SOME ASPECTS OF EXPERIMENTAL ARTERIOSCLEROSIS IN THE DOG

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Charles C. Middleton
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By

Charles C. Middleton

AN ABSTRACT

Submitted to the College of Veterinary Medicine
Michigan State University of Agriculture and
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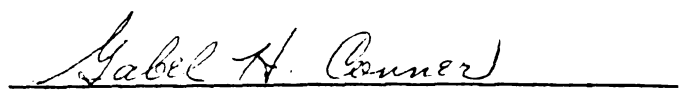
Approved 

ABSTRACT

Some aspects of experimental arteriosclerosis of the mature male dog were studied. Thirty-six dogs were thyroidectomized taking care to preserve the blood supply of the parathyroid glands. Vitamin D was administered at the rate of 100,000 units daily starting the first post-surgical day. Six control dogs were not thyroidectomized and received no Vitamin D.

Symptoms of Vitamin D toxicity became evident between the fifteenth and thirtieth day post-thyroidectomy. Debilitation was marked by the time of death which occurred by the seventy-third day post-thyroidectomy. Collateral circulation studies were attempted by transecting a pelvic limb and immediately reapposing the transected tissues. The femur, femoral artery and vein, and the femoral and sciatic nerve were left intact. This procedure was performed on the forty-ninth, fiftieth or fifth-first day post-thyroidectomy. On the eighteenth day following transection the femoral artery and vein were ligated. Limbs of the thyroidectomized animals that survived the transection did not survive the ligation as evidenced by necrosis distal to the site of transection in all but one dog.

Electrocardiograms were recorded. Serum calcium levels showed a significant increase in the thyroidectomized dogs during the course of the experiment. Serum cholesterol levels were progressively elevated in the thyroidectomized dogs. Limb temperatures were recorded at regular intervals.

Thyroidectomy coupled with vitamin D administration proved to be a relatively quick method of producing lesions of arteriosclerosis. Due to severe debilitation collateral circulation studies were not conclusive.

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CHAPTER I

INTRODUCTION

Cardiovascular disease found in ancient mummies would suggest that this condition is as old as recorded civilization (Moodie, 1923).

It was not until early in the twentieth century that the condition was found to be related to a dietary etiology or experimentally produced in animals (Duff, 1935). There has been a vast amount of theorization and research since that time but cardiovascular disease continues to be a perplexing problem.

Most of the early experimental work in the field of atherosclerosis and arteriosclerosis was done with the rabbit and chicken. Until the dog and other domestic animals were used for the experimental production of cardiovascular disease, very little attention was focused upon the spontaneous occurrence of the conditions in domestic animals. This brought to light the fact that arteriosclerosis and atherosclerosis do occur in domestic animals spontaneously and thus animals used for a controlled experiment must be within a selected age group.

The experimental production of arteriosclerosis and atherosclerosis is rather easily and rapidly accomplished in the rabbit and chicken but is met with more difficulty in the dog. It was found that depressing thyroid function (Smith and Jones, 1957) or removal of the gland (Smith and Jones, 1957) greatly enhanced the experimental production of the disease in the dog.

Since cholesterol and calcium aid in the formation of the lesions, attempts have been made to correlate an elevation of serum cholesterol and serum calcium with the spontaneous and experimental occurrence of atherosclerosis and arteriosclerosis.

Hypertension and electrocardiographic changes are frequently associated with cardiovascular disease and blood pressures and electrocardiograms are an important diagnostic and prognostic aid.

There has been an increased interest in recent years in revascularization of partially thrombosed areas and in the natural collateral circulation development to an ischemic area.

The purpose of this study was to experimentally produce arteriosclerosis in thyroidectomized dogs so that serum calcium and cholesterol levels could be studied in relation

to the histopathological changes produced. It was also hoped that blood pressure and electrocardiographic changes could be obtained. The development of collateral circulation in a transected limb of an arteriosclerotic animal was a point of considerable interest.

CHAPTER II

REVIEW OF LITERATURE

A. Introduction

The experimental production of arteriosclerosis and atherosclerosis has been accomplished in many species and by many methods. The main emphasis in the literature reviewed here will be on arteriosclerosis but due to the close relationship between the two conditions, some of the more pertinent literature on atherosclerosis will also be cited.

B. Paleopathology

That cardiovascular disease is not a new disease to man is well attested to by the findings of Moodie (1923). He reported on the histopathological and gross lesions of Egyptian mummies. The mummy of King Merneptah, the reputed pharoah of the Hebrew exodus, was found at Thebes, Egypt in the tomb of Amenhoted II who reigned in Egypt from 1448-1420 B.C. The ruptured aorta of Merneptah's mummy would dispute the theory that he was drowned in the Red Sea during the Hebrew exodus.

Histological study of the aorta showed the typical calcification of the aorta, the bony parallel elastic lamellae being perfectly preserved, and the interlamellar material thickly strewn with calcium phosphate.

Other mummies of the eighteenth to the twenty-seventh dynasties (1580-527 B.C.) also revealed lesions of arteriosclerosis which were exactly the same as are associated with the disease today. Williams (1948) examined two Peruvian mummies, probably between two or three centuries of A.D. 700. Microscopic examination revealed arteriosclerotic changes which included calcification of the aorta and a calcified thrombus.

C. Dietary Relationships

When Ignatovski, of the Russian Imperial Military Medical Academy, reported in 1908 that rabbits fed milk and egg yolks developed severe arteriosclerosis, he gave mankind the key to control of a disease which was becoming more prevalent as living standards improved (anonymous editorial, 1958).

