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AN EXPERIMENTAL HISTOLOGIC STUDY OF HYPERVITAMINOSIS D.*

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Soon after the discovery of the similarity of Vitamin D to irradiated ergosterol, several investigators became concerned with the action of excessive doses of irradiated ergosterol.[1-5] It was generally agreed that the compound exerted its effects by its influence upon calcium and phosphorus metabolism, presumably through some intermediary hormonal mechanism.[6,7] It was proved that the compound had no serious toxic action except when given in doses far in excess of those required for prevention or cure of rickets due to a deficiency of Vitamin D.[8] Finally, it was established that the principal pathologic changes attributable to excessive doses in man or animal were in the form of abnormal deposits of calcium in many organs and tissues.[9-11] Having settled practical matters concerning the clinical use of the compound, interest in hypervitaminosis D subsided, and except for the studies of Follis, little attention has been given to the subject since 1935.[12,13]

Our interest in hypervitaminosis D arose as a result of the emphasis of Wolbach, Bessey and Fell upon the use of avitaminoses and hypervitaminoses as tools for study of pathologic sequences and their relations to the locus or nature of action of vitamins.[14-18] Sequences in human disease with which we have been concerned relate to so-called degenerative changes which are especially conspicuous in connective tissues and more common in arteriosclerosis than in any other human disorder.[11,19] The lesions described in hypervitaminosis D seemed to offer a promising source of information in further study of these non-inflammatory degenerative sequences.[2-5,8] Hence, histologic studies of

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the distribution and evolution of lesions in rabbits given excessive doses of irradiated ergosterol were undertaken with special attention to changes in the vascular system.

Methods

The viosterol used in the experiments was a solution of irradiated ergosterol (15 mg. per ml.) in peanut oil. (Abbott Laboratories, Chicago, Ill.) The potency was assayed as about 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 for periods as long as 6 to 8 weeks. The regime was varied at lower dosages so that minimal and maximal pathologic effects could be defined and their evolution recognized over a period of several months.

Male albino rabbits from a single stock, about 5 pounds in weight and 3 months old, were used. Records of weight and analyses for serum calcium, phosphorus and cholesterol were made at intervals of about 2 weeks. The animals were fed a Purina Rabbit Pellet diet with fresh vegetables. For control purposes, complete microscopic studies were made of 75 normal rabbits from the same stock, of the same age and maintained on the same diet.

Since the initial plan was to produce maximal pathologic effects, the dose was regulated at levels just below the quick lethal range. Hence, most animals lost weight and died within 6 weeks. Later, the plan was to analyze minimal, sublethal and early pathologic effects, and animals were either sacrificed at any early stage or given viosterol at longer intervals and sacrificed at a later stage. Complete necropsies were done and a record of the gross findings made. All tissues and organs were fixed in formaldehyde (4 per cent U.S.P.) and studied microscopically. A series of 54 animals were suitable for the purposes of this report, with 49 additional animals on other dietary and viosterol regimes available for supplementary and confirmatory observations.

Results

Gross Pathologic Changes

The pathologic processes attributable to hypervitaminosis D varied with the dosage of viosterol, the length of the period between doses, duration of the regime, and the occurrence of intercurrent infections.

Minimal conditions for production of significant generalized calcinosis were a duration of 8 days and a total of 300,000 units given in 3 equal doses at intervals of 2 days. These conditions were usually complicated by intercurrent chronic purulent pyelonephritis of a type occasionally encountered in control animals. Among animals free

from extraneous disease, except hepatic coccidiosis, the total minimal effective dose was 500,000 to 600,000 units. This dose produced generalized calcinosis of increasing severity as the time of administration in periodic equal doses was extended to at least 3 weeks. The severity of the disease was further increased as the biweekly administration of doses of 100,000 units was continued beyond 3 weeks. This regime generally led to anorexia, loss of weight and death within about 6 weeks. Accurate control of the rate of development of pathologic processes was also obtained, so that prolonged participation of tissue elements in mechanisms of resorption, repair and regeneration might be studied, but this required careful regulation of dosage over a period of about 6 months.

Significant gross anatomic changes were encountered only in animals with considerable disease, manifest by changes in bones or deposition of calcium in numerous extra-osseous tissues. These could not be correlated with any consistent changes in values for cholesterol, calcium, or phosphorus in the serum.

The changes in bones varied with the severity and duration of disease. In general, the bones were more brittle than normal (Fig. 9). The increased fragility was due largely to resorption of cortical bone, often sufficient to allow mechanical deformity of bodies of vertebrae so that partial collapse and abnormal cortical contours were occasionally conspicuous. In these cases, the trabecular structure was compact and the marrow spaces reduced so that the internal structure of the vertebral bodies resembled that found in human osteosclerosis and Paget's disease (Fig. 10).

The amount and distribution of abnormal deposits of calcium in extra-osseous tissues also varied with the duration and severity of the disease. In mildly affected animals there were no gross changes. With increasing severity of the disorder, calcium salts began to appear as white streaks which acquired an opaque brittle or granular character. The deposits conformed to anatomic planes and structural outlines of affected tissues. They were most conspicuous in the aorta and its major branches, being maximal proximally and diminishing distally in a consistent pattern.[2,20] The deposits were in the media, usually in the form of continuous sheets or as a series of discontinuous rings with aneurysmal deformities of the wall. Calcium deposits were less conspicuous in the kidney, where bandlike granular deposits, were noted, first in the inner cortex and later, as the calcified zone of the innermost cortex spread toward the capsule, as one or more convex parallel bands in the periphery of the medulla (Fig. 7).[4]

The next most common location of calcium deposits was in muscle (Fig. 2). The deposits coincided with white streaks which followed the pattern of orientation of muscle cells or distribution of arteries. They were less common in skeletal than cardiac muscle, but frequently were clearly distinguished in the diaphragm and smooth muscle of special parts of the alimentary tract. The esophagus was never affected. The walls of the ileum and colon were occasionally altered, but this never occurred unless there were extensive deposits in the muscular wall of the stomach subjacent to the acid-secreting mucosa (Fig. 5). The acid-secreting gastric mucosa also commonly contained deposits of calcium but less frequently than the subjacent musculature. Calcium deposits were not found in the mucosa or muscle of those parts of the stomach which have no acid-secreting function.

Another common location of calcium deposits was in the respiratory tract. The tracheal and bronchial cartilages were often rigid and calcified, and thin plaques of calcium were at times recognized in the mucosa of the larger respiratory passages. Calcium deposits were rarely found in the media of large pulmonary arteries but were fairly common in the walls of pulmonary veins. In connection with these changes, pulmonary emphysema and decreased elasticity of the alveolar structure were usually demonstrable.

Other tissues and organs occasionally contained gross traces of calcium salts, but in many locations the presence of the mineral deposits was established only by microscopic studies.

Microscopic Pathologic Changes

Heart

Deposition of calcium in the heart was usually associated with a peculiar inflammatory reaction similar to that described in rats (Fig. 1).[1,8] In general, the calcification potential of various cardiac structures in order of decreasing intensity and frequency was as follows: internal elastic membrane of coronary arteries, smooth muscle of coronary arteries, fibroelastic tissue of the endocardium, and cardiac muscle. Calcium deposition in cardiac muscle cells was confined initially to the "A" disks of the myofibrils. As the amount of calcium increased, the "I" disks were involved, and later the interfibrillar compartment was affected so that the muscle cell appeared to be impregnated uniformly with calcium. At times, as much as one third of the myocardium was involved, but as a rule the calcified muscle cells were distributed irregularly around the coronary arteries or in the subendocardial region (Fig. 2). This localization seemed to be determined partly by the

common occurrence of a low-grade inflammatory reaction (Fig. 1). At times, the reaction was principally in the form of arteritis and periarteritis, characterized by swelling of collagen, perivascular proliferation of histiocytes, and formation of focal nodular lesions which resembled Aschoff bodies. Usually these lesions lay either adjacent to or around cardiac muscle cells in various stages of degeneration, calcification, and resorption. Their incidence increased with the level of dosage of viosterol and perhaps with the intrusion of intercurrent infections, though data concerning the role of infections were too meager to justify final conclusions. Suffice it to say that control animals dying of the same infections had no lesions of the type described.

The presence of an active periarteritis and myocarditis may have had something to do with the distribution of calcified cardiac muscle cells, but there seemed to be little relation between the inflammatory reactions and calcification of the media of coronary arteries. The main coronary arteries were free from inflammation, and calcium deposits in the internal elastic membrane or smooth muscle of the media were more conspicuous in these vessels than in the smaller intramyocardial branches which exhibited inflammatory reactions. In some instances, the deposition of calcium was so great that it was detectable in coronary arterial branches of all dimensions. This process was never uniform in arteries of equal dimensions but involved certain arterial branches more severely than others.

Calcification of the fibrous or fibroelastic stroma of the heart was never impressive in valves or in the myocardium. The endocardium, especially of the left atrium, was occasionally severely calcified, the process being more pronounced in elastic networks than collagenous fibrils.

Respiratory Tract

The sequences and pattern of calcification in the respiratory tract were of great interest. The earliest deposition, other than that occurring in the tracheobronchial cartilages and goblet cells, was in the fibroelastic stroma of the tracheal mucosa. The calcium deposits were distributed at regular intervals just below the basal layer of epithelial cells. As a rule, the basement membrane and underlying fibroelastic tissues were affected. In more advanced cases, the deposits involved the entire basement membrane and variable amounts of subjacent fibroelastic tissue, as deep as the plane of the mucosal vascular plexus (Fig. 3). Only rarely did the calcium deposits extend in continuity from basement membrane to the perichondrium of cartilaginous rings. Invariably, however, in these severe lesions there was excessive calci-

fication of the cartilaginous matrix. When this occurred, the calcified zones of cartilage lay adjacent to those of the mucosa, and at times there was erosion and stromal penetration of the margins of calcified cartilage. This was not followed by osteogenesis. On the contrary, the stromal invasion stimulated resorption of mineralized matrix and regional proliferation of young cartilage cells.

