# EXPERIMENTAL ARTERIOSCLEROSIS DUE TO HYPERVITAMINOSIS D

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Chronic progressive vascular disease resembling human athero-arteriosclerosis has not been produced in experimental animals. Fibro-calcific degenerative and reparative sequences similar to those occurring in the human disease may follow experimental arterial medial injury in animals.[1] Other sequences characterized by lipophage accumulations and atheromatous deposits resembling those in man may be induced by hyperlipemia and hypercholesteremia in animals.[2] Lesions which still more closely resemble those in man may be produced in animals by the simultaneous combination of local arterial medial injury, hypercholesteremia and hyperlipemia.[3,4]

These observations on locally induced lesions have indicated that experimental production of a generalized arterial medial disease combined with appropriate alterations in the lipid composition and dynamics of the blood might lead to a better understanding of the pathogenesis of human athero-arteriosclerosis.[5] It has been shown that generalized arterial medial disease follows the administration of excessive amounts of irradiated ergosterol or Vitamin $D_2$ in several animal species and man.[6-8] In undertaking the experimental plan outlined above, a systematic study of hypervitaminosis D in rabbits was carried out.[9] The present report is concerned principally with pathologic alterations found in the vascular system in hypervitaminosis D and comparison of these changes with those commonly found in human arteriosclerosis.

## METHODS

Male albino rabbits from a common stock, about 5 pounds in weight and 3 months of age, were used. They were maintained on a standard Purina Rabbit Pellet diet supplemented with fresh vegetables.

The irradiated ergosterol was dissolved in peanut oil (15 mg. per ml.; supplied through the courtesy of Abbott Laboratories, North Chicago, Illinois). The Vitamin D potency of this solution was $10^6$ U.S.P. units per ml. This was given intramuscularly in doses of 0.1 ml. ($10^5$ U.S.P. units) at daily, biweekly or triweekly intervals. The regime was varied so that minimal and maximal pathologic features could be defined and sequences in their evolution recognized. At first, the dosage was regulated at levels just below the quick lethal range. Pathologic changes developed rapidly, and

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most animals lost weight and died within 6 weeks. Later on, the regime was regulated to facilitate analysis of minimal, sublethal and early pathologic effects, so that animals were either sacrificed at an early stage or given a small dose and sacrificed at a later stage. Finally, the regime was modified so that rest periods of 2 or 3 weeks were alternated with dosage periods of 1 or 2 weeks. This allowed the development of chronic forms of the disorder, lasting several months.

Analyses for serum calcium, total phosphorus and inorganic phosphorus were done at intervals of 1 to 2 weeks.

At the end of each experiment a necropsy was done and a record made of the gross observations. Samples of all tissues and organs were fixed in formaldehyde (4 per cent, U.S.P.) and subsequently prepared for microscopic examination. Transverse sections were also made through the aortic arch, upper and lower abdominal aorta, common carotid artery, brachial artery, pulmonary artery, hilar pulmonary vessels, renal artery, common iliac artery, femoral artery and vessels at the hilus of the spleen, liver and kidney. Ninety-seven animals were useful for the purposes of this report, with studies of 82 additional animals on other dietary and viosterol regimes available for supplementary data. For control purposes, we made complete microscopic studies of 78 normal rabbits from the same stock, of the same age distribution and on the same diet.

## RESULTS

### HISTOLOGIC SEQUENCES IN THE WALLS OF ARTERIES

#### *Adventitia*

Involvement of the adventitia was uncommon. When lesions did occur, two independent sequences were recognized. The most important was essentially inflammatory. It began as an acute or subacute periarteritis or panarteritis of low intensity characterized initially by edema and nodose swelling of collagen in local areas.[9] These changes were accompanied or followed by a meager infiltration of the arterial wall and adventitia by heterophils and macrophages. The process ordinarily subsided in the media and intima without producing much structural damage but tended to persist in focal areas in the adventitia where collagen deteriorated in the midst of locally proliferating histiocytes. Occasionally, the proliferative reactions centered about degenerating cardiac or skeletal muscle cells adjacent to foci of adventitial inflammation. The deterioration of collagen, the histiocytic reaction and the incorporation of regional muscle cells in the periarterial response resembled alterations often encountered in acute rheumatic myocarditis in man. The arteritis, with rare exceptions, was encountered only around vessels in cardiac and skeletal muscle. Extramuscular, small arterial branches and large arteries leading to muscle were not involved.

The second type of lesion in the adventitia was a vascularizing stromal reaction (Figs. 4 and 8). This was secondary to medial degeneration and occurred especially when degenerative-calcific changes in the media extended to the adventitia. These changes stimulated stromal penetration

and early vascularization of the media by adventitial elements which grew in and around the degenerated calcified zones. Thereafter, there was fibrosis of the media and slow resorption of medial calcific deposits with occasional foci of osteogenesis. This type of reaction was confined principally to the external third of the aortic media and the degenerated calcified media of the iliac and femoral arteries (Figs. 4 and 8).

### *External Elastic Membrane*

The external elastic membrane of most arteries was usually uninvolved even though the adjacent adventitia and media showed lesions (Fig. 7). At times, however, the membrane was lightly basophilic. This alteration predisposed to mineralization, followed by transverse fragmentation of the membrane and stromal ingrowth into the media from the adventitia (Fig. 8).

The renal artery was the only vessel which showed conspicuous elective lesions in the external elastic membrane, but, even in this instance, the internal elastic membrane and media were always severely affected. There was evidence that preservation of anatomic continuity and structural integrity of the external elastic membrane in the presence of severe medial disease was a deterrent to stromal penetration and vascularization of the media by activated adventitial elements.

### *Media*

The patterns of involvement of the media varied from one artery to another. In general, however, they were similar in the walls of arteries with the same dimensions and structure.

*Elastic Arteries.* The elastic arteries showed two forms of medial alteration. The common form began with swelling of the spaces between the innermost elastic membranes (Fig. 5). These spaces, presumably swollen from an increment of ground substance, were traversed by a delicate meshwork of barely recognizable fibrils. Fine granules of calcium were deposited on these fibrils and in the ground substance between them (Figs. 3 to 6). The deposit was more conspicuous where fibrils were condensed along and in elastic lamellas. It appeared, therefore, as though the elastic lamellas were impregnated with calcium on their surfaces (Fig. 3). Close inspection, however, disclosed a delicate, intrinsic fibrillar pattern as a locus for calcium deposition throughout many apparently solid elastic membranes (Fig. 4). As calcium accumulated in these locations, the intervening fibrocytes and smooth muscle cells were either undergoing degeneration or being replaced by large pale cells with hyperchromatic nuclei resembling hypertrophied, immature smooth muscle elements (Fig. 5). The degeneration of cells was

closely related spatially and temporally to that of calcium deposition, and cell regeneration was stimulated only when the deposition of calcium was not excessive.

As the deposition of calcium increased, there were several different sequences. In one instance, the calcium deposits seemed to shift from the interlamellar ground substance to the surfaces of regional structures (Figs. 3 and 4). The order of increasing affinity for calcium among these structures seemed to be as follows: first, elastic tissue; second, margins and processes of smooth muscle cells; third, collagen; fourth, fibrocytes and their processes. In another instance, interlamellar cells disappeared and calcium was deposited almost exclusively in elastic lamellas (Fig. 3). In the third instance, all structure was solidly incorporated in a diffuse deposit of calcium (Figs. 2, 4 and 8).

As stated previously, these sequences in calcification in the elastic arteries were found in two locations. The more common distribution was in the inner third of the media. Here, as the disorder progressed, there was essentially a wave of calcium deposition spreading deeply from the intima into the media (Figs. 3 and 6). The process resembled a slow diffusion from the blood toward the adventitia with decreasing amounts of calcium being deposited as the distance from the blood increased.

The second common distribution in the media was in the middle third of the wall. Here, there were discontinuous deposits which tended to encircle the vessel and to extend in the long axis, eventually merging with one another (Fig. 2). These were in the nature of enlarging bands of calcium deposits, which had a complex arrangement following the distribution of smooth muscle.