Duff (1935), in a general review, further stated that Ignatovski was studying the effects of high protein diets in rabbits, when he noticed the occurrence of fatty plaques

on the intimal surface of the aorta of some animals. He attributed the lesions to the effect of an animal protein rich diet on a herbivorous animal. Starokadomsky the following year, 1909, repeated Ignatovski's experiment and drew the same conclusions. Stuckey reported in 1910 that by feeding various combinations of whole milk, egg yolk, egg white and meat juice, the diets containing egg yolk produced marked fatty changes in the aorta. He later produced identical lesions by feeding brain tissue. These findings led to the idea that the lesions were not due to the protein fraction of the diet but rather to some lipid common to the egg yolks and brain tissue.

Anitschkow and Chalatow in 1913 demonstrated that feeding pure cholesterol dissolved in oil could produce lesions in rabbits identical to those described by previous investigators (Duff, 1935).

Wacker and Hueck reported at about the same time that pure cholesterol added to ordinary food of rabbits could produce the lesions and that it also resulted in a marked increase in blood cholesterol levels (Duff, 1935).

It has been shown that while neither cholesterol feeding nor high fat diet had appreciable effects on plasma cholesterol in dogs, a diet containing both fat and cholesterol

had a decided effect, and the plasma level rose even higher if the diet was deficient in protein. Experiments by numerous investigators have gradually made it apparent that rabbits and chickens absorb cholesterol efficiently on diets low in fats, but dogs, monkeys, and man do not absorb much biliary or dietary cholesterol unless the diet contains sufficient fat. In man the bile provides daily as much cholesterol as would five to ten eggs and the absorption of this cholesterol is increased when the diet is rich in animal fat (anonymous editorial, 1958).

Recent work shows that no foods surpass eggs and butterfat in raising blood cholesterol of rabbits and man, and none have a more striking effect in acceleration of thrombus formation (anonymous editorial, 1958).

D. Spontaneous Arteriosclerosis and Atherosclerosis in Animals

Dauber and Katz (1942) reported that chickens show atherosclerotic lesions as early as the end of the second year of life.

Spontaneous arteriosclerosis was reported by Dauber (1944) to occur in the aorta in 45% of commercial roosters and hens over one year of age. There appeared to be no sex

differences in the incidence of macroscopic lesions. Only hens showed fatty lesions of the intima of the ascending aorta and aortic arch, while both roosters and hens commonly exhibited intimal lesions of the abdominal aorta. The lesions of spontaneous arteriosclerosis in the chicken resemble human arteriosclerosis lesions. If chickens are to be used for experimental arteriosclerosis studies they should be less than six months old since spontaneous lesions start to occur at this age.

Spontaneous arteriosclerotic lesions have been reported in cattle, dogs, swine and other species, but only rarely is the arteriosclerotic disease clinically significant (Smith and Jones, 1957).

While conducting an experiment on dogs unrelated to vascular changes, Morehead and Little (1945) noted degenerative and reparative processes in the vascular system which they concluded to be spontaneous in nature. This view was strengthened further by the fact that the vascular changes in the experimental animals were identical morphologically with those which have been described in a large percentage of human aortas removed from persons dying of various causes.

The above authors then obtained eleven mongrel dogs chosen at random for further study of spontaneous arteriosclerosis. These animals were sacrificed along with five puppies approximately six months old and three ten day old pups. Nine additional dogs from another terminated experiment that was thought to have no relationship to cardiovascular changes were also included.

Aortic lesions observed were:

1. Focal loss of elastic tissue with grouping of smooth muscle cells was most pronounced in the ascending aorta and arch. This was seen in varying degrees in all animals but was much less prominent in the ten day old pups.
2. Medial necrosis with cyst formation was pronounced in two dogs, but seen in varying degrees in eight other dogs.
3. Intimal thickening with splitting and reduplicating of the internal elastic lamina was present in fourteen animals ans was confined to the abdominal aorta. Seven dogs had nodular formations projecting into the lumen of the vessel. In every animal certain sections revealed a separation of the lamellae of the intima resulting in the formation

of irregular cystic spaces. This was best seen in the ascending aorta and arch of the vessel.

4. Localized areas of hyalinized collagenous tissue with calcification were observed in five dogs and was limited to the ascending aorta.
5. One dog had minimal sclerotic changes of the coronary artery.

Kollisch examined the aortas of 110 dogs and found arteriosclerosis in 23 of them. He included all degenerative vascular changes as lesions of arteriosclerosis (Morehead and Little, 1945).

Morehead and Little (1945) cited another group of dogs ranging from one to five years of age used to study spontaneous arteriosclerosis. Only one animal in the younger group was demonstrated to have true arteriosclerosis and in this instance the term was restricted to intimal degeneration with fatty deposits and plaque formation. In the older group of animals, arteriosclerosis was a fairly common lesion.

Hueper (1945) examined the aortas of fifty dogs between one and eight years of age. The majority of these animals were thought to be under four years of age. He concluded

that arteriosclerosis of the aorta is exceptional in dogs before the age of five. The arterial lesions encountered were predominately of the sclerosing type. He considers the highly edematous state of the inner part of the media of the ascending portion of the aorta frequently seen in dogs to be either a normal anatomical condition or an agonal phenomenon resulting from circulatory disturbances.

A study on 26 dogs, 14 of which were eight to seventeen years of age was done by Lindsay et al. (1952). This work states that the incidence of spontaneous arteriosclerosis of the aorta is high in dogs. The majority of the older dogs showed some evidence of cardiac disease, including coronary arteriosclerosis and myocardial infarction or fibrosis.

Medial aortic changes were observed in the majority of the dogs and were characterized by deposition of mucoid substance and collagen. This was often associated with focal proliferation of smooth muscle cells and focal alteration of elastic tissue.

The outstanding lesion of spontaneous canine arteriosclerosis is intimal fibrosis associated with deposition of a mucoid ground substance and collagen. The internal

elastic membrane may be altered without intimal disease and is almost invariably altered when intimal thickening is present.

Lipids appeared to play no part in the pathogenesis of the earliest intimal lesions, although lipids were present in later lesions. Lipid infiltration appeared to be a minor process even in the arteriosclerotic plaques of older dogs.