Calcification of the subepithelial tracheobronchial stroma was often preceded or accompanied by swelling and distortion of collagenous fibrils, but customary signs of inflammation were usually absent. However, once calcification had occurred, reparative sequences were at times recognized. These seemed to be initiated by two factors. One was the fragmentation of calcified fibroelastic tissue and basement membranes, leading to local proliferation of histiocytes and encapsulation of displaced degenerated calcified tissue by histiocytes and multinucleated giant cells. As this occurred, the subepithelial basement membranes and adjacent mucosal stroma were usually slowly regenerated. When the reparative process was unduly active, the epithelium regressed to an undifferentiated type of columnar cells which regenerated as either a characteristic respiratory epithelium or a metaplastic epithelium of stratified squamous type. This form of epithelial behavior called to mind the sequence described by Wolbach in the course of deprivation and restoration of vitamin A.[16-18] The second factor initiating reparative reaction was the deterioration of epithelial cells which followed calcification of mucus in the secretory vacuoles of goblet cells (Fig. 3). These cells, in the absence of any signs of diminished viability, inflammation, and undue calcification of subepithelial basement membranes or deeper stroma, were occasionally transformed into spherical, concentrically laminated, calcified crystalline structures. This form of primary epithelial calcification usually stimulated a regenerative reaction in which proliferation of basal cells led to atypical orientation of the new epithelium in relation to rigid calcified basement membranes. Ordinarily this activated the regional stroma which produced an entirely new structure with characteristics of the original basement membrane. The new membrane was interposed between the discontinuous calcified original basement membrane and the proliferating epithelium. As this occurred, the displaced calcified membrane became encapsulated by histiocytes or incorporated in the cytoplasm of multinucleated giant cells.

Despite the apparent interference of these changes with tracheobronchial function, little associated inflammation was provoked. A low-grade chronic tracheobronchitis, characterized principally by an

infiltration of plasma cells in the respiratory mucosa, was found in a few animals. This followed the most severe instances of stromal and epithelial calcification. It was never encountered in control animals and was regarded as a specific feature of hypervitaminosis D.

Not all parts of the tracheobronchial tree were equally affected by the calcific changes. The process developed more readily in the trachea and, as the disease became more severe, spread distally. Hence, in mild cases the principal changes were in the trachea. In moderately severe cases, the lesions were conspicuous as far distally as the terminal bronchi supported by cartilage. In the most severe cases, the process extended all the way into the walls of the alveolar ducts and alveoli. At times, the calcification of the terminal structures was very advanced, with involvement of pulmonary elastic tissue and the walls of interalveolar capillaries and pulmonary venules (Fig. 4). The alterations led to diminished elasticity with increased rigidity, relaxation, and fragility of alveolar structures. Thus, early stages of emphysema became conspicuous, and although peripheral pulmonary changes were usually associated with advanced tracheobronchial calcification, the relationship was not always encountered and was by no means quantitative. It was clear that factors which determined the locus and quantity of tracheobronchial calcification did not necessarily operate equally in determining the distribution and magnitude of calcification in alveolar walls and pulmonary vascular channels.

Alimentary Tract

The upper alimentary tract was studied more carefully than the lower tract so that conclusions concerning changes distal to the duodenum were only tentative.

The esophagus showed no evidence of disease.

The stomach was a common site of severe calcification but only in special locations; namely, the part of the gastric wall concerned with support, nutrition, and the specific performance of acid secretion (Fig. 5). This part encircled the cardiac orifice and occupied the principal part of the fundus and greater curvature. The mucosa of the lesser curvature and the pyloric region contain few if any parietal cells which are generally held to be responsible for secretion of hydrochloric acid. The parts of the gastric wall which were calcified are therefore spatially and presumably functionally integrated. Calcium in the muscular wall was usually most conspicuous, and there was concurrent calcification of the mucosa and arterial system. Deposits of calcium in the mucosa

usually appeared first in the *tunica propria* and basement membranes adjacent to the common junction of branches of the compound glands in the midmucosal regions. Occasionally, cells lining the glands were calcified, and at times calcified concretions lay in the lumens of glands. Later, stromal calcification also occurred in other locations, especially near the muscularis mucosae. In connection with the stromal deposits, calcified walls of small arterioles were conspicuous, and at the level of the muscularis mucosae and submucosa, calcification of walls of larger arterioles and small arteries was still more prominent. Walls of larger arteries deeper in the *tunica muscularis* and serosa were less severely calcified although calcium deposits in the internal elastic membrane and subjacent media were common. In these vessels, fibroelastic intimal proliferation was apparent, always overlying calcified or fragmented stretches of abnormal internal elastic membranes or subjacent media.

Calcium deposition in the *tunica muscularis* and the muscularis mucosae of the stomach occurred in a characteristic sequence and pattern. Major deposits lay in the cytoplasm of smooth muscle cells which seemed, initially at least, to be structurally normal. The smooth muscle cells were ordinarily affected in groups, and with increased calcification only the bare outlines of initial structure remained. In its place there were rows of curious calcified bodies which resembled strings of beads without recognizable origin in cytoplasmic structure (Fig. 6). Minor deposits of calcium appeared in tortuous curved structures which at times resembled fibrous or elastic elements and at other times minute canaliculi or autonomic nerve axons. Further study will be required for the identification of these calcified filaments, fibers and other structures.

The pattern of transmural calcium deposition in elective sites in the stomach disappeared with the transition of gastric mucosa to the type which secretes no acid. Indeed, the entire thickness of the gastric wall just beyond the level of mucosal transition was normal in all cases. In other words, all arteries, nerves, smooth muscle, mucosal glands and stroma in the non-acid-secreting part of the stomach were free from calcium deposition.

Immediately beyond the pyloric sphincter, which at times contained a few clusters of calcified smooth muscle cells, a different pattern of calcification was encountered. Here, the duodenal mucosa, *tunica muscularis* and muscularis mucosae were ordinarily spared while calcium deposits appeared conspicuously in the walls of small arteries and arterioles. Distal to the duodenum the only important calcium

deposits were in the *tunica muscularis* of the ileum and especially the colon. The deposits varied greatly in amount and position. Mucosal and arteriolar calcification was indistinct or absent, except in the colon, in the reticulum of lymphoid follicles or in the basement membranes of interglandular surface epithelium. There was a peculiar concentration of calcium in and around ganglionic plexuses in the *tunica muscularis* of the colon. Other plexuses were uninvolved. Mucus-secreting goblet cells, commonly calcified in the respiratory mucosa, were unaffected throughout the ileum and colon.

Urinary Tract

The kidney was also a common site of severe disturbance and was affected in all cases in which there was significant calcification in other tissues.[3,4,8] The renal calcification was confined principally to aggregates of functionally related units. For instance, nephrons were not involved equally or in a random manner. Those first affected were nearest the medulla, and from this region the process spread to involve the more peripheral nephrons but seldom reached those in subcapsular areas (Fig. 7). In connection with this curious wave of calcification there was a similar spread in the medullary tubular areas corresponding to the distribution of loops of Henle.

The sequence of changes in nephrons was also of interest. The epithelium of the convoluted tubules seemed most susceptible; the cells were frequently solidly impregnated with calcium often disposed in a spherical, concentrically laminated pseudo-crystalline fashion, occupying and expanding the original volume of the cytoplasm and nucleus. Intraluminal calcified protein casts were also encountered in these tubules. The basement membranes of the convoluted tubules had a conspicuous affinity for calcium so that at times they were more heavily calcified than the epithelial cells. This affinity was prominent beyond the limits of the convoluted tubules, especially in the stroma of Bowman's capsule. Less often, the subendothelial membrane of glomerular arterioles was affected.

No conspicuous calcification of tubular epithelium and basement membranes of the kidney appeared without calcification of the related arterial system. The large extrarenal arteries were least involved, although calcification of internal elastic membranes and subjacent patches of medial structure was usual. Where the extrarenal arteries branched to enter the cortex, near the corticomedullary junction, pathologic changes became more prominent. The walls of arteries and arterioles supplying the nephrons undergoing mineralization were fre-

quently solidly impregnated with calcium so that mural structure was hardly recognizable (Fig. 15). The process of arterial calcification ordinarily extended in continuity to the glomerular tuft (Fig. 8). Involvement of the glomerular arteriolar plexuses was uncommon except when calcium was deposited in spherical masses scattered throughout the tuft in swollen glomerular endothelial cells. The postglomerular arterioles and venous channels were spared, except in the most severe instances of diffuse calcification. This was equally true of all arterial and venous systems connected with the nephrons of the peripheral half of the renal cortex.

The most remarkable feature of the renal lesions was their distribution. Of interest also, however, was the absence of expected signs of inflammation. Nor was there ever more than a trace of the reparative reaction one would anticipate in response to the observed magnitude and duration of renal damage. The disease was essentially indolent, progressive and degenerative.

The remainder of the urinary tract was not studied carefully, but ureters and renal pelves were often included in the sections. The ureters were always normal. The pelves ordinarily contained subepithelial deposits of calcium, occurring at intervals in the fibroelastic connective tissue and among bundles of smooth muscle cells. There was no inflammatory reaction preceding calcium deposition although the stromal fibers were often swollen and nodular. Following calcium deposition, there was in some instances a proliferation of histiocytes resembling the reaction encountered in similar calcified tissues in several other locations.

Vascular System

Particular attention was given to a study of the vascular system.[2,5] The earliest evidence of hypervitaminosis D was in the proximal aorta and changes were always found here, whenever there were calcific deposits in other locations such as the tracheobronchial mucosa, gastric wall and kidneys. As the disease became more severe and prolonged, calcium deposits appeared in the midaorta, then in the distal aorta, and at about the same time, in various aortic branches.

Microscopically, the initial calcium deposits in the aorta were in and along the innermost elastic membranes and delicate intermembranous fibrils or matrix (Fig. 11). With increasing duration and severity of the disease, calcification increased in depth and magnitude. In advanced cases the inner third of the media of the proximal aorta was calcified in continuity, and there were discontinuous annular zones of calcification in the middle third of the media. The depth of pene-

tration of calcification in continuity decreased slightly with increasing distance from the aortic valve but not in proportion to the decrease in thickness of the media. Hence, in advanced cases, the full thickness of the media of the distal aorta was calcified, while not more than the inner half of the media of the proximal aorta was similarly involved.