The best examples of elastic arteries with these lesions were the aorta, carotid arteries and pulmonary arteries. The changes were most conspicuous in the aorta. The inner wave of calcium deposition seldom spread into the outer third of the media of the proximal aorta (Fig. 3). With increasing distance from the aortic arch, the actual depth of penetration diminished slightly, but because of the decreasing thickness of the aortic wall, the relative depth of penetration increased. Hence, whenever calcium deposition extended beyond the inner third of the media of the aortic arch, the entire thickness of the wall of the lower abdominal aorta was usually calcified. The bandlike deposits of calcium were usually more prominent in the upper aorta. Though generally more common in the middle third of the media, the bands at times spread internally to the intima and externally to the adventitia (Fig. 4). In the latter instance, adventitial stromal reactions with vascularization of the media often occurred. Vascularization from the intimal side seldom occurred and was never conspicuous.

The pulmonary artery, except in the main trunk, was usually unaffected. Here, there were occasional focally distributed inner medial calcific plaques, rarely extending beyond the innermost 4 or 5 elastic lamellas. Extension of calcification into the secondary branches was encountered only in cases with severe alveolovenous calcific disease which in turn predisposed to chronic progressive emphysema.

The carotid arteries were usually severely affected. Though there was a recognizable combination of the inner and central patterns of medial disease, the inner pattern predominated and not infrequently spread, as in the lower aorta, throughout the thickness of the wall to the adventitia. We have no data on the changing pattern of the disorder along the course of the common carotid artery and its branches. However, the ophthalmic and cerebral arteries were always spared while the arteries to the salivary glands were usually severely affected.

*Musculo-elastic Arteries.* The pattern of lesions in the musculo-elastic arteries showed a gradient of change from the type found in elastic arteries to that encountered in large muscular arteries. In the axillary, common iliac, and upper femoral arteries, the inner and central patterns of medial alteration tended to merge with one another. Here, as the internal elastic membrane and smooth muscle became more conspicuous and the elastic tissue lamellas in the media less conspicuous, the pattern of medial alteration changed. The continuous wave of calcium deposition in the inner media was replaced by a periodically spaced local process which followed a spiral or bandlike distribution (Fig. 2). The earliest evidence of this process was either in or just beneath the internal elastic membrane (Figs. 6 and 7). It began locally as an interstitial swelling and fusion of fibrils. Calcium was deposited, and the deposits spread in depth as well as in the long axis of the vessel, with muscle cells often showing a remarkable persistence while the intercellular spaces and tissues were being occluded or impregnated with calcium. As the deposits spread, the contiguous calcified bands enlarged in depth and breadth, finally fusing to form a more or less continuous sheet of calcium from internal elastic membrane to adventitia (Fig. 8).

*Muscular Arteries.* The principal difference between different systems of musculo-elastic and muscular arteries was due to variation in the degree to which the media of the artery was affected by progression of the above process. For instance, the intensity of the process fell off very rapidly with successive divisions of the two primary coronary arterial branches, even though there was no significant change in vascular structure. On the contrary, the process increased in intensity along the course of arteries to the kidneys, the acid-secreting portion of stomach, the salivary glands, duodenum, colon and thyroid (Figs. 1 and 9). In other

systems it increased or decreased in an unpredictable manner in different animals. In still other systems, arteries were never affected, though they were of the muscular type with essentially the same structure or dimensions as arteries which elsewhere were regularly affected.

*Arterioles and Capillaries.* These vagaries of distribution were still more accentuated when attention was directed to the lesions in the media of arterioles. The distribution in arteriolar walls was essentially the same as in larger muscular arteries. As a rule, though, there was a greater degree of alteration from one arteriole to another in a given system than from one muscular artery to another in the same system. The earliest lesions, however, were in the same location, namely in a focal subintimal position. From here, the changes spread so that the walls of most affected arterioles became diffusely impregnated with calcium and were thicker than normal, with a corresponding reduction in the diameters of their lumens (Fig. 10).

The frequency and magnitude of involvement of arteriolar systems in different locations were in the following diminishing order: inner third of the renal cortex, acid-secreting part of the gastric wall, wall of the first part of the duodenum, salivary glands, spleen, skeletal muscle, cardiac muscle, renal pelvis, outer two thirds of the renal cortex, bone marrow, thymus, thyroid, colon, adipose tissue and pancreas. Arteriolar systems in the following locations were never involved: brain, eye, spinal cord, pituitary, adrenal, testis, liver, non-acid-secreting part of the gastric wall, esophagus, ileum, lung, ureter, fascia, tendons, synovial membranes, intervertebral disks and skin of the ear.

At times, there were lesions in the distal arterial system beyond the larger muscular arterioles. The changes, not necessarily associated with severe alteration of the larger arterioles, consisted of calcific deposits in the subendothelial tissues and membranes. Because of the thinness of the vascular walls, it was often impossible to recognize calcium deposits in capillaries beyond the terminal arteriolar system. Suffice it to say that such deposits were at times easily recognized in skeletal muscle, spleen, thyroid, acid-secreting gastric mucosa, bone marrow, thymus, and retroperitoneal fat tissue. It is possible that calcium deposits were usually there but were beyond the limits of detection by the microscopic methods used. The arteriolar-capillary deposits were especially interesting in the glomerular tufts (Figs. 9 and 10). Here, isolated arteriolar loops were primarily affected. Occasionally, most of the glomerular arteriolar structure was involved so that subendothelial membranes were sharply outlined by calcium deposits, but glomerular alterations occurred only when the arterial system leading to the glomerulus was also calcified. In the spleen the calcium deposits occasionally extended

beyond the limits of arterioles and impregnated the reticular structure of lymphoid follicles while the large vascular sinuses of the red pulp were spared. In the thyroid, skeletal muscle and other previously specified locations, the walls of capillaries were occasionally outlined by thin subendothelial lines of calcium, but this was usually recognizable only in animals with severe generalized disease, especially in small arteries and precapillary arterioles in tissues showing capillary calcification.

### *Internal Elastic Membrane*

There were two divergent alterations in the internal elastic membranes of musculo-elastic and muscular arteries or arterioles. The usual type of change was characterized by the following sequence. First, the customary regular undulation of the membrane was replaced by an irregular undulation with linearity along short stretches (Figs. 5 and 6). These zones, which were usually discontinuous but often regularly spaced in the circumferential and long axes of the vessel, acquired an affinity for hematoxylin, interpreted as evidence of early calcification (Figs. 6 and 7). This was followed by a tendency for transverse discontinuities or fractures to develop, and calcium deposits became increasingly conspicuous in or on the membrane (Figs. 5 to 7).

The second type of lesion in the membrane resembled the one just described up to the point of modification of the staining affinities. Then, rather than acquiring an increasing affinity for hematoxylin, the membrane developed an increased affinity for eosin (Fig. 5). At the same time, its optical properties changed and it became more homogeneous and refractile. This modification was not accompanied by development of sharp transverse fractures. On the contrary, the discontinuities which appeared as this process increased in severity were the result of attenuation and fraying of the membrane (Fig. 5).

A detailed analysis of the distribution of the two types of lesion in the internal elastic membrane has not been made. Some arterial and arteriolar systems were more susceptible to one type of change than the other. It was expected that the nature of the alteration in the internal elastic membrane might reflect the nature of changes in the elastic membranes in the media. This was not found. The lesions in the media were essentially the same, irrespective of the direction of change in the internal elastic membrane. There was, however, a fairly consistent spatial relation between the deteriorative alterations in the membrane and degenerative-calcific changes in the subjacent medial structure (Figs. 5 to 7). This was easily recognized in the femoral, brachial, splenic, gastric and other large muscular arteries.

### *Intima*

Pathologic changes in the intima were spatially related to alterations in the internal elastic membrane and subjacent media. The sequences in the development of these lesions were as follows: The first recognizable changes were in the internal elastic membrane and subjacent media as previously described. (Fig. 5). These were usually focal and spaced rather regularly. As they increased in severity, proliferative reactions often occurred in the intima. The early proliferative reactions were essentially fibroblastic in character (Fig. 5). The final product of the reaction was usually a diffuse or focal avascular fibrous structure which at times was as thick as the original vascular wall (Figs. 6 to 8). In some instances, newly formed elastic tissue appeared among the collagenous elements. At other times, there was a differentiation of the proliferating fibroblasts into smooth muscle cells. This was common in the large renal, iliac and femoral arteries. At still other times, the thick avascular intima showed degeneration in cells and stroma, with accumulation of lipids and calcium. As a rule, however, the proliferative reactions were essentially in the direction of the formation of fibrous plaques of increasing thickness, apparently limited by a surprisingly consistent refractoriness to vascularization (Fig. 8).