Cholesterol was not found in older lesions even where other lipids had infiltrated the intimal fibrous plaques (Lindsay et al., 1952).

The work of Detweiler (1959) showed that spontaneous arteriosclerosis was fairly frequent in dogs and increased in incidence with advancing age. It was also associated with chronic renal disease and was especially extensive in dogs with renal disease in which postmortem evidence of arterial hypertension was also observed.

The prominent lesions of canine arteriosclerosis, in the aorta, according to Detweiler, consist of intimal fibrosis with deposition of mucoid ground substance and collagen. The internal elastic membrane is usually altered by reduplication and fragmentation. Lipids do not occur in

the early intimal lesions and are not important in older ones. Recent observations reveal a high incidence of intimal changes in the posterior abdominal aorta and iliac arteries in dogs more than eight years old. Intimal lesions were frequently observed in coronary arteries associated with myocardial damage. Most older dogs were found to have medial sclerosis of the aorta, characterized by deposition of mucoid substances and collagen. This recent work indicates that arterial disease plays an important role in the pathogenesis of myocardial damage in older dogs and in some animals is associated with arterial hypertension and renal disease.

E. The Effects of Hypervitaminosis D on Man and Animals

Vanderveer (1931) used a group of rabbits varying in age from three months to one year for the production of arteriosclerosis. This was accomplished by feeding irradiated ergosterol biweekly until death. The earliest calcareous deposits in blood vessels were in the form of incrustations about the elastic fibers in the media. Smooth muscle degeneration of the media appeared later.

Pathological calcification may be divided into two broad groups according to Ham (1932). The first group depends upon degenerative changes in the recipient tissues. The mechanism may be that described by Klotz (1905) which depends on the breaking-down of fats with the formation of soaps and later more permanent calcium deposits, or that described by Wells (1925) which depends on the tissues assuming characteristics more physical than chemical, which enables them to bind calcium. The second group of pathological calcification is etiologically different according to Ham (1932). In this instance the blood becomes unable to retain all its calcium in solution. Ham (1932) was concerned with studying the sequence of events in tissues in an attempt to classify the calcifications. Rats were given enormous single doses of irradiated ergosterol which resulted in massive calcification of the aorta, coronary vessels, and cardiac musculature as soon as forth-eight hours following administration. Tissues examined 24 hours after administration showed nothing that would presage what was observed at 48 hours, so that the calcification did not appear to depend on degenerative changes in the recipient tissues. However, the rapidity

and massiveness of calcification suggested that the prime factor in this process was the inability of the serum to retain all of the calcium in solution. It was also suggested that a change in carbon dioxide tension, addition of other ions which would force precipitation of calcium salts, or a continued liberation of ions from the non-diffusable calcium after the diffusable calcium had reached its point of saturation may bring about these changes.

Goormaghtigh and Handovsky (1938) studied the effects of pure calciferol, administered orally, on the vascular and renal systems of the dog. They stated that vitamin D, a biological substance, given in adequate doses, caused almost simultaneously hypertension and hypertrophy of the arteriolar media. Four factors were thought to be responsible for the hypertension: (a) the thyrotropic effect of calciferol (vitamin D_2), (b) the increased tone of the hypertropic muscle cells, (c) the increased sensitivity of the arteriolar wall to epinephrine, (d) a central excitation. This hypertensive effect was elicited by daily dosage of irradiated ergosterol ranging from 70 to 90 micrograms of vitamin D per kilogram during a period of from 15 to 25 days. Hypervitaminosis D_2 with marked hypercalcemia led to glomerular, tubular and interstitial

lesions of the kidney with a distinct tendency toward uremia.

With thyroidectomy, thyroid aplasia, or marked hypothyroidism, the dosage causing the renal damage will also cause gross lesions of the aorta. A value of 13 milligrams per hundred cubic centimeters is the blood calcium level which is the prelude to massive calcium excretion, tubular distention, and subsequent glomerular regression (Goormaghtigh and Handovsky, 1938).

Follis (1955) administered acutely toxic amounts of vitamin D to rats and rabbits. This led to profound biochemical and anatomical changes. The serum calcium, and to a lesser extent serum phosphorus, concentrations rose abruptly. Calcium values reached their peak on the third or fourth day and then fell. Serum phosphorus followed a similar pattern. Alkaline phosphatase values began to fall and low levels were noted at the time of death. There was an unexplained rise in the arterial blood pH. Values as high as pH 7.8 were observed from the fourth to sixth day of the experiment. Renal damage was increasingly evident both microscopically and by chemical evidence of rising calcium and phosphorus concentrations in the renal tissue. Concentrations were also greatly elevated in the myocardium. Rachitic changes were noted in the bones but

no change in calcification of the epiphyseal cartilage was observed. Due to inanition, growth ceased, however, wide osteoid borders appeared which indicated that osteoblastic activity continued. The reason for the lack of calcification in such osteoid tissue in the presence of increased concentrations of calcium and phosphorus was not clear.

Lesions resembling human arteriosclerosis were experimentally produced in rabbits by Hass et al. (1960). They used irradiated ergosterol dissolved in peanut oil. This was administered at daily, biweekly or triweekly intervals. Initially the dosage was regulated at levels just below the quick lethal range. Pathological changes developed rapidly accompanied by weight loss and death within six weeks. The dosage and interval of dosage in later groups of rabbits was varied to facilitate studying the pathological vascular changes at various stages.

Serum calcium, total phosphorus and inorganic phosphorus were determined at one and two week intervals. The histopathological lesions produced by this method resembled the Mönckeberg type of changes seen in human clinical cases. There were no persistent or consistent elevations in the average levels of serum calcium, however, there was an upward trend in serum inorganic phosphate. There was

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no correlation between the serum calcium and inorganic phosphate levels and the degree of calcific medial disease. Abnormal deposition of calcium salts were found in many extra-osseous tissues. The calcium deposition in the vascular system was in the direction of arterial blood flow and extended further from the heart with extended periods of experimentation (Hass, 1960).