Each aortic branch had individual susceptibility to mineralization, and each had its own pattern of calcium deposition. In general, branches of similar size and structure had similar susceptibilities and patterns, but this was not an invariable rule. Without undue description of deviations, the trends in the different systems may be given as follows. As the elastic structure which dominated the composition of the upper aorta was gradually replaced by an increased amount of smooth muscle in the distal aorta and carotid, renal or iliac arteries, calcification of the internal elastic membrane became more conspicuous and local deposits of calcium appeared in and between the subjacent smooth muscle cells (Fig. 14). The deposits in the beginning were discontinuous and distributed in an annular manner (Fig. 12). As they increased in number and extent, they tended to fuse to form annular rings and to spread in the long axis of the media, eventually occupying it entirely. The process was especially conspicuous in the iliac and femoral arteries. A similar lesion but with less annular and axial discontinuity of calcification was common in the carotid, axillary and brachial arteries (Fig. 13).

Changes in the smaller and more peripheral branches of these arteries did not follow any uniform pattern which could be related to the dimension or structure of the vessel. For instance, the immediately extrinsic and intrinsic vessels of the cerebral vascular system were always normal. The main pulmonary artery and major branches occasionally contained discrete calcified lesions of the inner media, but the minor branches down to the level of the alveolar capillary network were usually normal. At the alveolar level, calcium deposition became conspicuous and extended in continuity to involve the media of vessels of the outflow venous system into the left atrium (Fig. 4). Arteries to special organs such as the submaxillary glands, kidney, spleen, thymus, duodenum, acid-secreting part of stomach and thyroid were often very severely affected, while those supplying the eye, testis, ileum, adrenal, pituitary and liver were spared from significant alteration. Arteries to skeletal muscle, pancreas, bone marrow and skin contained lesions to a variable degree among different animals and from place to place in the same animal. These general statements also pertain to arterioles and capillaries.

Veins, except those carrying blood from pulmonary alveolar spaces to the left atrium, were never electively involved, though venous channels in the kidneys, bone marrow and elsewhere were occasionally secondarily affected in connection with massive calcification of regional tissue.

Not only were there unexplained vagaries in localization of calcium in different arteries and arterial systems, but the patterns of reaction associated with the localization were equally variable. For instance, fibrous intimal proliferation was negligible in the proximal aorta. It increased in magnitude in the aorta with increasing distance from the aortic valve. It was maximal in the systemic arteries of large caliber which showed severe calcification and fragmentation of internal elastic membranes supported by medias composed largely of smooth muscle rather than layers of elastic lamellae. It was limited exclusively to areas covering abnormal internal elastic membranes and subjacent medial structures in most animals, and predominantly so in all animals. The fibrous intimal plaques formed sluggishly but at times within 6 weeks acquired a thickness equal to that of the arterial wall (Fig. 14). In smaller arteries, the intimal proliferation was less conspicuous so that in arterioles, despite extensive transmural calcification, intimal proliferation was scarcely recognizable, except in the presence of active or healed arteritis (Fig. 16).

The occurrence of arteritis was another unexplained variable. The evidence indicated that the arteritis was related to exceedingly high dosage levels and perhaps to acute intercurrent infection. Suffice it to say that arteritis of the type occurring in animals with hypervitaminosis D was never encountered in the control animals, many of which also died of acute intercurrent infections. Whatever the eventual explanation, the arteritis and periarteritis had no specific connection with the degenerative calcifying disease of arteries in general. There was good evidence that it was connected with the occurrence of calcified deposits in distal arterial systems lying within muscle and concerned principally with the nutrition of cardiac and skeletal muscle (Figs. 1, 16). Seldom was arteritis or periarteritis encountered elsewhere and then only in isolated vessels and never in the conspicuous generalized form noted in the muscles.

Not only was the anatomic distribution of the arteritis a matter of considerable significance, but the pattern of inflammation of arterial walls and perivascular tissues was equally interesting. The most severe forms were in the nature of an indolent panarteritis, which resembled reactions occurring in periarteritis nodosa, lupus erythematosus, rheumatic fever, and occasionally rheumatoid arthritis. In less severe cases,

the signs of inflammation were principally in the collagenous adventitial tissues. These signs varied from a mild nodose swelling of the fibrils to a more pronounced response characterized by aggregates of histiocytes, neutrophils and lymphocytes in and around foci of degeneration of perivascular interstitial tissues. At times, especially in the myocardium, several persons who have studied these sections have remarked the similarity of the lesions to Aschoff bodies. Similarity was all that could be claimed, however. The lesions were entirely unlike the random inflammatory foci encountered occasionally in the myocardium of rabbits in the control series.[1,8]

Muscular Systems

The preceding descriptions have disclosed that the muscular tissues of the body were usually affected. There was no evidence that this involvement was secondary to alterations of arteries, although arterial lesions customarily accompanied muscular abnormalities. Smooth muscle was more generally affected than cardiac or skeletal muscle. Changes in smooth muscle of the media of systemic arterial and pulmonary venous systems, the *tunica muscularis* of the alimentary tract and the tracheobronchial tree, and the mucosa of the renal pelvis have already been described. Similar changes were less common in such other locations as the corium of the skin and septa of the spleen. Though successive stages of development of the lesions in smooth muscle were similar in most locations, this was not true of all locations, and there were wide differences in the susceptibility of various smooth muscle cells. In general, cardiac and skeletal muscle were less susceptible than smooth muscle, whereas skeletal muscle in most locations was less susceptible than cardiac muscle. In some instances, manifestations of inflammation and cellular degeneration preceded the microscopic signs of calcification of muscle. In other instances, however, the intracellular deposition of calcium seemed to occur in muscle which showed no definite evidence of inflammation or degeneration (Fig. 6). Whether these occurred or not seemed to be related to the rapidity of development and severity of the disease. At times, the stromal elements around smooth muscle cells were affected before there was conspicuous calcification of the cells. At other times, the cells were initially altered, and the stromal elements resisted calcification. This resistance of the stroma was generally apparent when cardiac muscle was undergoing calcification, but less so when calcium was being deposited in skeletal muscle cells. In the latter instance, the sarcolemma of some fibers was calcified before the cells had acquired much calcium.

Skeletal and Hematopoietic Systems

The dominance of this disease in many tissues concerned with motion is well illustrated by the remarkable changes which occurred in the skeletal system.[12,13] The first effects occurred early and were characterized by a resorption of bone, a decrease in the prominence of osteoblasts, and an increased prominence of osteoclasts (Fig. 9). These changes were more conspicuous in cortical than trabecular structures. A significant amount of pathologic calcification of the viscera did not occur unless these alterations were demonstrable. The development of abnormal calcium deposits in the viscera was also accompanied or followed by abnormal basophilic deposits of osteoid tissue in the skeleton. These deposits occurred not only in locations notable for the degree of osseous resorption but also in the bone marrow, along the margins of persistent trabecular and cortical bone (Fig. 10). Though there was some local increase in stroma which either preceded or accompanied the abnormal massive osteoid deposits, any close resemblance to normal sequences of osteoblastic orientation, osteoid production or calcium deposition was lacking. The osteoid tissue seemed to engulf old stroma, reticulum cells and other structures in a spreading wave beginning at the margin of pre-existing bone and progressively obliterated the adjacent bone marrow. When this process ceased, the customary form of normal eosinophilic osteoid tissue with incorporated osteocytes began to appear as local islands in the midst of the widespread basophilic osteoid deposits. This indicated onset of repair and, with passage of time, the deposits were largely replaced by new bone. There resulted an osteosclerosis characterized by excessive reformation of bone along lines which did not reproduce the initial or normal pattern of bone growth. The final result was a deformed skeletal structure, usually with a porous cortex. This was accompanied by a thick layer of periosteal new bone, especially excessive at the margins of some articular surfaces, and an excess of trabecular bone which had encroached upon an abnormal bone marrow (Fig. 10). As a rule, the response of the bone marrow to calcification and osteogenesis was fairly consistent in the areas which remained free from fibrosis. The marrow which had not been replaced by fibrous tissue, abnormal osteoid tissue, or new bone, was depleted of fat cells and was densely cellular. Hematopoietic elements varied considerably in their relative proportions and stages of maturation in different animals. The commonest abnormalities were maturation arrest and diminished numbers of megakaryocytes associated with an increase of cells resembling megaloblasts or plasma cells. Conspicuous cytologic changes in the marrow were

usually accompanied by calcification of the media of blood vessels which appeared to belong to the arterial system. As yet, no connection between the changes in hematopoietic elements and the severity of the disorder in other parts of the body has been established though a correlation with gastric disease is strongly suspected.

The spleen was usually not severely disturbed. The earliest deposits of calcium occurred in the interiors of the septa. Here, fibroelastic tissue and smooth muscle were electively calcified, but the bulk of the calcium was deposited in collagen. There was no consistent evidence of any inflammatory or degenerative change prior to calcification, although collagen fibrils often were swollen and irregular in outline. In some instances the arterial system was more heavily calcified than the trabecular structure. Here, the main splenic artery and its major branches regularly showed calcification with fragmentation of the internal elastic membrane overlying foci of medial calcium deposition. Fibrous intimal proliferation was seldom conspicuous but, when found, always was superimposed on degeneration of the vascular wall. As the splenic arterial branches entered the spleen and decreased progressively in diameter, transmural calcification became increasingly conspicuous so that the walls of many follicular arterioles were uniformly calcified. Nor did the impregnation of vascular structure cease here, for in some cases it continued into the walls of the sinusoids and outlined the reticular framework of lymphoid follicles.

Endocrine and Other Glands

The principal calcium deposits in endocrine glands were in the thyroid and thymus. In the thyroid the small arteries were usually severely affected in animals with advanced generalized disease. The vascular process was associated with atrophic changes in glandular epithelium and diminution in the amount of colloid. This in turn was related to variable calcium deposits in basement membranes of follicles and interfollicular stroma.

The thymus gland showed conspicuous atrophy in severely diseased malnourished animals. Calcium deposits were restricted principally to Hassall's corpuscles although occasionally there were deposits in the media of small arteries and arterioles.

The pituitary never contained calcium deposits. There were unexplained variations in the ratios of cell types in the anterior lobe. The parathyroid glands were normal in size and in histologic characteristics. The adrenal glands were resistant to calcification with deposits occurring only in the most severe cases. These appeared only in occasional

swollen endothelial cells of the vascular sinuses in the cortex. Large vessels were spared.

