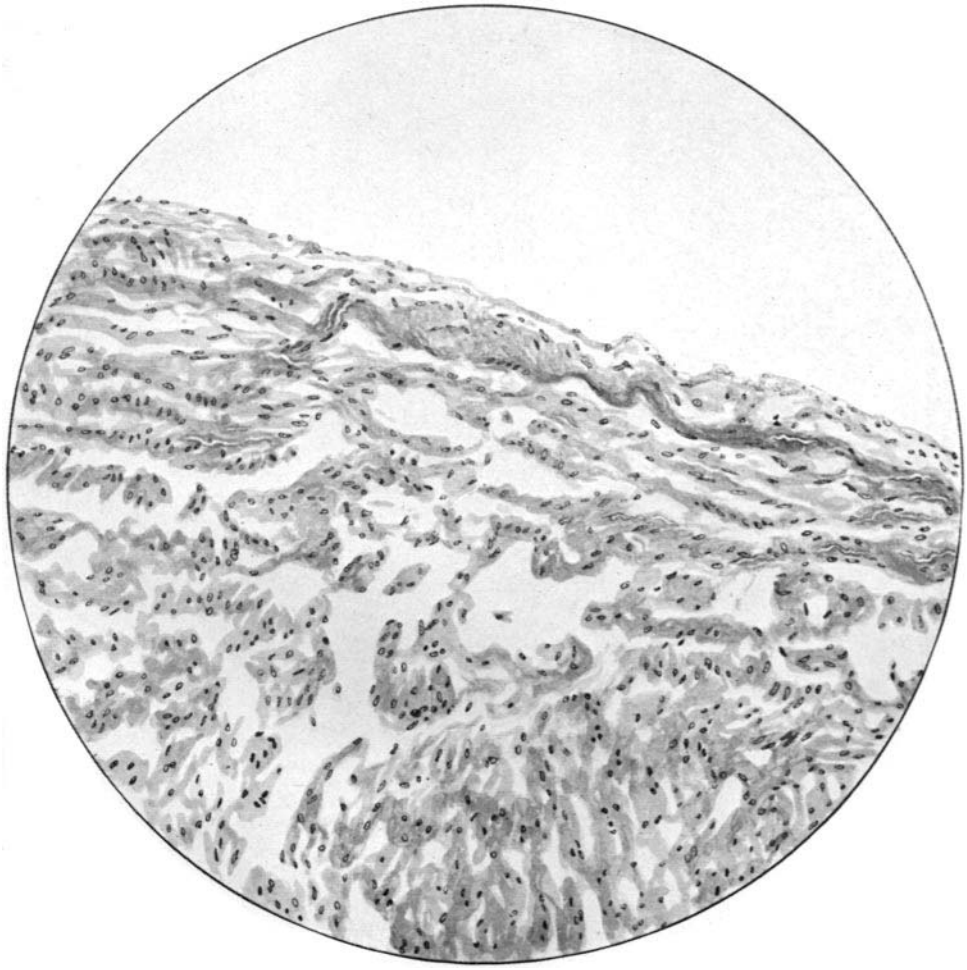

FIG. 4.

(Adler: Experimental atherosclerosis.)

FIG. 5.

(Adler: Experimental atherosclerosis.)