Overdosage with vitamin D in pups resulted in bloody diarrhea, anorexia, listlessness, greasy hair, vomition and loss of body weight. Necropsy revealed severe calcification of the stomach, heart, lungs, kidneys and muscles. The blood became more viscid with increased non-protein nitrogen and serum calcium. This would indicate progressive renal failure (Steiner, 1946).

Smith and Jones (1957) stated that massive doses of vitamin D caused an abnormally high level of blood calcium with metastic calcification especially of the arterial media.

A total surgical thyroidectomy and massive dosage of vitamin D orally was employed by Crisp et al. (1958) to produce arteriosclerosis in dogs. All of the animals under experimentation developed severe gross arteriosclerotic

lesions. The microscopic lesions included medial deposition of calcium and internal proliferation.

Hawk et al. (1954) gave the chief symptoms of vitamin D toxicity due to overdosage, as anorexia and polyuria. In advanced stages, calcification of soft tissues, particularly of the renal arterioles and the aorta was seen, hypertension was not encountered. Vitamin D overdosage is indicated by a rise in serum calcium.

In rats, Sodeman (1956) listed the effects of prolonged excessive vitamin D intake as loss of weight, anorexia, nausea, vomition, muscular weakness, apathy, hypercalcemia and phosphatemia and the calcification of cartilage, blood vessels and organs including the kidneys. The same conditions have developed in arthritic humans treated with 200,000 to 1,000,000 units of vitamin D. In an extensive literature review of arteriosclerosis, Hueper (1944) reviewed the changes associated with excessive doses of vitamin D.

1. Vitamin D, synthetic or natural, if given in massive doses caused toxic symptoms and arteriosclerotic lesions. Hypertension is reported in dogs and cats maintained on toxic doses of vitamin D. This is attributed

to the renal arteriosclerosis that develops. Severe myocardial damage resulting from vitamin D poisoning may cause hypertension.

2. There is general agreement that the toxic functional and anatomical effects elicited by excessive doses of vitamin D are related to the ensuing hypercalcemia and hyperphosphatemia. An elevation in serum cholesterol has been observed in cats, rabbits and man.

3. The arterionecrotic action of hypervitaminosis D has been demonstrated in monkeys, dogs, cats, rabbits, guinea pigs, rats, mice, chickens and to a limited extent in man. The lesions are identical in their extent, anatomic character, and organic distributions, as well as in their causative mechanism to those associated with parathyroid hormone poisoning.

4. The majority of investigators agree that the arterial and metastic organic calcification are of a secondary nature, i.e., they follow the primary degeneration and necrosis of the tissues brought about by the circulatory disturbances. However, there have been some workers reporting calcification in the absence of degenerative changes in the rat.

5. Low toxic doses of vitamin D, moreover, show degenerative vascular changes without calcification. These changes consist of loosening and swelling, glassy vesicular cells, disintegration of cells and fibrils, hyalinization of the media and fibrous thickening of the intima. These lesions are in contrast with extensive medial calcification and necrosis in the aorta and the coronary arteries seen in response to massive doses of vitamin D.

6. Hypertrophy of the renal arterioles and medial calcinosis of the afferent arterioles were described in dogs subjected to hypervitaminosis D by one group of workers. Another group of investigators reported gross aortic changes in dogs only after thyroidectomy.

7. It has also been reported that vascular injury, due to vitamin D, was transmitted from the mother to the young rabbits. Other reports failed to confirm this observation in rabbits, guinea pigs and rats.

8. Exposure to ultraviolet rays, such as those contained in the solar spectrum and those produced by ultraviolet ray lamps, elicit in the skin of man and animals photochemical reactions. These include the production of vitamin D, the generation of melanin from a precursor of epinephrine, tyrosine, the destruction of vitamin C,

and the release of histamine. Following solarization, there is a gradual drop in blood pressure due to peripheral vasodilation, which lasts for several days. The action of vasopressor proteolytic substances and a drop in blood tyrosine, indicate a deficiency of epinephrine. With repeated exposure to ultraviolet rays and increased pigmentation, the blood pressure rises and parallels the degree of melanosis, while the blood tyrosine remains low. There may be an increase in blood calcium.

9. Children exposed to solar radiation for several months evidenced no signs of hypervitaminosis D as a result of this treatment. Similar results were noted with mice exposed to ultraviolet rays in amounts suitable for the production of actinic cancer.

10. Rabbits fed nonirradiated ergosterol and subsequently exposed to ultraviolet rays or intense solar rays exhibited mild lesions of arteriosclerosis.

11. In a group of rats which were exposed to carcinogenic doses of ultraviolet rays, an excessive incidence of hyaline and calcified medial lesions in the aorta, coronary, pulmonary, pancreatic, renal, adrenal and testicular arteries were observed. These lesions increased in frequency, extent and severity with the duration of treatment.

12. Hypercalcemia resulting from excessive intake of calcium salts or from acidosis producing chemicals mobilizing the calcium in the osseous structures or from extensive destruction of bone tissue results in calcification of the arterial walls and certain organs similar to those found with hypercalcemias of parathyroid hormone and vitamin D origin. The same vascular changes occur in osteoporosis such as the cancers, multiple myeloma, endocrine disturbances of pregnancy and athyreosis and nephrosclerosis (Hueper, 1944).

F. The Interrelation of Elastic Tissue and Calcium in the Genesis of Arteriosclerosis

It has been shown by Blumenthal et al. (1950) that the splitting and fragmentation of the internal elastic lamella are the initial changes in the aging vessel. It has also been shown that the intensity and the rate of calcium deposition are directly proportional to the intensity and rate of the elastic tissue changes.

The association of elastic tissue changes and calcium deposition is not limited to man. The condition has been described in several species and is more pronounced in the rabbit, cow and bird.