The testes were seldom affected, except insofar as the illness led to reduced spermatogenesis. There were occasional calcified concretions in tubules and insignificant calcium deposits in the media of large arteries leading to the testis; these were minor late manifestations of severe generalized disease.

Other glandular structures had their individual changes. The liver was never affected, even though the arterial branches at the hilus showed the same types of calcium deposition as other branches of the celiac axis. Intrahepatic vessels were free from lesions.

The large branches of the splenic artery to the pancreas were affected to about the same extent as the arteries to the spleen. The vascular changes decreased with diminishing size of the arteries so that in contrast to splenic arterioles the arterioles in the pancreas were usually normal. In occasional lobules of the pancreas of severely affected animals, the process extended into the arterioles, and the extension was accompanied by atrophy of acinar cells with calcification of the delicate basement membranes of glands. The pancreatic islets were not affected.

The arteries and arterioles supplying the submaxillary glands were among the most susceptible peripheral vascular structures. The walls of these vessels were often densely calcified. The process varied from one lobule to another. Accompanying the most severe arteriolar lesions, there was a tendency for other degenerative and calcific changes to occur; these were epithelial atrophy, calcification of inspissated secretion in the acini of glands or small ducts, and heavy impregnation of basement membranes with calcium. The sequence of these changes was not clearly defined, but the evidence indicated that vascular and epithelial changes preceded changes in the intervening stroma.

Central Nervous System and Eye

The eye was regularly spared except for small deposits of calcium in the sclera. This comment excludes any consideration of the lens because it was not examined microscopically. Retinal arteries were never calcified.

There were no deposits of calcium in the brain or spinal cord. This was a consistent observation and applied to all structures ordinarily associated anatomically with the brain and spinal cord. The level to which calcified deposits in the carotid, vertebral and spinal arteries extended from their points of origin has not been determined, but no vascular alteration has yet been found in the intracranial or intraspinal

divisions of these vessels. Furthermore, the vascular system within the confines of the pia mater and gray or white matter was always spared from calcification even around foci indicative of chronic intercurrent meningo-encephalitis which was encountered in a few animals of our current stock.

Skin and Adipose Tissue

The skin showed changes which need not be described in detail. Beneath the epithelium and at a slightly deeper level in the corium, the lesions resembled those occurring in fibroelastic tissues elsewhere. In connection with these alterations in their advanced form, there was mild calcification of the tributary arteriolar and precapillary walls. Other histologic features resembled the early modifications in human cutaneous fibroelastic tissue designated "senile elastosis." In the deeper tissues, lesions were insignificant except where short stretches of fascia were lightly calcified and where skeletal muscle showed degeneration of the type described elsewhere.

Adipose tissue in most locations was unaffected by the disorder. However, in the retroperitoneal tissues, especially around the larger arteries and adrenals, severe atrophy of fat cells was encountered, usually accompanied by a deposition of calcium. The principal deposits were either adjacent to or in the cytoplasmic membranes of fat cells. No massive deposits were encountered in the interior of fat cells.

Discussion

A major problem in analysis of the pathogenesis of many generalized diseases is an inability to explain the vagaries of distribution of the anatomic or functional manifestations. In one patient the manifestations of a disease may be referable to a single system or some part thereof. In another, the manifestations of the same disease may be referable to more than one system or more than one part of any system. In still others, the manifestations may be dissociated so that the expected quantitative relations between the anatomic and functional changes are not found. Concepts which guide thinking in these matters have no solid foundation in structural or functional pathology.

It was our belief that an experimental study of an easily controlled metabolic derangement which produced widespread structural changes of a simple degenerative nature rather than a complex inflammatory reaction might lead to formulation of more suitable concepts.[19] Wolbach demonstrated with well-planned experiments and great interpretive insight that the use of deficiencies and excesses of vitamins was an elegant method for approaching problems of this kind.[16-18] After surveying this subject, it was decided that a systematic experimental

study of hypervitaminosis D might be helpful for our purposes, and that this eventually might be compared with experimental hyperparathyroidism.[11,21,22]

The widespread changes disclosed by this study may be summarized as follows. First, degenerative changes with calcium deposition occurred in many organs and tissues. Second, the pattern of distribution of the degenerative changes was not specifically related to any common physical, chemical, or other characteristic of the affected elementary tissues or their combinations. Third, the sequences in development of these changes were not the same everywhere but were determined in part by unknown factors characteristic of the affected organ or tissue. Finally, the pattern and sequences of degeneration occurred in a reproducible fashion which could not have been predicted from a knowledge of factors which ordinarily determine the local characteristics of generalized disease processes.[19]

The widespread distribution of pathologic changes was largely dependent upon the amount of irradiated ergosterol given, the interval between doses, and the duration of the experiment. The data indicated that a sufficient quantity of the vitamin would produce minimal changes if it were given in a single dose or in a series of small daily doses over a period of several days. Maximal subacute changes due to the same quantity of the vitamin occurred when it was given in equal divided doses at intervals of 2 or 3 days. The best regime for obtaining maximal, chronic, slowly progressive changes has not been established, but good results followed the interposition of prolonged rest periods between brief dosage schedules designed to produce bursts of maximal active changes.

The pattern of distribution of pathologic alterations was constant under a given regime but varied somewhat with variation of the experimental conditions. In general, however, the earliest evidence of degeneration appeared in a particular location in each affected tissue or organ. Then, the process spread to involve other tissues or the same tissue in a succession of locations, each of which was resistant to change until the antecedent pattern of degeneration was set. Each affected type of tissue in each affected location in each affected organ had a definite level of susceptibility to the degenerative changes. This curious order of susceptibility to degeneration with calcification led to the conclusion that each type of cell or tissue in a functionally integrated system had a characteristic degeneration-calcification potential and that the level of the potential varied among different integrated systems. Hence, the succession of tissue alterations might be regarded as a result of the operation of four mechanisms. The first mechanism

raised the calcification potential of the tissue. The second raised the calcifying potential of the environmental fluids. The third was concerned with adaptation, and the fourth with restoration.

It is generally accepted that the best way to increase the calcification potential of a cell or tissue which does not normally calcify is to reduce its viability without bringing about rapid structural disintegration.[11] In the present experiments, it was clear that there was a reduction in viability of certain cells and stromal elements. It was not clear whether the reduction preceded or followed the deposition of calcium salts.[8] If reduction in viability preceded calcification, there was at this stage little evidence of customary findings of lessened viability. In other words, the earliest evidence of modification of stroma or cells did not definitely precede evidence of calcification in many locations. Furthermore, in most pathologic conditions, the reduction of viability of stroma and cells ordinarily initiates a sequence of inflammatory and regenerative reactions before calcium deposition becomes conspicuous. In the present study, this was not encountered as an early reaction in any location, except perhaps in certain muscular tissues, and then only in connection either with intolerably high dosage or intercurrent infection. However, under these conditions, conspicuous inflammatory reactions did not occur prior to degenerative calcifying changes, and the reactions were restricted principally to the arterial systems in cardiac and skeletal muscle. Usually, the reactions were confined to the media and adventitia of arteries, but in the severe cases there was a spread into neighboring muscle. The distribution of the inflammatory reaction, when it occurred, coincided with the distribution of the degenerative calcifying changes which developed without signs of inflammation in muscle of other experimental animals. From this it may be inferred that some factor increased the degeneration-calcification potential in all instances, but an inflammatory reaction was not elicited except when it was excessive or when other factors, perhaps related to intercurrent infection, were introduced. In any event we must admit the possibility of a more complex form of local tissue potential which might be called an inflammation-degeneration-calcification potential. Thus, under the same systemic conditions local factors may induce various combinations of inflammation, degeneration and calcification to occur at the same time in different organs or in the same tissue types in different organs.

The second mechanism seemed to be related to the composition of oxygenated or arterial blood and to special units of tissue integrated together in the performance of some particular function. For instance, emphasis has often been placed upon the deposition of calcium in tis-

sues concerned with acid secretion.[3,5,11] The explanation has been that the secretion of acid led to a local alkalosis which in turn enhanced the likelihood of local precipitation of calcium phosphate and carbonate. Inasmuch as arterial blood is ordinarily more alkaline than venous blood, the view may be taken that through diffusion, systemic arterial walls may be more alkaline than venous walls, the reverse being true in the pulmonary system. The view may also be taken that the alkalinity of oxygenated systemic arterial blood decreases progressively with increasing distance from the alveolar capillaries and venules. Hence, if the interstitial fluids of the vascular wall reflect the local alkalinity of the blood, the deposition of calcium and associated degenerative changes of constituent tissues might be expected to fall off progressively in the direction of flow of the arterial blood. The microscopic observations disclosed that this was not always so. In a further analysis of this matter, the lung, kidney and stomach may be considered separately as the three principal acid-secreting organs.

Theoretically, it might be predicted that the region of maximal alkalinity in the lung would be in connection with the blood in the postalveolar pulmonary venules, and that maximal degenerative calcifying changes would occur in the walls of these vessels. In general, this was the case (Fig. 4). There were negligible lesions in the pulmonary arterial system, and the conspicuous changes were initially found in the walls of interalveolar capillaries and adjacent interalveolar stroma. Later, the venules and small pulmonary veins were affected. Insofar as the respiratory passages are concerned, if we accept free diffusion of gases into tissues, the level of maximum alkalinity would be in the upper trachea (Fig. 3). Hence, it might be predicted that the changes would be more conspicuous here than in the terminal bronchi. In general, this was found. The tracheal structures showed the earliest changes, and as a rule calcification along the respiratory passages decreased progressively in identical tissue types in identical locations with increasing distance from the larynx. In a consideration of this rule, however, other factors have to be considered. First, the lesions around the circumference of any respiratory passage were not uniform at a given level. The earliest changes were either in goblet cells of the epithelium or in the subjacent stroma. With increasing time and severity of the disease, the changes spread circumferentially and also in a very specific way in depth. The circumferential spread finally led to involvement of the entire basement membrane zone and an increasing span of epithelium. The spread in depth tended to skip the loose collagenous tissues of the mucosa and to appear in the walls of mucosal vessels nearest the subepithelial zone of calcification, the smooth

muscle in the neighborhood, and the part of bronchial cartilages contiguous to the maximal mucosal changes. Thus, it seemed that the distribution, the sequences, and the magnitude of lesions were determined by the operation of local influences, as follows: alkalinity of environmental gases and fluids, type of tissue, type of cell, and the locally integrated functions of susceptible types of tissues or cells.