A curious feature of this reaction was the lack of correlation between the degree of intimal proliferation and the degree of abnormality of the internal elastic membrane and media. In other words, though the intimal proliferation was always spatially related and temporally secondary to the subjacent disorder, the occurrence of severe changes in the internal elastic membrane and media was often unaccompanied by any intimal proliferative reaction. Several factors seemed to govern this matter. First, some vessels were more reactive than others. For instance, intimal proliferation in the arch of the aorta was much less than the proliferative reaction in the low thoracic and abdominal segments (Fig. 3). Also, proliferative reactions were conspicuous in large muscular branches such as the iliac, renal, femoral, splenic and gastric arteries (Figs. 2 and 6 to 8). The reactions were usually minimal or absent in small muscular arteries and arterioles even in the presence of severe changes in the internal elastic membrane and the media (Figs. 9 and 10). Second, the distribution and rate of development of degenerative-calcific alterations in the vascular wall seemed to influence the intimal reactions. Local changes were a greater stimulus than general changes. Slowly progressive lesions were a greater stimulus than acute transient lesions. Finally, the development of sharp discontinuities in the internal elastic membrane, especially of muscular arteries, was an important stimulus

to intimal proliferation (Figs. 5 to 7). In these instances the fractures in the membrane widened, and the defects so created were filled in by fibroblasts and collagen which spread progressively in an apparent attempt to bridge the defects (Fig. 5). This led to the formation of separate or fused continuous intimal plaques which required several weeks to reach maximum thickness. In addition to the factors mentioned, intimal proliferation was regulated by other conditions which are still obscure. One of these was the alternation of periods of high viosterol dosage with periods during which viosterol was not given. A chronic intermittent state of hypervitaminosis D was a more effective stimulus to intimal proliferation in small arteries than a chronic persistent state. In no case, however, did the newly formed intima exceed the thickness of the arterial wall (Figs. 6 to 8).

One other form of intimal reaction should be mentioned. It consisted of enlargement of single isolated endothelial cells. These cells had a strong affinity for hematoxylin, and as this affinity increased, each cell was transformed into a series of concentric sphero-crystals with the nucleus of the cell at the center and the cytoplasmic membrane at the periphery. These cells were conspicuous in renal glomeruli and often were the only part of the glomerulus involved by calcium deposits. They were found at times in other small vascular channels, especially in the lungs. Here, they were most easily recognized in the mucosal vessels of the trachea and large bronchi. In this location they were usually in the endothelium of the vascular wall adjacent to stretches of calcium deposits in the subepithelial tissues of the respiratory mucosa. The significance of these cells was not clear. Their location and structure indicated that they belonged to the reticuloendothelial system. Presumably, they had been activated to engulf calcium and had progressively accumulated calcium in sufficient quantities to undergo crystallization as the cell became nonviable. There was no clue as to why the reticuloendothelial system should have these activities restricted to only a few special locations.

### DISTRIBUTION OF THE DISORDER IN DIFFERENT ARTERIAL SYSTEMS

The pathologic alterations appeared first in the proximal aorta. With increasing duration and intensity of the disorder, the changes spread along the aorta and became more conspicuous in its principal branches.

The same general pattern of spread was encountered in the coronary arterial system. Lesions appeared first near the arterial orifices, and here medial disease was often very pronounced. The magnitude of the process decreased in the direction of the blood flow in most instances, but in some cases with active or healed arteritis and myocardial calcification, the intramyocardial branches were more severely affected than the larger vessels.

The common carotid arteries were regularly involved whenever there was a significant amount of aortic abnormality. Pathologic changes usually decreased in the direction of the blood flow. Somewhere along the course of the internal carotid artery, lesions disappeared, because no abnormality was found in the ophthalmic or cerebral arteries. The disorder persisted to a variable degree along the course of the external carotid artery and its branches. This persistence was selective because alterations in the arteries of the submaxillary gland were far more severe than those encountered in other branches of the external carotid arterial system.

The branches of the thyroid axis were not examined in detail, but the thyroid artery was always involved in moderately severe cases. The vertebral arteries were not examined distally, but in no case was there any abnormality of the basilar artery.

Lesions in the axillary, brachial, iliac and femoral arteries were similar. They decreased in severity in the direction of the blood flow. The magnitude of disease at comparable levels was greater in the arteries of the lower than upper extremities. As a rule, evidence of the disorder decreased to zero along the first or second divisions of the muscular branches of the brachial and femoral arteries. However, in most instances of arteritis or calcific myositis, alterations in arteries again became conspicuous as the extramuscular branches passed into the muscle. Successively smaller intramuscular divisions then showed lesions of increasing severity.

The large branches of the descending aorta were usually involved although the intercostal arteries seldom had significant changes. The branches of the celiac axis were about equally affected at their origins. The severity of the process increased along the course of the left gastric and splenic arteries while it decreased along the course of the hepatic artery. Divisions of the hepatic artery entering the liver and all subsequent branches were normal. Divisions of the splenic artery leading to the pancreas showed diminishing alteration and a transition to normal vessels at the lobular level. Divisions of the splenic artery entering the spleen frequently showed increasing severity of lesions down into the terminal arterioles in lymphoid follicles. Divisions of the left gastric artery showed a persistence of moderate abnormality which increased greatly in severity along the branches which penetrated the muscular wall of the acid-secreting part of the stomach. With subsequent divisions down to arterioles passing just beyond the muscularis mucosae into the mucosa, the disorder increased progressively in severity but again diminished in the smaller mucosal vessels between the glands. The distribution of these vascular changes was precisely defined. They did not

extend beyond the acid-secreting mucosa or the subjacent submucosa, muscularis mucosae, and tunica muscularis. All arteries in the remainder of the stomach, especially of the pyloric region, were normal. However, just distal to the pylorus, the mucosa and subjacent wall of the first part of the duodenum were involved by arterial lesions similar to and only a little less severe than those in the acid-secreting part of the gastric wall.

The superior and inferior mesenteric arteries were not regularly examined microscopically, but there were often gross alterations which diminished along the course of the branches. A study of the smaller divisions in sections of the ileum and colon showed minimal changes or normal structure. No arteriolar involvement was recognized in the ileum, but in the colon severe lesions were occasionally conspicuous.

The main adrenal arteries were not examined regularly, but there was no arterial abnormality in the adrenal glands.

The main renal arteries were less severely affected than other major branches of the aorta. The principal divisions usually showed a slight to moderate alteration, but as these branches divided to enter the kidney near the corticomedullary junction (Fig. 1), severe arterial lesions became conspicuous and spread along the first intracortical branches and arterioles leading to the proximal glomeruli (Figs. 9 and 10). As the nephrons adjacent to the corticomedullary junction became inactivated, the disorder spread in continuity along the main trunks, branches and preglomerular arterioles toward the peripheral third of the cortex but seldom affected it except in instances of prolonged chronic disease.

Arterial systems of the testes, ureters, skin, bone, skeletal muscle, brain, spinal cord, salivary glands and other structures were regularly investigated. There was no abnormality of arteries in or at the hilus of the testis. The arteries and arterioles along the ureters were normal. The arterioles and precapillary arterioles in the skin of the abdomen were occasionally involved but only in the most severe cases. Only the large central artery of the ear ever showed abnormality. The arteries and arterioles in the bone marrow were regularly affected while those to synovial membranes and intervertebral disks were spared. Changes in the vessels in skeletal muscle were usually found in many locations. The brain, spinal cord and their membranes had no trace of vascular disease. The large arteries leading to the salivary glands were often either normal or only slightly involved by the process. As these divided into multiple branches within the gland, the severity of the disorder often increased distally so that many small arteries and arterioles along the minor ducts were solidly calcified, and the lesions could occasionally be traced into the walls of the smallest vascular divisions between the

secreting glands. Under these conditions atrophic changes in the lobules of the gland became conspicuous, and, at times, basement membranes of atrophic glands were outlined by deposits of calcium.

The general distribution of lesions in the pulmonary vascular system also deserves some comment. The main trunk of the pulmonary artery occasionally had a few scattered superficial foci of calcification in the inner media. These were less common in the two main branches. Except when there was chronic pulmonary emphysema, the intrapulmonic branches remained normal to the level of the interalveolar capillaries. Then, in some animals, calcium deposits appeared in the walls of the capillaries. These deposits were irregularly distributed and were conspicuous only in animals with severe generalized disease. The capillaries were tributaries to small venules and thence to veins which also had a variable but often severe degree of degenerative-calcific medial alteration. Ordinarily, these lesions continued along the main pulmonic veins to the left auricle of the heart. The venous changes were reactionless and, in contrast to arterial lesions, were unaccompanied by intimal proliferative responses.