Calcification of the arterial wall becomes visible before the formation of intimal plaques in man. This is thought to be the case in animals as well. If this is true, then if sufficiently old animals are used, the ease with which athreomatous plaques are produced by cholesterol administration is dependent upon the state of development of the spontaneous process of elastic calcification.

The alterations that constitute arteriosclerosis depend chiefly on the colloidal properties of elastin, and that the changes in the elastic elements of arteries are in striking agreement with the behavior of aging colloids in general. The alterations with age noted are: (a) reduced capacity to bind water, (b) decrease in elasticity, (c) decrease in permeability, and (d) a tendency for the gel to be transformed into a granular state with a marked decrease in the colloidal properties. Water loss is considered the primary process, with a resulting decrease in the capacity of the colloids to hold soluble constituents such as cholesterol and calcium in solution.

G. Vascular Degenerative Changes in Hypothyroidism

Kountz (1950) reported that persons with low basal metabolic rates as well as athyroid states had a definite

tendency toward degenerative changes in the smooth muscle of the blood vessels. Elderly persons that had a normal basal metabolic rate over a period of years prior to death did not have as advanced arterial changes.

It was also noted that calcification of the media proceeded intimal plaque formation and that the elastic fibers were primarily involved. The mechanism involved was thought to be a primary factor in the destructive effect on capillaries since the media of larger vessels is well supplied with vasa vasorum and this degeneration of the media could be related to a decreased blood supply or stagnated blood in the vasa vasorum. The thyroid may also play an important part in muscle metabolism.

H. Theories of the Genesis of Arteriosclerosis

In a general review of arteriosclerosis by Hueper (1944), several factors were listed which must be explained if a theory on the genesis of the disease is to be valid. These are:

a. The absence of appreciable arteriosclerotic lesions in people is fairly frequent.
b. The infrequent occurrence of degenerative and sclerosing lesions in young persons.

c. The absence of arteriosclerotic lesions in certain species of aged animals and the relatively early incidence of the lesions in other species.

d. The irregular topographic involvement of the arterial system by these changes.

e. The pronounced differences in anatomical types of arterial lesions and their relationship to structural vascular locations.

f. The relation of certain degenerative and sclerosing changes to sex and perhaps to race.

g. A correlation of these vascular changes to definite endogenous or exogenous causal factors in many cases.

h. The experimental production of various types of arteriosclerotic lesions in various species by markedly different chemical or physical means.

The Senescence Theory

The senescence theory of arteriosclerotogenesis assumes that the degenerative and sclerosing changes observed in arteries are in general the result of the physiological process of aging. This may be altered in individual cases by additional vasoactive factors. This is supported in

part by the increase in incidence of arteriosclerosis with increase in age. Additional support for this theory is derived from the demonstration of physical and histological changes in arteries, both large and small, related to aging. Observations have established the fact that the relative width, circumference of the vascular lumen, the length of the vessels, and thickness increase with age. Microscopic investigations have revealed that a degeneration of the elastic elements accompanied by a peculiar axial crystallization of the elastic lamellae during the fourth and fifth decade. These lamellae later undergo granular disintegration and develop discontinuities filled with collagenous tissue. This results in a reduction of tensile strength. These so-called senile vascular changes are the basis for subsequent alterations of the lipoidal, hyaline and calcifying types. These factors, according to the supporters of the senescence theory of arteriosclerosis, are an integral and important preparatory process in the formation of arteriosclerotic lesions.

The physiochemical reactions in the tissues underlying these morphologic senile changes and their secondary arteriosclerotic sequela consist of the colloidal gels composing the cellular and the intercellular matter. In

youth, these are finely dispersed hydrates of alkaline reaction. They become dehydrated with increasing age, denser, more coarsely dispersed and more acid. During this period, processes of precipitation and disintegration associated with the separation of complex colloidal material favoring the formation of deposits occur. Simultaneously, the mucin content of the vascular wall increases, the extracellular collagenous and elastic fibrils and hyaline membranes undergo impregnation and disimpregnation with various chemicals. This results in molecular transformation of the cement substances and physical and chemical changes of the elastic substances. Nitrogenous materials increase and an accumulation of waste metabolites such as calcium and cholesterol, in the aging dehydrated tissues leads to precipitation of these products. The diffusion process in such bradytrophic tissues, having no vessels of their own, depend, moreover, on the presence of large surfaces and decrease therefore with the density of the tissues. The localized nutritive disturbances in the tissues affected by these physico-chemical reactions are aggravated by the precipitates of calcium and cholesterol, especially since these are usually located near the vessel lumen.

Atherosclerosis, according to these concepts, is essentially the direct result of physiologic senescing processes of a physicochemical nature affecting the vessels and not the reaction product of alimentary hypercholesteremia or disturbances in cholesterol metabolism.

Investigations have shown that these physicochemical and morphologic vascular changes are not related to any appreciable quantitative disturbances in the cholesterol and calcium contents of the blood since they are not consistently elevated during old age.

The appearance of arteriosclerosis has been connected with a physiologic decrease in function of the thyroid gland during advanced years. Cessation of functional activity of the sexual glands is thought to be related to arteriosclerosis. This is evidenced by the incidence of arteriosclerosis found in women after oophorectomy and menopause, and sometimes in men after castration and during climacteric.

The fact that appreciable arteriosclerosis is not a consistent finding in old age and occasional demonstration of local as well as generalized arteriosclerotic, arteriocalcinotic and atherosclerotic lesions in babies, children

and young adults precludes the validity of the senesence theory of arteriosclerosis (Hueper, 1944).

The Mechanical Trauma Theory

The mechanical trauma theory considers trauma of the muscular walls resulting from the presence of exogenous physical forces or of physiologic or pathologic hemodynamic forces as a principal or an important contributory cause of the development of chronic degenerative arterial disease. The evidence, however, indicates that such physiologic or pathologic influences play a minor and secondary role, if any, in the development of arteriosclerosis (Hueper, 1944).