An analysis of alterations in the stomach may also be attempted in terms of relative alkalinity of parts of an acid-secreting organ. One might postulate that maximal alkalinity would be in relation to the venous blood flowing from the acid-secreting mucosa and that maximal calcification would occur in the tissues supporting the acid-secreting glands and in the walls of vessels leading from these tissues. This was not found even though, when mucosal changes did occur, they were restricted to the acid-secreting mucosa and to the *tunica propria* or cells of glands responsible for this function. Such changes, however, were inconspicuous in the early stages and often followed the development of prominent changes in the walls of arteries, the *tunica muscularis*, and muscularis mucosae (Fig. 5). Veins or venules were seldom affected. It might be assumed, therefore, that if relative alkalinity governed the distribution of the lesions in the stomach, it would have to operate in a curious way throughout the gastric wall. Consequently, it seemed that an inquiry should be made into the role of locally integrated functions of susceptible types of tissues or cells. It was apparent that the calcification was not a disorder of the acid-secreting gastric mucosa alone but a disturbance of the full thickness of the gastric wall concerned with acid secretion. For present purposes, the acid-secreting part of the stomach should be considered transmurally as a functional unit, separate and distinct, pathologically, from the remainder of the stomach. We have no explanation as to why the arterial system, the *tunica muscularis*, the muscularis mucosae and, to a lesser degree, the mucosa of only this part of the stomach were so severely affected by this degenerative calcifying disease. It offered a puzzling example of localization of pathologic changes in all susceptible tissue types in a functionally integrated system in a special location. The remainder of the intestinal tract offered other problems in this connection; namely, the variable pattern of involvement of the *tunica muscularis* at different levels, the variable resistance of different mucosal types to the disorder, and the restriction of severe arteriolar disease to the duodenal and, to a lesser extent, colonic segment of the intestinal tract.

Changes of the type found in the kidney have also been attributed to a local alkalosis, secondary to secretion of acids by the kidney.[10,11] It might be predicted therefore that changes would occur in that part

of the kidney just distal to the postabsorptive tubulovascular structure or in some special region concerned with selective absorption of alkaline elements or selective secretion of acidic elements. Actually, the earliest alterations occurred in the cells of the proximal convoluted tubules, soon thereafter in the basement membranes of these tubules, and somewhat later in the arterial supply to the affected nephron rather than the venous return from the nephron (Fig. 7). Thereafter, the arterial tree was usually progressively involved with increasing severity (Figs. 8, 15). It was concluded that within the limits of present knowledge of renal function, other factors than local alkalinity determined the distribution and sequence of the changes. As in the instance of the lungs and stomach, these factors were concerned with the interrelationships between susceptible tissue types in a functionally integrated system in a given location. The spread of changes in the kidney demonstrated this very well. In the early stages only a scattering of total nephrons as complete units showed lesions, but these were the nephrons with short arterial connections close to the medullocortical junction. As more lesions developed, the intervening nephrons with similar arterial connections were affected. With increasing time and severity of the disease, there was a spread of the changes distally toward the capsule, but seldom did the animal live long enough to show much alteration in the nephrons of the peripheral third of the cortex. As cortical spread developed, there was a similar spread of changes in an ever-broadening medullary zone within which the medullary loops of the successively affected nephrons were presumably located. These sequences are presumably related to the distribution of functional load rather than of a specific secretory function among a system of nephrons.

The preceding comments have touched upon local factors such as alkalinity, type of tissue, type of cell and locally integrated functions of susceptible tissues or cells. Muscle cells were among the least resistant, the order of decreasing susceptibility being smooth, cardiac, and skeletal muscle. Furthermore, the most readily affected component of these cells was the contractile protein, especially the "A" disk. Among the stromal elements, the severest lesions appeared in elastic tissue, basement membranes, the matrix of cartilage and the sarcolemmal investiture of muscle cells. Cellular and stromal elements were not equally susceptible in all locations. Hence, the development of this systemic metabolic disorder did not result in a generalized modification of a given element, and this is analagous to the effects of certain "toxins."[23] On the contrary, it led to conspicuous modifications of these elements in specific locations without changes in identical struc-

tures in other locations. The extent to which this selectivity may be attributed to unrecognized differences in elements assumed to be identical, remains an intriguing problem. Certainly there are good reasons for believing that the composition of structures such as smooth muscle cells, collagen, elastic tissue, basement membranes and cartilaginous matrices is not the same everywhere. By the same token, the functional burden to which these elements are subjected is not the same everywhere.

The third mechanism was concerned with adaptation. Three adaptive activities deserve mention: the intimal proliferative reactions in arteries; the excessive production of atypical osteoid matrix following extensive resorption of bone; and the regression of the disease despite continuation of the same dosage regime which produced it. The discussion of these and other less obvious adaptive reactions awaits the completion of more prolonged experiments.

The fourth mechanism was concerned with restoration, principally the resorption or elimination of abnormal mineralized tissue, and the regeneration of tissues to replace those attacked by the disease. The salient feature of this mechanism was its indolence. In the respiratory mucosa, desquamation of calcified goblet cells and regeneration of basal cells to replace them were sluggish processes. At times, the regenerative sequences took a metaplastic trend so that the degenerated epithelial layer was replaced by one or more layers of stratified squamous epithelium. Wherever this occurred, there were local defects in the basement membrane and chronic inflammation in the subjacent mucosa. The mineralized basement membranes also tended to persist, being very slowly resorbed (Fig. 3). With increasing mineralization, there was increased fragility of these structures. Fractures developed and discontinuities thereby created seemed to stimulate local proliferation and accumulation of histiocytes. These commonly formed multinucleated giant cells which slowly resorbed the mineralized stromal structure, often encapsulating it completely. As this proceeded, the mineralized basement membrane seemed to migrate to a deeper level, and a regenerated stromal support appeared as a new structure between it and the respiratory epithelium. Similar mechanisms of encapsulation and resorption were encountered as far distally as the alveolar ducts but were seldom detected in connection with the calcified components of alveolar walls or pulmonary vascular channels. Alveolar walls seemed to break down spontaneously with the production of emphysema, but it was not clear that these mechanisms participated in the rupture (Fig. 4).

The behavior of the mesenchyme adjacent to abnormally mineralized

cartilages along respiratory passages was also interesting. Intense mineralization was usually conspicuous on the mucosal side of the cartilage. The perichondrium became less distinct, and the margin of the calcified matrix acquired a moth-eaten appearance. Capillaries occasionally invaded the marginal lacunae, and in some instances there was active formation of new cartilaginous matrix by proliferating chondrocytes. These activities resembled those which normally occur at provisional zones of endochondral bone formation. The overall result here, however, was the slow resorption of the heavily calcified bronchial and tracheal cartilages without osteogenesis.

Resorption and regeneration were still more sluggish in the kidney. Calcified tubulovascular components of the nephron seemed to endure without resorption or regeneration for at least several weeks. Evidence of regeneration of tubular epithelial cells of affected nephrons, compensatory hyperplasia of tubules of spared nephrons, interstitial fibrosis, inflammation, arteritis, glomerulosclerosis or other responses were never significant. The principal reaction occurred in the precortical and large cortical arteries. Here, there was intimal proliferation, excited less by degeneration and calcification of the media than by discontinuities due to fractures of calcified internal elastic membranes (Fig. 15). Whatever lesser reactions occurred in the subepithelial stroma of the renal pelvis were similar in most respects to those described in connection with the basement membranes of the respiratory tract.

Resorption and regeneration in relation to the acid-secreting part of the gastric wall were also very sluggish. The mucosal structures were not very responsive to the degenerative changes. The intimal reactions in the arterial tree were more active than in other organ systems, and the proliferative reactions also extended further into the smaller arterioles. The muscularis mucosae and the *tunica muscularis* showed the most conspicuous evidence of resorption and regeneration. In both locations, the degenerated calcified smooth muscle and intervening stroma often stimulated local histiocytic and giant cell reactions. These cells assisted in the resorptive process. Also, at times, there were many large pale elongated immature elements which appeared to be regenerating smooth muscle cells. In no case, however, was there significant leukocytic infiltration, local vascularizing reaction or fibrosis.

The complexities of resorption and regeneration in the skeletal system were difficult to interpret. Initially, the resorption of bone was accelerated, the number of osteoclasts was increased, and the number

of osteoblasts was decreased (Fig. 9). A curious proliferative process followed, resulting in the formation of a large amount of abnormal osteoid matrix beneath the periosteum and in the marrow around the residual cortical and trabecular bone (Fig. 10).[12,13] Though this matrix may be called "osteoid," it was more fibrillar, less homogeneous and much more heavily stained with hematoxylin than normal osteoid tissue. Furthermore, there was no relation between the voluminous deposits of matrix and any particular array of cells which resembled osteoblasts. The tissue looked more like collagen which had as much affinity for hematoxylin as any of the extra-osseous calcified stromal or cellular elements. With onset of repair, normal eosinophilic osteoid tissue and bone appeared in the midst of this voluminous basophilic fibrillary material which was resorbed. It was conspicuous only when extra-osseous calcium deposition had essentially ceased or in the period following termination of ergosterol administration. The prompt and rapid formation of bone was more pronounced in connection with the peritrabecular matrix and in the subperiosteal regions adjacent to the margins of articular cartilages. This led to a deformed osseous framework with depleted porous cortical bone, an excess of periosteal new bone, an excess of endosteal bone, and a coarse dense trabecular structure displacing the marrow. The final result had some resemblance to osteosclerosis of a pagetoid type with myelofibrosis (Fig. 10). The development of atypical hematopoietic elements as these changes encroached upon the bone marrow is beyond the limits of this discussion.

Finally, it was clear that a moderate dosage regime was most effective in the first few weeks. As the regime was continued, the expected progression of pathologic changes failed to occur, and evidence of the disease slowly disappeared. This was attributed to the activation of obscure mechanisms which provided increasing tolerance, resistance or immunity to the action of excessive vitamin D. In the light of certain degenerative calcifying human diseases, an inquiry into the nature of these adaptive and restorative mechanisms would seem to be of first importance.