## Discussion

Generalized arteriosclerosis in man is represented by many combinations of different types and distributions of chronic vascular lesions. As a rule, the lesions are noninflammatory in nature and they exhibit a mixture of chronic progressive degenerative and reparative sequences which become more conspicuous with advancing age.[10–12] The disorder is never identical in any two people. This lack of uniformity has stimulated experimental work designed to segregate and analyze its elementary aspects. One aspect is intimal atheromatosis, and this has been investigated in several animal species with hyperlipemia or hypercholesteremia induced by different methods.[2] A second aspect is intimal fibro-elastosis; this has been studied principally by creating conditions which cause local inflammation or degeneration of the arterial wall. Still another aspect is medial degeneration. This has been produced locally and systemically by many methods.[1,4,12,13] As a rule, however, published descriptions of the distribution and evolution of experimental vascular lesions have not been given in sufficient detail to justify comparisons with analogous lesions in the common forms of human arteriosclerosis. We are confronted, therefore, with many vascular alterations which can be produced experimentally but with little evidence that these have any relation to generalized arteriosclerosis in man. The deficiency of evidence is not only in absence of proof of a common pathogenesis but also in lack of proof of a common pattern of structural change.

The occurrence of arteriosclerosis in animals or man with hypervitaminosis D does not justify an assumption that this disorder is connected with the pathogenesis of the usual forms of human arteriosclerosis. The occurrence may justify an assumption, however, that a metabolic derangement similar to that in hypervitaminosis D but of another origin may be a factor in the pathogenesis of human arteriosclerosis. This assumption may be strengthened by emphasizing the similarity and minimizing the dissimilarity of pathologic changes in the experimental and human disorders.

The first basic point of similarity is the tendency for both disorders to be generalized throughout the major divisions of the systemic arterial system. This tendency was less pronounced in the experimental disorder than is the rule in the common forms of human arteriosclerosis. It must be remembered, however, that the duration of the experimental condition was brief, and had it been extended throughout the normal life span of the animal, a more widespread distribution might have occurred. At least, long duration seems to be an important factor in the evolution both of human arteriosclerosis and experimental hyperlipemic atherosclerosis.

A second point of similarity is the tendency for lesions in this experimental disease and human arteriosclerosis to be of maximal severity in certain arterial systems and minimal or absent in other arterial systems having essentially the same dimensions and structure. In man, the relative magnitude of the disease in different systems follows no consistent pattern from patient to patient.[11] In the experimental disorder the relative magnitude of disease in different systems was surprisingly uniform in animals subjected to the same regime. Even though the relative magnitudes of involvement of different arterial systems in man varies from person to person, there are certain systems which are statistically more susceptible to arteriosclerosis than others. In general, the aortic, coronary, renal, cerebral, femorotibial and splenic systems are among the more susceptible ones. In the experimental animal, all these except the cerebral and tibial arteries were also quite susceptible. In man, the intrahepatic, pulmonic intra-adrenal, intramyocardial, dermal, hypophysial and bone marrow systems are among the least susceptible. In the experimental disorder this was also true with the possible exception of the intramyocardial and bone-marrow systems.

Although these comparisons indicate some differences in the elective distribution of the experimental and the human lesions, they fail to emphasize that, exclusive of arteritis, the major pathologic changes in the peripheral arterial systems of animals were in the kidney, the wall of the acid-secreting part of the stomach, the wall of the duodenum,

the spleen, the salivary glands and the pulmonary venous system. Such a distribution of major peripheral vascular changes never occurs in man, except perhaps in rare instances of hyperparathyroidism, hypervitaminosis D, or other conditions productive of the syndrome known as "calcium metastasis." A careful examination of this syndrome, however, discloses that the magnitude and distribution of lesions cannot be accounted for by the theory intended to explain them.[14] Furthermore, by appropriate control of the degree and duration of hypervitaminosis D, a pure vascular disorder without resemblance to "calcium metastasis" in its distribution can be produced.

The third point of similarity between the experimental and human disorders is the tendency for the intensity of the vascular lesions to vary greatly among similar branches of an affected arterial system. This variation is always present in vessels of all dimensions in human arteriosclerosis where two arterioles of identical structure arising from a single vessel are commonly seen. One arteriole may show severe alterations along its course, and the other may retain a normal structure. This characteristic of human arteriosclerosis was equally conspicuous in the experimental disease. It was well shown in small arteries and arterioles in cardiac muscle, skeletal muscle, renal cortex, salivary glands and gastric wall. It was especially impressive in the arborization of radial arteries in the renal cortex. Here, in the same microscopic field, normal and severely affected arteries, arterioles, and glomerular tufts were encountered side by side, the overall degree of involvement diminishing as the periphery of the cortex was approached.

A fourth point of similarity between the two disorders is that the degenerative sequences and processes of repair are occasionally complicated by inflammatory reactions. In human arteriosclerosis these reactions are seldom conspicuous in the smaller divisions unless some unusual factor is superimposed. In the larger divisions, inflammatory reactions are common, especially in the adventitia of the aorta and coronary arteries, when the mural degeneration is severe and accompanied by more than the usual amount of necrosis or mural thrombosis with vascularizing reaction. Secondary inflammatory reactions were also occasionally encountered in the experimental disorder, but the common primary reactions which either accompanied or preceded the degenerative-calcific changes in arteries in the myocardium and in skeletal muscle are not a feature of human arteriosclerosis. These intramuscular arteritic and periarteritic reactions were not a necessary feature of the experimental disease, for they were dependent on an excessive dosage regime and perhaps an intercurrent pulmonary infection. Hence, there was no evidence that inflammation in the usual

sense was any more significant in the pathogenesis and evolution of the experimental disorder than in human arteriosclerosis.

The final important point of similarity is the close resemblance between the microscopic sequences in the Mönckeberg type of human arteriosclerosis and sequences in the evolution of the experimental disease in larger muscular and musculo-elastic arteries.[15] The Mönckeberg type of deterioration and calcification of the internal elastic membrane and media with subsequent intimal proliferative reactions was reproduced in close detail in the experimental animals. There was, however, one conspicuous difference between the degenerative-calcific condition of the media of human arteries and that of the experimental animals. In man, the degenerative-calcific changes tend to gradually decrease as the arterial branches of an affected system decrease in dimensions. Calcium deposits become hardly recognizable in small branches, and the degenerated walls of small arteries and arterioles often acquire a homogeneous hyaline property characterized by an affinity for eosin and an accumulation of lipids. In the experimental disorder there was a tendency for the degenerative-calcific lesions to spread beyond the large divisions and involve the small arteries and arterioles. Although the walls of these small vessels underwent structural alterations similar to those commonly encountered in human arterio-arteriolosclerosis, they acquired an affinity for hematoxylin, rather than for eosin, as is characteristic in human disease. At times, however, the affinity for hematoxylin was changed to an affinity of the degenerated structure for eosin. This change was always accompanied by regional resorption of calcium deposits. At other times, the internal elastic membranes of certain arteries seemed to be transformed directly into eosinophilic hyaline structures which differed sharply from the subjacent medial elastica, deeply stained with hematoxylin. Despite these suggestive trends, the degenerated eosinophilic hyaline medial structure, characteristic of human arteriolosclerosis, was never found in the experimental disorder.