The Toxin Theory

The toxin theory is favored by some investigators according to Hueper. This is the concept that certain substances, of bacterial origin or of endogenous or exogenous chemical nature, exert a specific and direct cytotoxic effect on the vascular wall and thereby give rise to atherosclerotic, arteriosclerotic and arteriocalcinotic lesions.

This theory, as originally proposed by Virchow, assumes that bacteriotoxins present in the blood injure the intima and thus predispose it to subsequent atheromatous changes

by increasing its permeability to cholesterol and fats contained in the plasma. It further considers arteriosclerosis and hypertension as sequelae of the toxins' action.

This theory is not generally applicable as there are other mechanisms by which arteriosclerotic lesions especially those of the intima and atheromatous types are produced. The fact that the toxins do not always produce vascular changes would also question the validity of this theory (Hueper, 1944).

The Anoxemia Theory

The anoxemia theory of arteriosclerotic genesis, as reviewed by Heuper (1944), contends that the common fundamental action through which the various types of causal agents and their different causal mechanisms affect the vascular walls is represented by a severe and short, or a moderate and prolonged, but frequently recurring or persistent interference with the oxidative metabolism and nutrition of the vascular wall.

The validity of this concept on which the classification of the causal agents are based is supported by evidence obtained from an analysis of the degenerative

organic lesions found in association with the vascular changes. The organic degenerative manifestations are elicited by an appreciable number of agents in the brain, heart, stomach, blood, thyroid gland, and the testes, where they are caused by a local anoxemia related to the arteriosclerotogenic mechanism. These include such agents as carbon monoxide, lead, manganese, mercury and others which lead to softening in the basal ganglions in the brain which frequently give rise to symptoms of the Parkinsonion complex. Ischemic myocardial degeneration is characteristic of poisoning with epinephrine, nicotine, digitalis glucosides, parathyroid hormone, vitamin D, histamine, nitrites, and reduced oxygen pressure. Peptic ulcers on the basis of local ischemic vasospasm or stagnant anoxemic, are found in mercury, carbon monoxide, carbon disulfide, cyanides and nicotine. Chronic anoxemia of various types and genesis are responsible for the degeneration of the spermatogenic epithelium of the testes in exposure to reduced atmospheric oxygen pressure and in poisoning such as carbon monoxide, carbon disulfide, nitrites, nicotine, etc.

The thyroid gland, an organ which has an important function in oxygen metabolism of the body, becomes

hyperactive after poisoning with carbon monoxide, mercury, lead, manganese, cyanides, thiourea and sulfonamide.

Some factors producing anoxemia such as hypotonia of the vessels due to vasodilating agents, followed by stagnation anoxemia, disturb the metabolic processes since the media of large arteries depend entirely on diffusion processes for their nourishment and gaseous exchange. The outer two-thirds of the vessel receives oxygen and nutritive substances from the vasa-vasorum and with severe hypotonia, stasis develops in these small vessels.

Many toxic chemicals will bring about vascular hypotonus as will traumatic shock, hypopituitarism, hypoadrenalism and hypothyroidism. Chronic pathologic hypotensive states are not common, but it is well established that with prolonged and intensive relaxation of the arterial walls with a resultant fall in blood pressure and slowing of blood flow, an intimal proliferation and medial degeneration ensue. Endogenous agents as histamine, acetylcholine, anaphylactic shock, orthostatic shock, and anoxia are all factors in hypotonia and stagnation anoxemia.

Exogenous agents include nitrates and nitrites, carbon monoxide, arsenic, aromatic hydrocarbons, manganese, mercury

as well as agents producing traumatic vasoparesis, lead to hypotonia and stagnation anoxemia with resulting arteriosclerotic vascular changes.

Another type of anoxemia is hypertonia followed by vasoconstriction which results in anoxemia of the vessel wall. Hypertonic agents elicit a contraction of the muscular elements of the vascular wall and thereby a reduction of the vascular bed. This results in a compensatory acceleration of blood flow due to an increase in blood pressure. Prolonged or frequently recurring excessive vasomotion results in a disturbance of the nutritive conditions and oxygenation of the tissues. This results in degenerative vascular changes. The mechanism entails the following events: (a) inordinate vasoconstriction, (b) reduction of the blood flow of the vasa vasorum, (c) contracting media, with ischemic hypoxemia, (d) accumulation of metabolic waste products. The increased functional activity of the muscle cells results in an increased production of these metabolic waste products. The increased density of the constricted vascular structures hinders the movements of the interstitial fluid and thus reduces diffusion.

The validity of the hypertonia anoxemia etiological concept is attested by the myocardial and arteriosclerosis lesions in animals subjected to lethal poisoning by parathyroid hormone, vitamin D and digitalis glucosides. In evaluating the causative factors responsible for the hemorrhages and myocardial necrosis observed under these conditions, it is significant that the topographic relations on the myocardial arteries and arterioles to the surrounding muscular tissue of the heart are approximately the same as those of the vasa vasorum to the muscle tissue of the arterial media. The cardiac effect, of excessive doses of the three aforementioned substances, consists of an extraordinary prolongation and accentuation of the systolic contraction and an incompleteness and shortening of the diastolic dilation of the ventricles. This creates a circulatory stasis in the myocardial vessels which results in myocardial anoxemia. This is most pronounced in the myocardial tissue located near or beyond the end of the blood vascular supply, i.e., the subendocardial and papillary regions where the ischemic necrosis and hemorrhages are seen. Similar myocardial changes occur in connection with epinephrine poisoning.

Before concluding that hypertension and arteriosclerosis are related, one must determine if the hypertension is due to hypertonia or if it is due to increased cardiac output, increased blood volume, increased blood viscosity or reduction of elasticity of the vascular walls. It must also be remembered that not all hypertensive cases due to hypertonia result in arteriosclerosis.