Summary

A microscopic study of the evolution of hypervitaminosis D in rabbits disclosed generalized pathologic sequences which were due to partly reversible inflammatory, degenerative and mineralizing processes. The participation of these processes in the pathogenesis of the disease in a given location was governed principally by the level and duration of dosage with the vitamin. Administration of the vitamin in amounts just sufficient to produce pathologic changes in 2 or 3 weeks

led to mineralization of certain tissues which do not normally calcify. Under these conditions the deposition of calcium occurred without significant preliminary local structural modifications attributable to inflammatory or degenerative processes. When somewhat larger doses of the vitamin were used, abnormal mineralization of tissues was often preceded and accompanied by structural changes of a degenerative type. In order to excite significant early inflammatory reactions, still larger doses of the vitamin and perhaps other factors were required. These reactions occurred only in cardiac and skeletal muscle. They were localized to the walls of arteries, periarterial tissues and adjacent muscle, in which there were signs of progression of degeneration and mineralization.

Studies of restorative phases of the disorder disclosed evidence of reversibility and resistance to progression of these processes. The inflammatory reactions in muscle subsided, and mild indolent reparative reactions appeared in many degenerated mineralized tissues. Furthermore, additional degenerative changes seemed to progress very slowly, if at all, while, at least in some locations, the process of mineralization was slowly reversed even though the dosage was maintained. The reversal was characterized by the resorption of abnormally calcified matrices. At times, this occurred spontaneously, but as a rule the resorption was associated either with mobilization of macrophages and giant cells or penetration of the calcium deposits by vascularized stroma. The signs of reversal were usually preceded or accompanied by resumption of osteogenesis in the abnormal osteoid matrices in bone.

The same type of tissue or cell in different locations was not equally affected by the inflammatory-degenerative-mineralization mechanisms, presumably because of a difference not only in the composition of tissue gases and fluids from place to place but also in the susceptibility of a particular tissue in different locations. The latter was governed primarily by participation of a cell or tissue in a functionally integrated and spatially related system which had in one or more of its parts a high calcification potential.

The designation of function as adding to or detracting from the inflammatory-degenerative-calcification potential of a given structure led to a better understanding of patterns of the disease, especially in the respiratory tract, kidney, stomach, muscles and arteries. It may also assist in the future analysis of the reasons for the resemblance between certain structural changes in this experimental disease and those encountered in some presumably unrelated human disorders which are indolent, fundamentally degenerative, and ordinarily attributed to ill-defined metabolic derangements of advancing age.

Among these, senile osteoporosis, hypertrophic arthritis, pulmonary emphysema and arteriosclerosis deserve more than fleeting consideration, so long as it is recognized that identity of structural change does not imply identity of pathogenesis.

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LEGENDS FOR FIGURES

FIG. 1. Multiple, focal, inflammatory-degenerative-calcification changes in the myocardium of a rabbit given 600,000 units of viosterol in 2 weeks. The inflammatory reaction is located principally in and around the walls of small arteries and arterioles which in turn are surrounded by cardiac muscle showing degeneration with early calcification. Hematoxylin and eosin stain. × 200.

FIG. 2. Massive calcification of cardiac muscle in a rabbit given 1,200,000 units of viosterol in 7 weeks. The degenerated calcified muscle cells have multiple transverse fractures through which proliferating stromal elements have grown. The isolated calcified fragments of cells have undergone partial resorption. There is no inflammatory reaction. Hematoxylin and eosin stain. × 150.

FIG. 3. The tracheal mucosa of a rabbit given 1,200,000 units of viosterol in 7 weeks. Intra-epithelial calcification is in the form of concentrically laminated pseudocrystals which seem to develop exclusively in the goblet cells. The subepithelial calcification is concentrated in the darkly stained fragmented basement membrane. Note the absence of inflammation. Hematoxylin and eosin stain. × 500.

FIG. 4. A peripheral field of the lung of a rabbit given 1,200,000 units of viosterol in 6 weeks. Hematoxylin has a strong affinity for calcified tissue, demonstrated here as deeply-stained black segments of an emphysematous alveolar wall and the entire thickness of the wall of a small pulmonary vein. These changes in alveolar and venous walls regularly occurred together and were independent of inflammation. Hematoxylin and eosin stain. × 500.

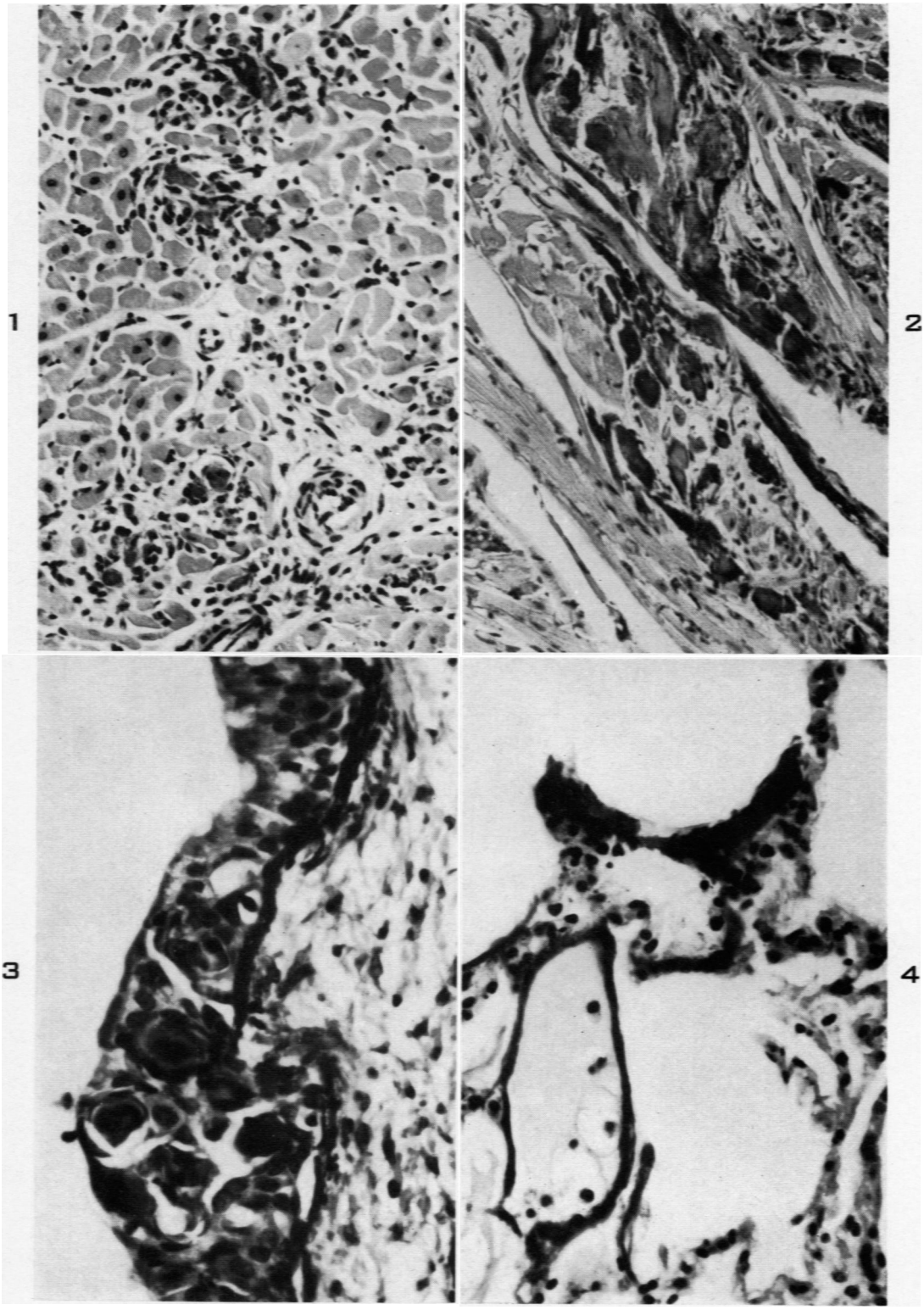
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FIG. 5. A cross-section of the wall of a part of the stomach concerned with secretion of hydrochloric acid. The rabbit from which this section was made was given 1,600,000 units of viosterol in 14 weeks. Hematoxylin has an affinity for calcified tissues, shown as irregular small dark areas in the mucosa, in the walls of submucosal arteries, in the bundles of smooth muscle of the inner half of the *tunica muscularis* and in the wall of a small serosal gastric artery. Hematoxylin and eosin stain. × 40.

FIG. 6. A high-power photomicrograph of one of the small darkly stained calcified areas in the *tunica muscularis* of the wall of the stomach shown in Figure 5, showing degenerated smooth muscle cells in the midst of granules and filaments of calcified protoplasm demonstrated by its strong affinity for hematoxylin. The small calcified granules are in the cytoplasm of the muscle cells. The tortuous calcified filaments are external to smooth muscle cells and seem to consist largely of interstitial fibrous or reticular elements. Hematoxylin and eosin stain. × 600.