It would seem from the foregoing comments that hypervitaminosis D, as well as pyridoxine deficiency, may be useful as a tool for investigating the pathogenesis of some elementary arterial structural lesions similar to those occurring in human arteriosclerosis.[12,13] Among these, the degeneration and calcification occurring in the media of arteries are conspicuous. It has been assumed that these changes in animals are a direct result of an increase in serum calcium and a "toxic" action of Vitamin D.[6-8] If so, all vessels are not equally affected. Furthermore, animals used in these experiments had no persistent or consistent rise in the average levels of serum calcium, though there was ordinarily an upward trend in the serum inorganic phosphorus. The magnitude of calcific

medial disease was not related to alterations in the levels of serum calcium and inorganic phosphorus. This points out the desirability of seeking for other factors responsible for development of calcific degenerative arteriosclerosis in hypervitaminosis D. It is possible that these factors, when fully disclosed, may also bear upon the pathogenesis of senile calcific degenerative arteriosclerosis in man.[16]

This abnormal metabolic state also offers an opportunity to analyze another aspect of arteriosclerosis, namely the variable intimal proliferative reactions. In the experimental animal these followed degenerative-calcific changes in the internal elastic membrane and subjacent media. These alterations, however, were not the sole determinants of the rate or amount of fibro-elastic intimal proliferation.[12] This is also true in human arteriosclerosis. In other words, there is no parallelism between the magnitude of calcific medial disease and the degree of intimal proliferation secondary to medial degeneration in man or animal. Though these differences may be due to a variation in the deposition of fibrin on the intima, the deposits, if present, were not recognized by the methods used.[17,18]

Finally, this experimental disorder provides a model system for a study of relations between medial calcific degeneration, intimal fibro-elastic proliferation, atheromatous deposition and thrombosis. One approach might properly include a study of the effects of combinations of hypercholesteremia, hyperlipemia and hypervitaminosis D.[19] A second approach might be concerned with chronic states involving prolonged repair of the effects of these combinations, for it seems probable that the occurrence of occlusive arterial thrombosis in human athero-arteriosclerosis is usually secondary to disturbance of vascularizing stromal repair of chronic degenerated intimal lesions.[20,21] Hence, by prolonging the experiments, it might be possible to induce a state of intimal vascularization and thrombosis, neither of which has occurred in these experiments despite very severe intimal arterial disease with extensive medial vascularization (Fig. 8). A third approach deals with a larger problem. This is concerned with a study of mechanisms by which the pattern of distribution of organic vascular disease may be governed by the pattern of distribution of units of tissue structure whose coordination is required for the performance of function.[9] This is clearly evident in hypervitaminosis D and there are reasons for believing that the variations of organic vascular disease in different arterial systems among different people may have a similar pathogenesis. In any event, a means is hereby provided for assessing the role of function and regulation of function in the etiology of organic disease of arterial sys-

tems which supply tissues whose coordinated activity is required for carrying out the function.


## Summary

Rabbits given excessive doses of irradiated ergosterol developed a generalized disorder characterized principally by resorption of bone and abnormal deposition of calcium salts in many extra-osseous tissues. The circulatory system was particularly susceptible to calcification, but there was a wide variation in lesions in different parts of the system. On a moderate dosage regime, calcium appeared first in the inner media of the aortic arch. With increasing time the deposits spread in depth and in the direction of the blood flow along the aorta and into the major branches. Usually, arterial changes decreased in severity in successively smaller branches, but there were two principal exceptions to this rule. The first exception occurred in animals on high dosage regimes. These animals developed a conspicuous arteritis and periarteritis in cardiac and skeletal mucle. These inflammatory reactions either accompanied or served to augment the development of intramuscular degenerative-calcific disease of arteries and arterioles. The second exception occurred in special arterial systems where the calcific vascular disorder increased in severity in the successively smaller divisions. This was conspicuous in the spleen, the inner third of the renal cortex, the duodenum, the salivary glands, and the part of the gastric wall concerned with acid secretion. In the pulmonary circuit the arterial system was usually unaffected while the pulmonary venous system from the interalveolar capillaries to the left auricle was often affected by the disease.

The basic histologic alterations formed a complex of inflammatory-degenerative-calcific sequences with subsequent reparative reactions. The principal locus of the deteriorative-calcific lesions was in the internal elastic membrane and media while the fibro-elastic intimal proliferative reactions and vascularized stromal resorption of the media were the principal manifestations of repair. Combinations of these changes led to the development of structural forms of vascular disease essentially identical to those encountered in the Mönckeberg type of human arteriosclerosis. This similarity, together with the remarkable distribution of the disorder, characterized by conspicuous susceptibility of certain arterial systems and absolute resistance of others, indicated that the disease should be useful in the study of factors which may contribute to the pathogenesis of human arteriosclerosis. Not the least important among these factors would seem to be the mechanisms which regulate the blood supply to functionally coordinated units of structure.

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[ *Illustrations follow* ]

## Legends for Figures

Photographs were prepared from sections stained with hematoxylin and eosin.

Fig. 1. Several extraparenchymal branches of a renal artery in a rabbit given 200,000 units of viosterol for 6 weeks and sacrificed at the end of 6 additional weeks on a normal regime. There is extensive calcification of the internal elastic membranes and subjacent media as indicated by the darkly stained parts of each vascular wall. An associated secondary intimal proliferation can be detected in several places. × 20.

Fig. 2. Iliac artery in a rabbit given 200,000 units of viosterol each week for 6 weeks. The deeply stained areas in the media are discontinuous concentric rings of calcium deposit. Over each of these and restricted to them is a plaque of thickened fibrous intima, in places equal in thickness to the thickness of the normal arterial wall. × 40.