Some of the endogenous and exogenous vasoconstricting agents, which have been employed in the experimental production of arteriosclerosis, include the following: vitamin D, epinephrine, physostigmine, nicotine, epinephrine derivatives and precursors (ephedrine, amphetamine, tyramine, pyrocatechin, tyrosine etc.). Also included are: psychic strain which is based on life insurance studies of various groups of people: adrenal cortical hormone which have a vasoconstrictor action as does the synthetic desoxycorticosterone, pituitary hormones, as pitressin, are associated with hypertension, and arteriosclerosis is noted in numerous cases having basophilic adenoma encroaching upon the posterior pituitary gland: parathyroid hormone in excess, but not toxic levels, results in hypercalcemia, hyperphosphatemia, bradycardia and hypertonia.

Other vasoconstricting agents which have been observed clinically to produce vascular sclerotic changes are hydrastine, ergot, barium chloride, lead, thyrosin and iodine, cold, physical trauma, renal hypertension and essential hypertension. These arteriosclerotic vascular changes are due to anoxemia according to this theory (Heuper, 1944).

With the increasing interest in space medicine recent workers have studied, among other things, the effect of altitude on the cardiovascular system. Highman and Altland (1949) studied a group of 14 day old rats exposed to 25,000 feet of simulated altitude for four hours daily. An important effect of this procedure was the development of polycythemia. Pathological observations consisted of gross cardiac hypertrophy, varying degrees of myocardial damage, and nodular thickening of the heart valves. There were also renal infarcts and other lesions not involving the cardiovascular system.

Aortic insufficiency was produced by Altland and Highman (1959) in one group of dogs and mitral insufficiency in other. Following thirty days post surgical rest, these animals were subjected to simulated high altitudes. The dogs were gradually taken to thirty thousand feet and maintained at this altitude for four hours daily. After a

ten day interval, surviving animals were then exposed to 32,000 feet, to 34,000 feet, and to 36,000 feet respectively.

The most constant pathological findings were visceral engorgement, increased cellularity of the bone marrow, cardiovascular lesions, and renal hemosiderosis. The severity of the lesions varied markedly. The cardiovascular lesions included thickening of the mitral and aortic valves with a large amount of metachromatic material and material staining like acid mucopolysaccharide. The myocardial arteries frequently had a thickened adventitia and occasionally cellular proliferation of the intima. Some animals showed intimal hyaline changes, necrosis, or hyalinization of the media. Some plaques were seen in the aorta and pulmonary artery and occasionally the coronary arteries. The thoracic and abdominal aorta showed arteriosclerotic lesions. The arteriosclerotic plaques were attributed to hypoxia.

Rabbits were fed a high cholesterol diet and exposed 23 hours daily to 16,000 feet simulated altitude for a maximum of 17 weeks by Altland and Highman (1960). The lesions produced were compared with identically fed rabbits maintained at ground level. The high altitude group showed much more severe atherosclerotic lesions in the

pulmonary vessels than did the ground level control group. However, lesions in the descending and abdominal aorta were milder. The high altitude group also showed a much higher incidence of calcification. It was thought that, at least in part, the greater severity of lesions in the high altitude group could be attributed to hypoxia.

Rinehart and Greenberg (1948) reported the occurrence of arteriosclerotic lesions in Rhesus monkeys subjected to pyridoxine deficiency. These same workers (1949) reported in more detail the investigative findings of pyridoxine deficiency in arteriosclerosis production. In 1951, Rinehart and Greenberg again reported on 18 Rhesus monkeys in which arteriosclerosis was experimentally produced in the same manner as their earlier work. They state that the sclerotic arterial lesions are related to pyrodoxine deficiency without a doubt, and that lesions so produced have not been found in other deficient states.

Calves, in which low magnesium levels were produced, developed arteriosclerotic lesions which were not associated with elevations of either serum calcium or phosphorus. This work was done by Hartman (1959) to further investigate work reported in the 1930's on calves on low magnesium diets.

White muscle disease, which has as its etiology a vitamin E deficiency, is associated with metastic calcification of the heart, skeletal muscle, kidneys, pancreas, fundal portion of the abomasum, duodenum, small intestine and lungs. This disease and the metastic calcification associated with it occurred in thirty lambs out of a flock of 400 in western Australia (Gardiner, 1961).

I. Collateral Circulation

It was observed by Plummer years ago that patients with exophthalmic goiter exhibited widespread dilation of the peripheral blood vessels. This prompted Herrick et al. (1933) to administer graduated doses of desiccated thyroid to dogs to see if the same results could be produced. This procedure resulted in an increase in blood flow from 200 to 300% in the femoral artery.

Ferris and Harvey (1925) using dogs, placed ligatures around the upper part of the femoral artery. Limb temperatures fell abruptly, about four degrees centigrade, following ligation. In about one hour there was 1.5 degree centigrade rise. The second hour the temperature fell 1.5 degrees centigrade and then rose suddenly to slightly above normal. During the following six hours the

temperature gradually decreased 12 degrees centigrade. This was at eleven hours post-ligation. There was a gradual rise during the next 75 minutes when suddenly within 15 minutes a 10.5 degree centigrade rise was recorded. There was a further abrupt rise of two degrees centigrade and it remained at this plane for the next 17 hours at which time recordings were terminated. One animal had only a slight temperature drop in the limb following ligation of the femoral artery. This was considered to be due to a very efficient collateral circulation already in existence.

These investigators concluded from rapidity of the temperature rise, that the first step in the establishment of a collateral circulation is a surprisingly abrupt vasomotor reaction, the mechanism of which was not determined.

Ligation experiments and temperature studies conducted on dogs by Mulvihill and Harvey (1931) consisted of (a) ligation of external iliac artery, (b) ligation of both external iliac arteries, (c) ligation of both external iliac arteries and unilateral removal of the tributary sympathetic ganglia.