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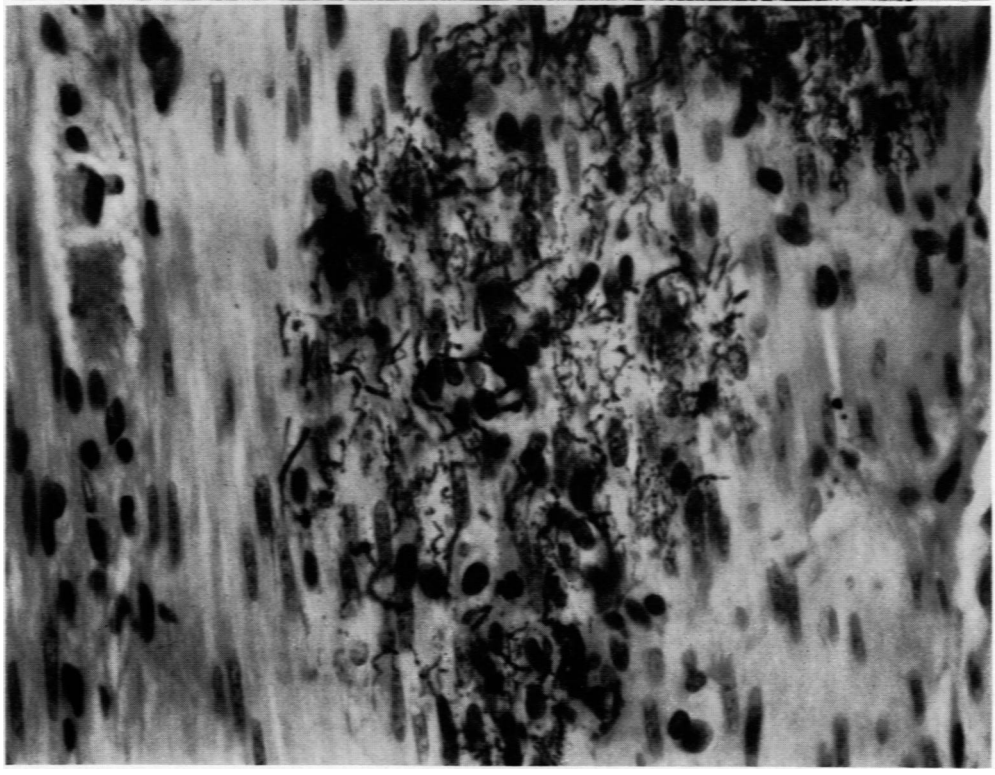

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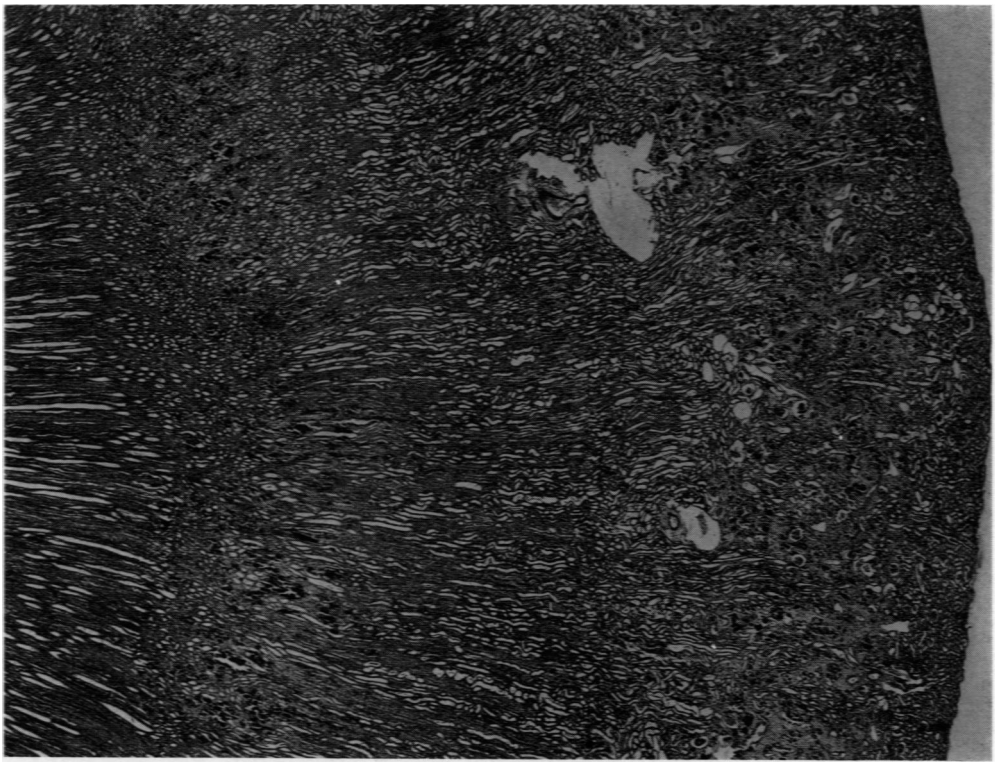
FIG. 7. The cortex and medulla of a kidney from a rabbit given 1,000,000 units of viosterol in 5 weeks. Six weeks later the animal was sacrificed. The loss of normal structural detail is apparent in the inner half of the cortex and in a narrow transverse zone in the middle of the medulla. The small dark spots represent calcified structures in these 2 locations. The intervening lightly stained areas which are more conspicuous in the cortex are places where proliferating connective tissue has replaced degenerated tubulovascular units of nephrons. This represents an early stage of resorption of mineralized tissue with repair and regeneration. Inflammation is negligible in the kidney at all times during the development or regression of the disease. Hematoxylin and eosin stain. × 23.

FIG. 8. A field in the inner third of the cortex of a kidney of a rabbit given 800,000 units of viosterol in 4 weeks. This shows early calcification of glomerular structure with minimal associated alterations in tubular structure. The calcified elements have an affinity for hematoxylin. There is a tendency for calcium to accumulate in isolated glomerular loops. In one glomerulus the accumulation is in continuity with the calcified wall of the arteriole entering the glomerulus. Hematoxylin and eosin stain. × 220.



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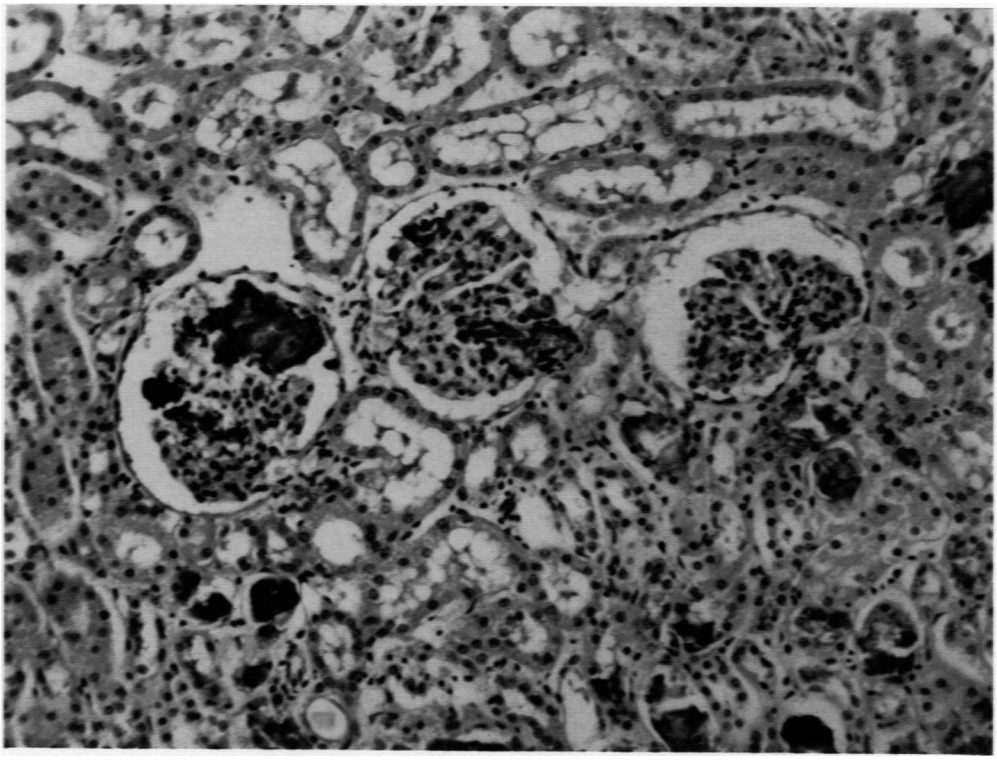

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FIG. 9. A longitudinal section of a rib from a rabbit given 1,200,000 units of viosterol in 4 weeks, showing widespread resorption of bone with conspicuous porosity of cortical bone, fibrosis of the marrow, and early subperiosteal deposition of osteoid tissue. The changes are characteristic of the disorder between the third and fifth weeks. After about the fifth week, the changes illustrated in Figure 10 become increasingly dominant. Hematoxylin and eosin stain. × 60.

FIG. 10. A longitudinal section of the vertebral column of a rabbit given 1,200,000 units of viosterol in 12 weeks. The resorption of bone, typical of early stages of the disease (see Fig. 9), has led to partial collapse of a vertebral body which is at this later stage undergoing an osteosclerotic pagetoid change. This is characterized by excessive deposition of osteoid tissue which has a conspicuous dark color due to its affinity for hematoxylin. Much of this abnormal osteoid tissue has been converted to bone, producing a dense trabecular structure which has seriously encroached upon the marrow. Also, note the proliferative changes which have produced "spurs" of new bone at the margins of the intervertebral disks. Hematoxylin and eosin stain. × 10.

FIG. 11. A cross-section of the wall of the thoracic aorta of a rabbit given 600,000 units of viosterol in 2 weeks. The inner third of the elastic tissue framework, stained deeply with hematoxylin, is uniformly impregnated with calcium. Note the absence of any inflammatory reaction though only a trace of residual cellular structure remains between the calcified elastic lamellae. Hematoxylin and eosin stain. × 250.

FIG. 12. A cross-section of an iliac artery of a rabbit given 1,000,000 units of viosterol in 5 weeks. The segmental darkly stained areas represent subintimal bands of calcific deposits in the inner half of the media. These discontinuous deposits tend to spread in all directions until they occupy the entire media. Meanwhile, there is a reactive proliferation of the intima which in time tends, as shown, to be equal in thickness to the part of the subjacent media inactivated by calcification. Hematoxylin and eosin stain. × 30.