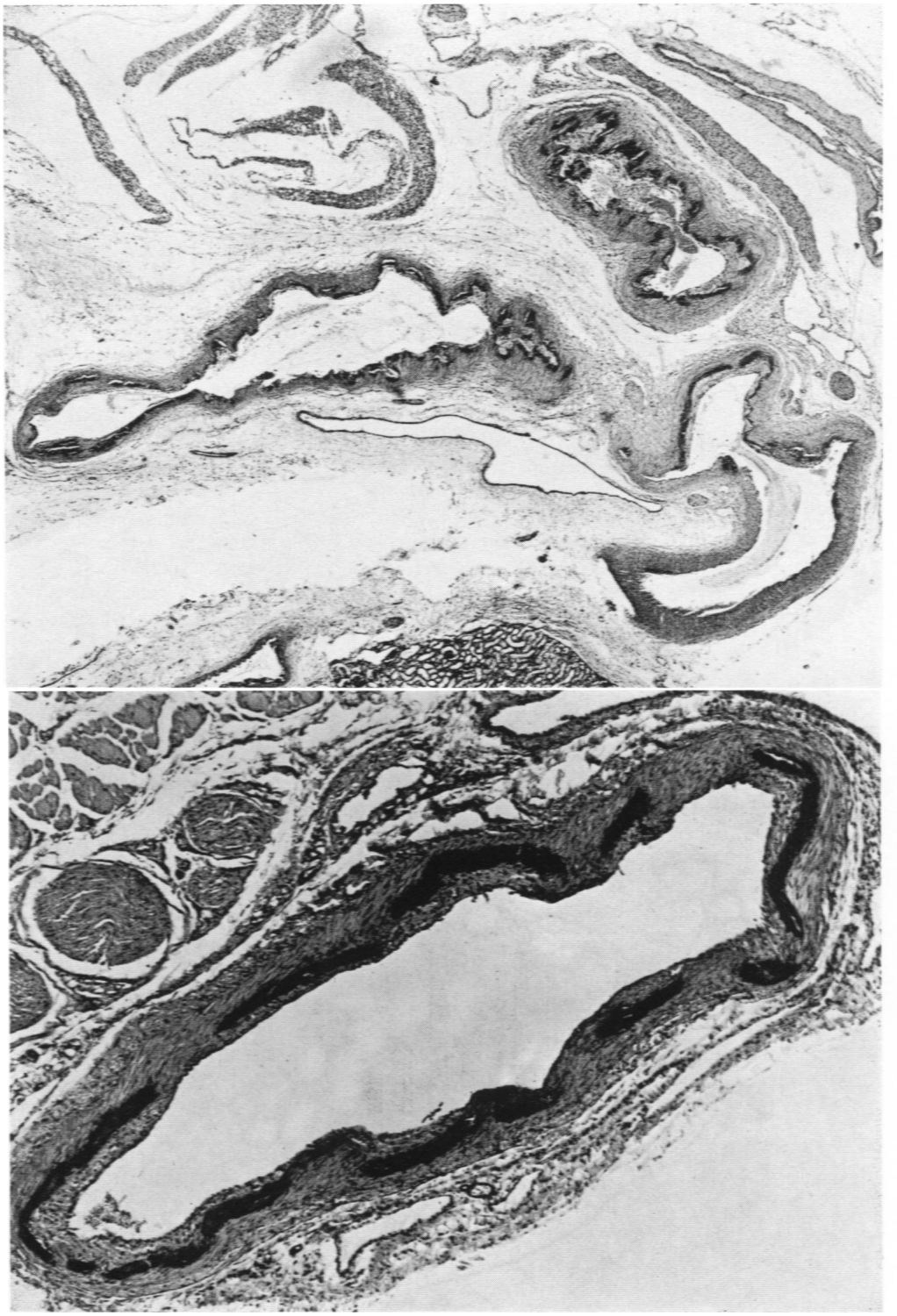

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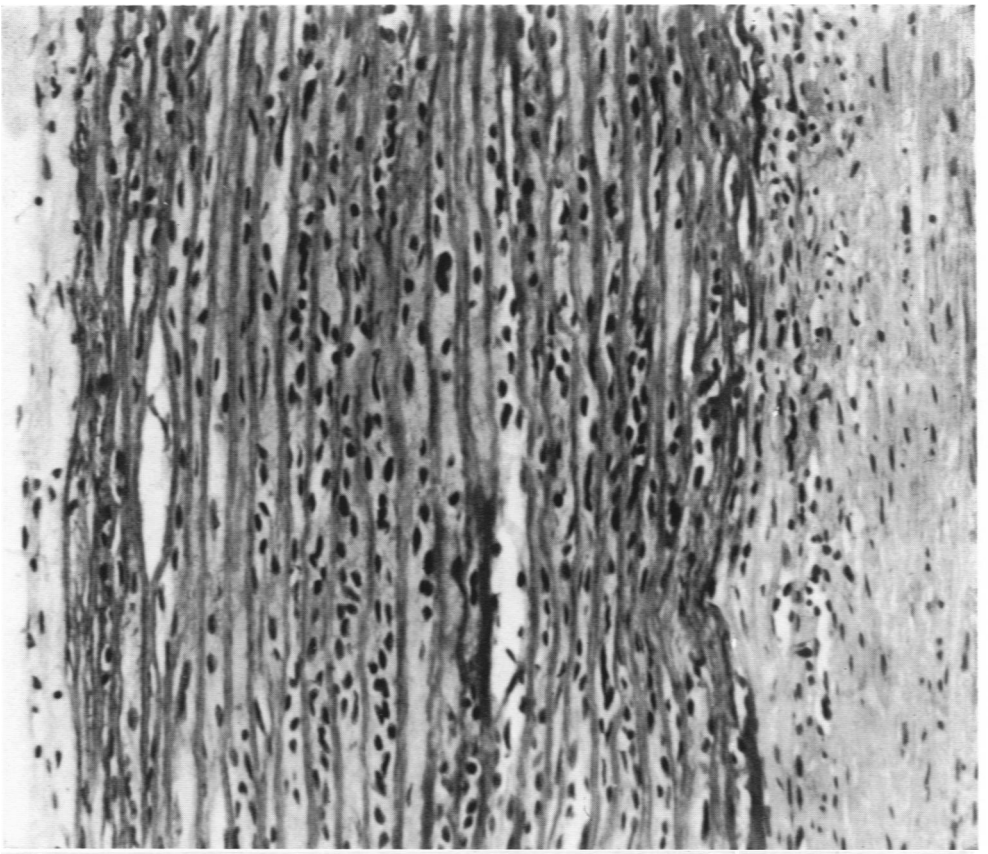
FIG. 3. Full thickness of the wall of the thoracic aorta in a rabbit given 200,000 units of viosterol every fourth week over a period of 33 weeks. The inner two thirds of the media shows calcium deposits restricted largely to the elastic lamellas. There is minimal intimal proliferation. Structural relations and cellular elements are well preserved throughout the media. × 180.

FIG. 4. Full thickness of the media and intima of the abdominal aorta in a rabbit given 200,000 units of viosterol every fourth week for 42 weeks. An area of medial calcification similar to that shown in Figures 2 and 8 has been penetrated by vascularized mesenchyme, arising in the adventitia and extending through the media to the thickened intima. Resorption of calcium and degenerated medial structure is advanced. These medial vascularizing sequences seldom, if ever, originated from the intimal aspect of arterial lumen. × 420.



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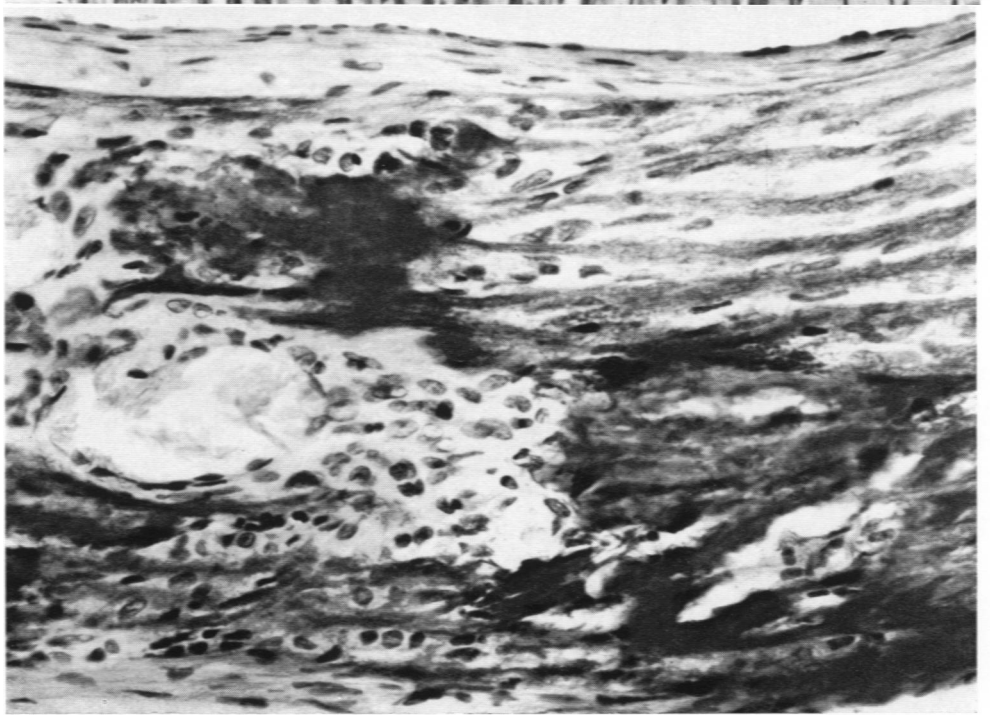

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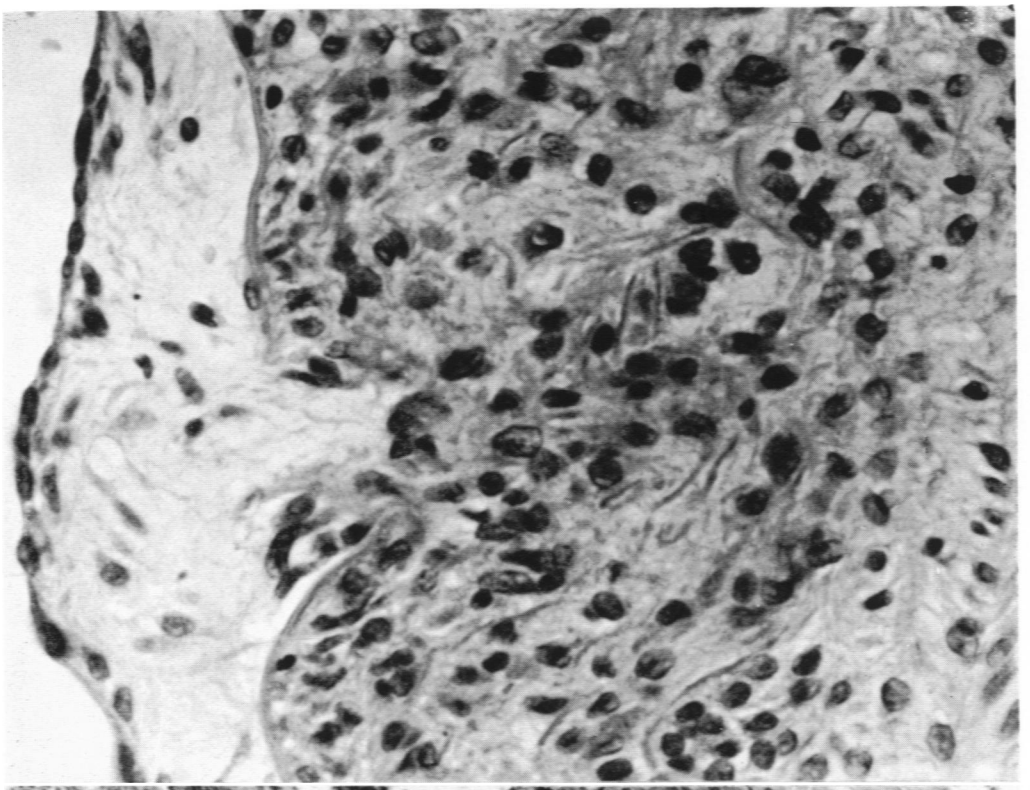
FIG. 5. Carotid artery in a rabbit given 200,000 units of viosterol each week for 4 weeks. There is focal subintimal medial degeneration with early calcification and mesenchymal activation. The internal elastic membrane is hyalinized and has an affinity for eosin. It is fractured and frayed over the site of medial degeneration. At this point there is a "splint" of proliferating intima covered with a regenerated endothelium. The fibroblasts in the immature plaque are separated by a myxomatous nonfibrillar matrix. It is possible that a small thin layer of fibrin may have provided the initial lattice for support of the migrating fibroblasts. If so, it must have been promptly resorbed. This represents one of the earliest stages of primary medial degeneration and secondary intimal proliferation. × 48.

FIG. 6. Femoral artery in a rabbit given 200,000 units of viosterol each week for 4 weeks. All but a short segment of the internal elastic membrane and subjacent media has undergone degeneration and early calcification. Though there is some cellular reaction in the media, the principal response is an intimal mesenchymal activation with fibrocellular intimal thickening, almost equal to the thickness of the media. At the angle, the media, internal elastic membrane and intima retain normal structure. × 150.



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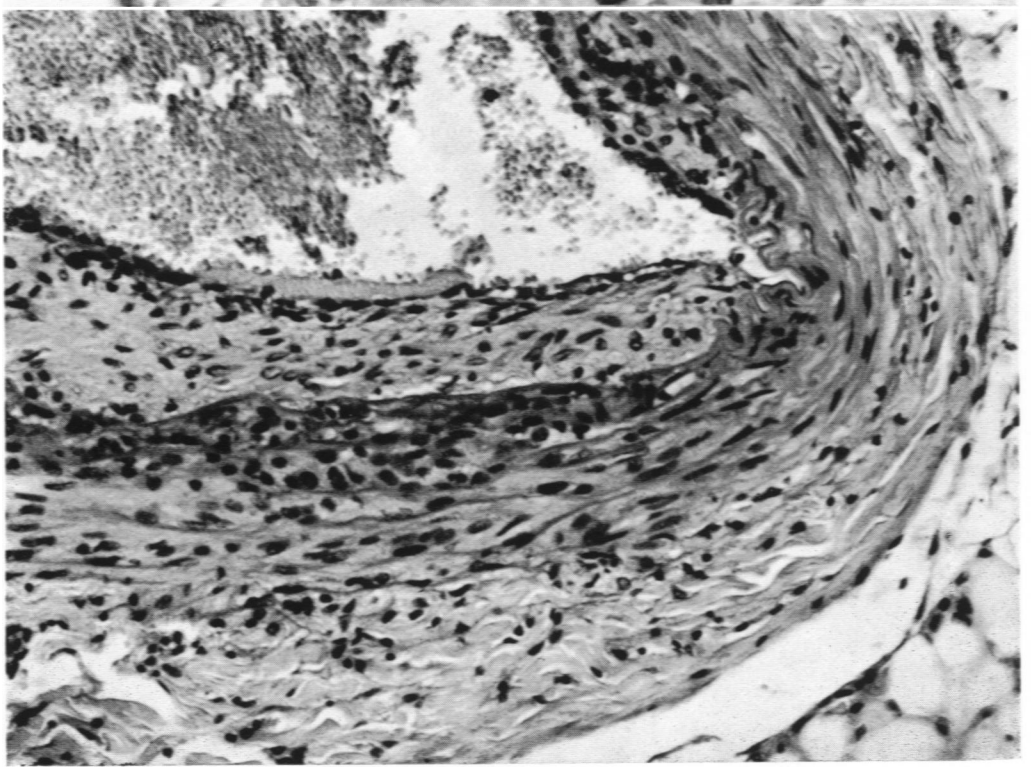

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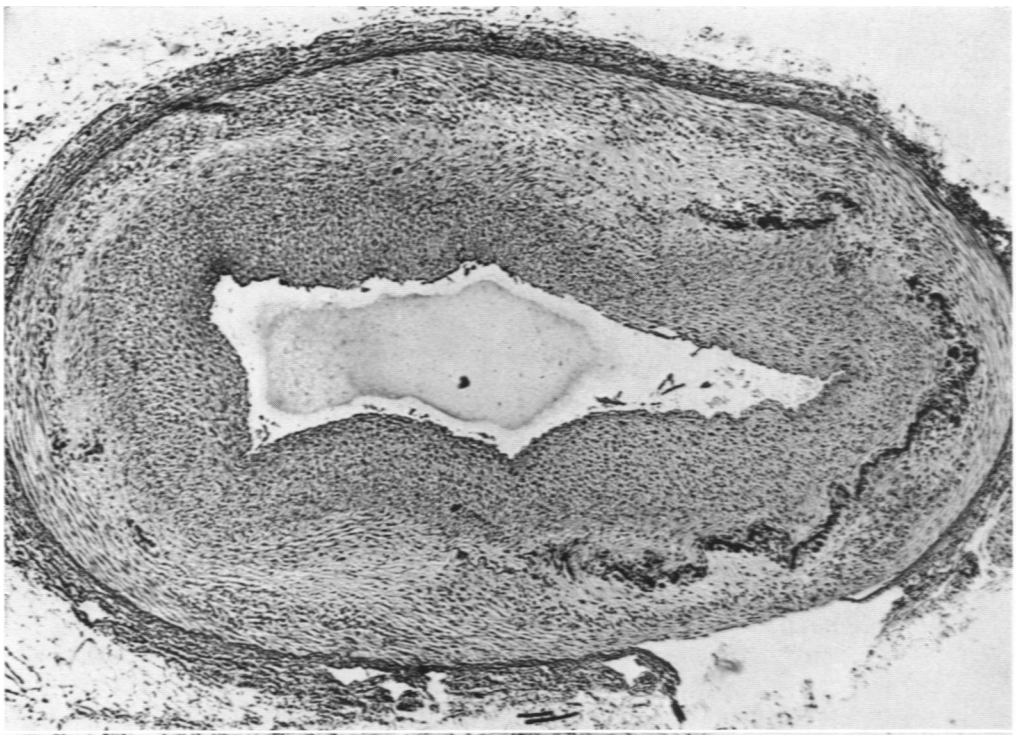
FIG. 7. A main renal artery in a rabbit given 200,000 units of viosterol every fourth week for 54 weeks. The remnants of the irregular, discontinuous, darkly stained, calcified internal elastic membrane now lie in the middle of the arterial wall. Internal to this is a greatly thickened fibrocellular intima, which has encroached upon the lumen. External to the fragmented membrane is a partly degenerated scarred media with remnants of calcium deposits undergoing avascular resorption. The external elastic membrane, though calcified, as shown by its affinity for hematoxylin, is intact and has acted as a barrier to adventitial mesenchymal penetration of the media. As usual, the thick, fibrocellular, newly formed intima is refractory to calcification despite prolonged viosterol dosage. These changes closely resemble those often found in medium-sized muscular arteries in the human being. × 50.

FIG. 8. Cross-section of a femoral artery in a rabbit given 200,000 units of viosterol every third week over a period of 33 weeks. The lumen is partly filled with a clot. It is lined by regenerated endothelium supported by thick dense fibrocellular intima, resistant to calcification. External to the thick intima is a porous, mottled zone once occupied by a medial smooth muscle and now by densely calcified, darkly stained degenerated medial structure being resorbed by highly vascular mesenchyme which has penetrated the media from the adventitia. Despite the intense vascularization and associated resorption of the degenerated calcified media, not a single capillary could be found in the thick fibrous intima. × 40.