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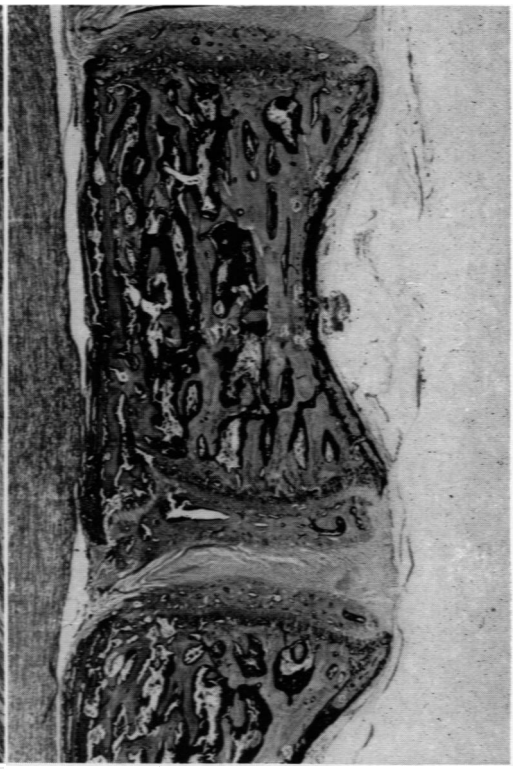

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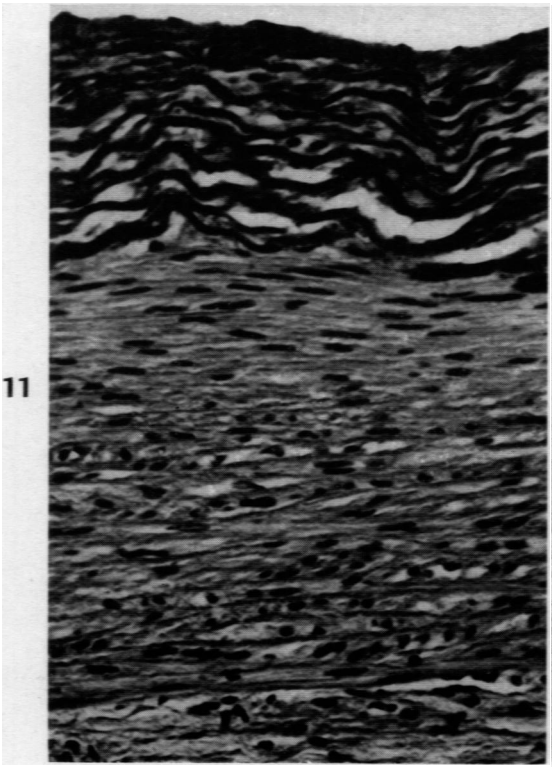

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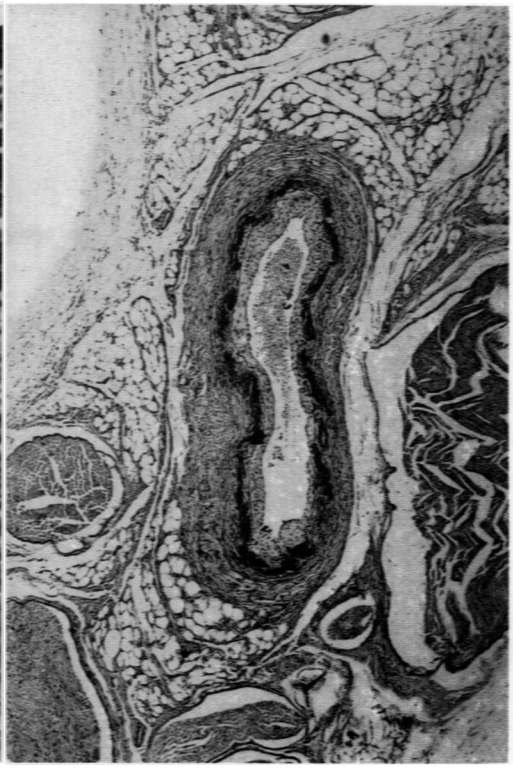

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FIG. 13. A cross-section of a carotid artery of a rabbit given 1,000,000 units of viosterol in 15 weeks. From the lumen outwards 3 distinct zones are shown. They are of almost equal thickness. The inner zone is composed of proliferating fibrocellular intimal tissue. The middle zone, deeply stained with hematoxylin, is the inner half of the media which has been uniformly impregnated with calcium. The outermost zone is the external half of the media which has been unaffected by the disease. Hematoxylin and eosin stain. × 32.

FIG. 14. A pancreatic artery of a rabbit given 1,600,000 units of viosterol in 14 weeks. The darkly-stained material in the vascular wall is calcified tissue with a strong affinity for hematoxylin. The deposits of calcium involve most of the internal elastic membrane and a modest amount of the media subjacent to the calcified parts of the internal elastic membrane. Three short stretches of the internal elastic membrane and adjacent media are normal. The loose-textured fibrocellular tissue internal to the calcific deposits is proliferating intima. Note that intimal proliferation did not occur over the 3 stretches of internal elastic membrane which were unaffected by the calcific disease. Hematoxylin and eosin stain. × 225.

FIG. 15. A small artery and adjacent arteriole in the renal cortex of a rabbit given 1,500,000 units of viosterol in 2 weeks. The walls of these vessels are heavily calcified as indicated by the strong affinity of the media for hematoxylin. Swollen intimal cells with their nuclei projected away from the calcified media toward the lumen of the larger artery represent the earliest intimal reaction to degenerative calcifying medial disease. Hematoxylin and eosin stain. × 600.

FIG. 16. A tiny arteriole in skeletal muscle of a rabbit given 1,200,000 units of viosterol in 7 weeks. The media of the arteriole, stained deeply with hematoxylin, is uniformly calcified. The swelling of the intima has reduced the size of the lumen. In the adventitia there are a few inflammatory cells representing a mild form of periarteritis often found in cardiac and skeletal muscle of rabbits dying of pneumonia during the course of a high-dosage viosterol regime. Hematoxylin and eosin stain. × 500.

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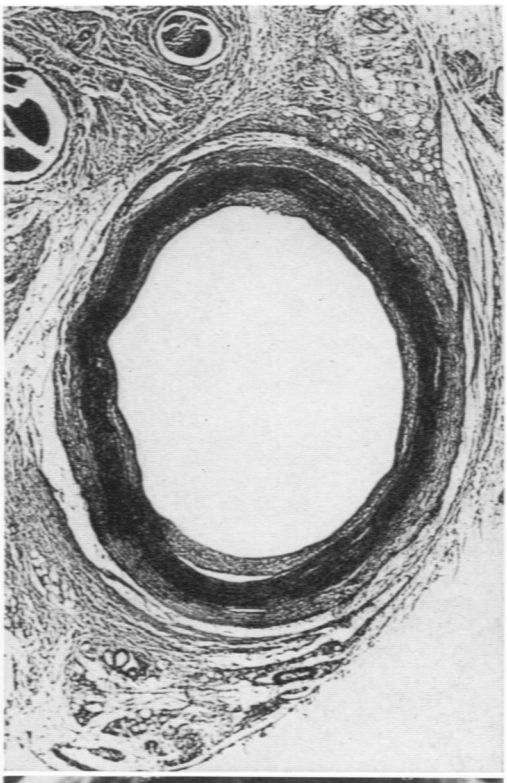

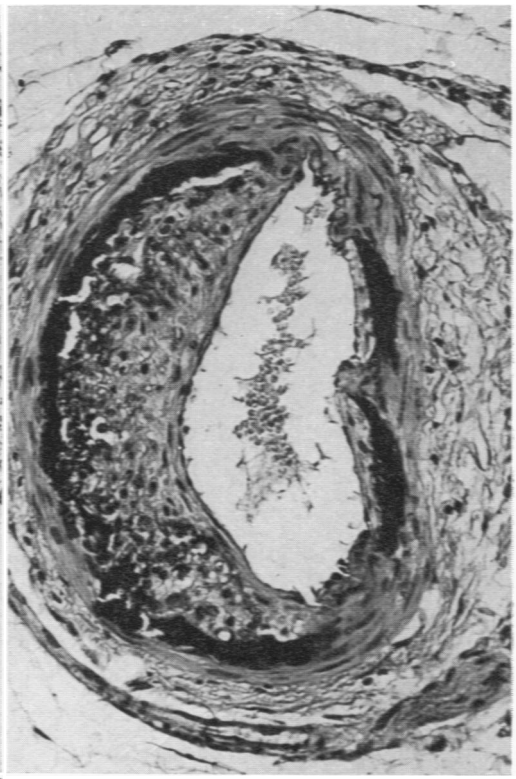

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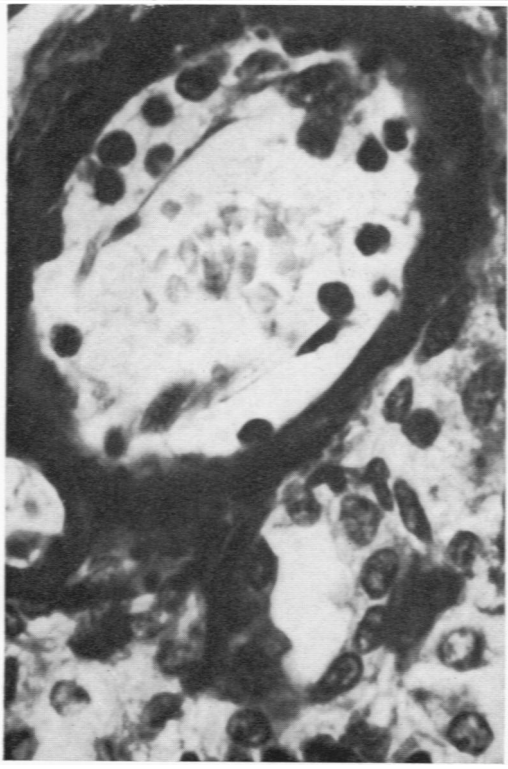

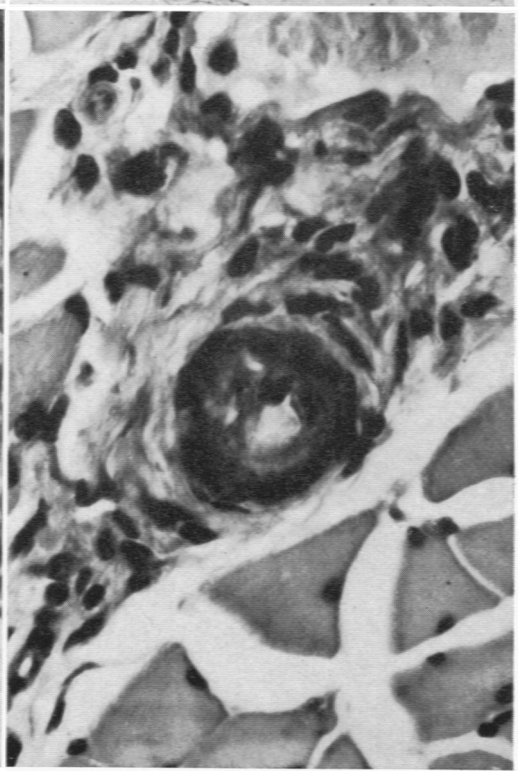

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