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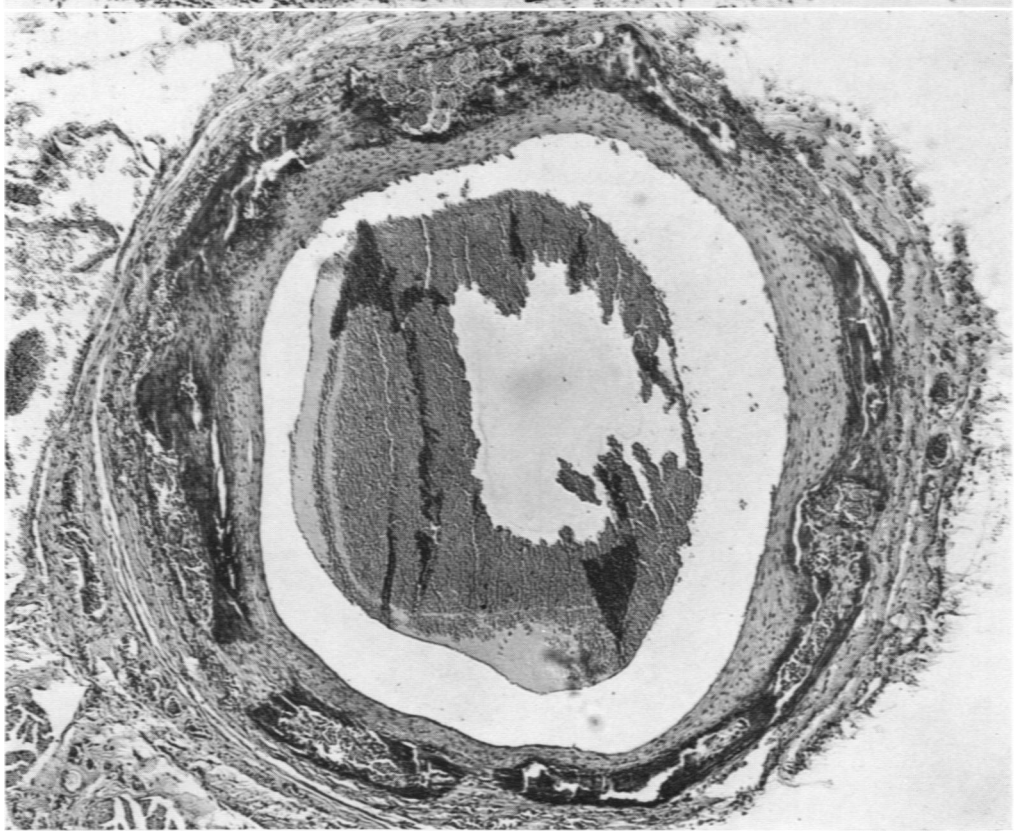

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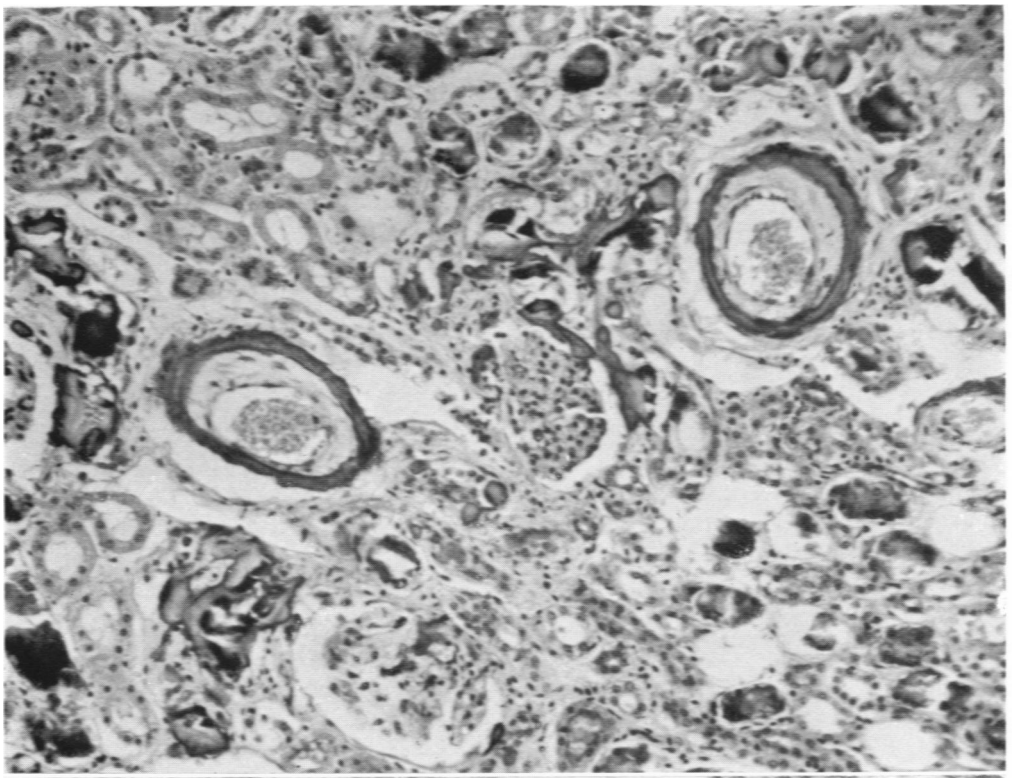
FIG. 9. Renal cortex in a rabbit given 100,000 units of viosterol daily for 24 days followed by a normal regime for 21 days. There is widespread degeneration with calcification of tubules, glomeruli and arteries. The two medium-sized arteries illustrate a high degree of medial calcification, a secondary fibrocellular intimal proliferation with narrowing of the vascular channels and the early stages of avascular resorption of calcified medial structure as indicated by the irregular contour of the margins of calcific deposits in each artery. × 160.

FIG. 10. Renal cortex in a rabbit given 100,000 units of viosterol 5 times each week for 4 weeks. The tubules are normal. The glomerulus is adherent by capsular proliferation to a degenerated calcified zone of Bowman's membrane. The afferent arteriole is heavily impregnated with calcium, as indicated by the dark homogeneous stain with hematoxylin. × 380.



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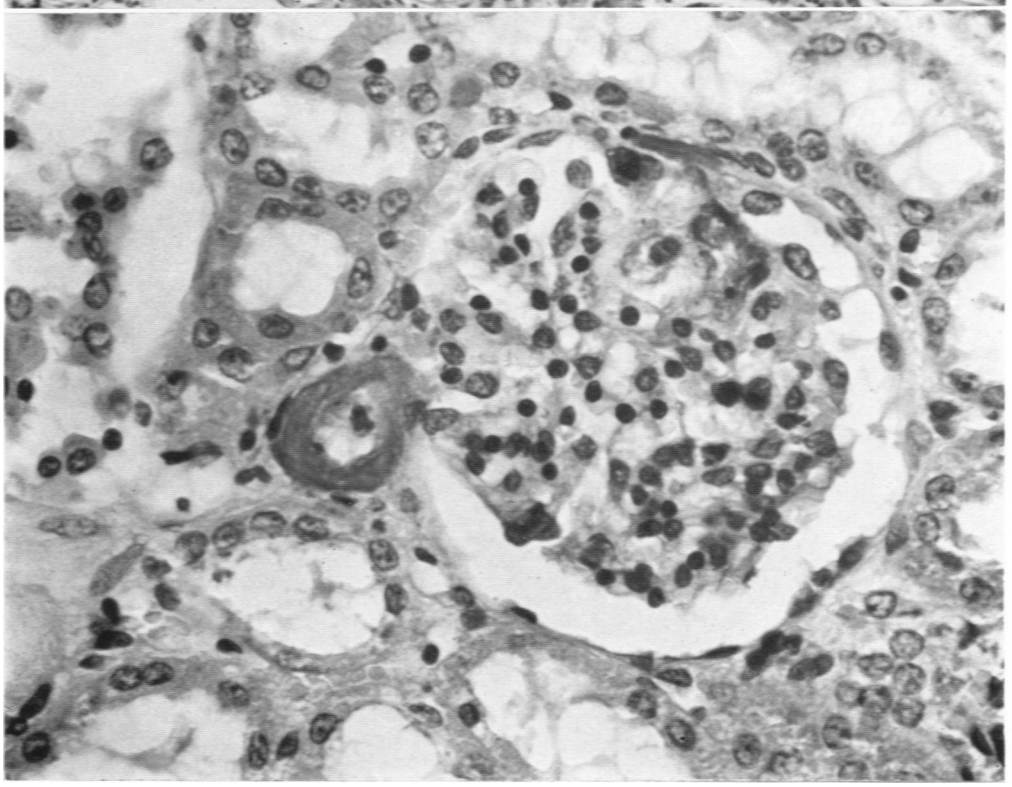

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