Ligation of one or both external iliac arteries, close to the aorta, was always followed by a temperature drop in

the corresponding foot of from ten to thirty degrees. In a majority of the dogs, a rise in temperature and subsequent return to the previous level was noted in about 13 hours post-ligation.

It was the opinion of Mulvihill and Harvey (1931) that anatomical channels sufficient to care for the circulation is a vasomotor phenomenon. This was further substantiated in that the temperature of a limb which had dropped to room temperature post-ligation of the external iliac, rose to normal soon after removal of the tributary sympathetic ganglia and that simultaneous removal of tributary sympathetic ganglia with ligation of the artery prevented the lowering of the temperature of that limb.

In dogs with external iliac ligations, Harvey and Halpert (1931) studied the effects of: (a) transection of the spinal cord at the lumbar level, (b) transection of all posterior roots on one side below the level of the first lumbar vertebra, (c) removal of the tributary sympathetic ganglia on limb temperatures. This procedure caused a prompt rise in the temperature of the corresponding foot from room temperature to nearly that of the limb prior to ligation of the external iliac. This rise also occurred in animals with the external iliac artery ligated and the

spinal cord transected and also in the dogs with all the posterior roots cut below the level of the first lumbar vertebra.

Eckstein et al. (1941) designed an experiment to study the magnitude, time of development and pressure relations of collateral circulation following femoral, carotid and coronary artery ligation.

The femoral artery was ligated in the upper third and a canulla was placed in the main artery or in a side branch peripheral to the point of ligation. Immediately after occlusion, the flow and pressure values are small, however, within a minute retrograde pressures and flow increase and continue to do so for hours. Within 15 seconds following femoral artery ligation the peripheral pressure drops from the control level of 180/130 mm. Hg to 20/20 mm. Hg and the pulse disappears. After 90 minutes the pressure increased to 98/93 mm. Hg. Blood flow rises from 10 cc. per minute 15 seconds following ligation to 50 cc. per minute in 90 minutes. Once open, collaterals tend to remain open since after one hour when the ligature was removed, the retrograde pressure remained at about 98/93 mm. Hg and replacement of the ligature resulted in a drop

to 72 mm. Hg compared to 20 mm. Hg following the first occlusion. Two hundred and fifty days following ligation, the retrograde pulse was 160/125 mm. Hg and the flow was 200 cc. per minute.

These workers, Eckstein et al. (1941), concluded from this experiment that collateral circulation development is influenced by: (a) the openings of pre-existing but non-functional collaterals, (b) by a combination of differential pressures, metabolites, nerve action and the formation of new collaterals.

Collateral circulation development in the presence of an arteriovenous fistula, is not due to the Lewis Theory--that the tissue needs beyond the arteriovenous fistula excite development of collateral circulation, because amputation of the potentially ischemic tissues beyond the fistula does not interfere with collateral circulation development. The important condition for collateral circulation development was found to be the ready access of blood to the site of the fistula by retrograde flow through a widely patent distal artery and its branches. The most efficient deterrent to the development of collateral circulation is to ligate the artery just distal

to the fistula, leaving no branches intervening between the fistula and the site of ligation (Holman, 1949).

The collateral circulation which develops following ligation of a large artery depends upon transforming high end pressure in the parent artery into lateral pressure which directed into its branches, results in an increased volume flow through them. This volume flow distends the collaterals and opens up their prearteriolar and arteriolar beds (Holman, 1949).

Applying Poiseuille's Law of flow $Q = \frac{(P_1 - P_2)r^4}{L\eta}$, the most important value is r, for if the radius of the capillary is doubled, the flow within it is increased 16-fold. When 6% of the total blood volume is accommodated in the capillaries, 94% of the blood volume is either approaching or returning to them. This is the basis for Learmonth's (1950) approach to collateral circulation. The therapeutic objective is to keep the pressure as high as possible on the arterial end of the capillary and keep its caliber as wide as possible. To do this it is necessary to maintain adequate blood volume and normal systemic arterial pressure which is not due to vasoconstriction. Another consideration is the architecture of collateral circulation which falls into two groups: (a) after a short

course in alternative channels the arterial blood is returned into the main artery or arteries of the part, (b) arterial blood never returns to the main arteries, usually because they have been blocked by extensive thrombosis. In the normal arterial tree 20 mm. Hg are expended in forcing blood into arterioles, but with occlusion, and only small branches available, 60 mm. Hg are expended. These two groups make it obvious that to prevent ischemia the main and terminal arterial branches must be kept dilated to allow as much blood as possible to reach the arterioles. The arterioles must also be kept widely dilated to increase the amount of blood reaching the capillaries. These objectives are accomplished by the following methods: (a) use of drugs such as the dihydrogenated alkaloids to produce peripheral vasodilation, (b) blocking vasoconstrictor impulses at their synapses in the sympathetic ganglia with such drugs as tetraethyl ammonium bromide or chloride, (c) stimulation of vasodilator nerves with such agents as butylsympatol, (d) heat application to the part, (e) sympathectomy of vasoconstrictor nerves in the precarious region.

Collateral circulation is divided into three types of distribution, whether or not the main collateral branches

or accessory collateral branches are participating in its formation (Krahl et al., 1954).

1. Areas of hypervascularization--These areas are mainly formed by accessory collateral branches, very small in size, which carry blood to an area in which the normal blood supply has been cut off.
2. Supplementary branches--These may arise either from main or accessory collateral branches and are large enough to be identified by arteriogram. They usually run parallel to the occluded main artery but vary in size and distribution.
3. Bridging collaterals--Most of these are formed by main collateral branches. They delimit a segmental obstruction of a main artery and are actually responsible for the functional diversion of blood to the main artery distal to the obstruction. The blood flow is reversed in those bridging collaterals which originate from the main artery distal to the block.

The formation and development of new vascular channels may be modified or controlled by factors other than purely mechanical or reflex adjustments (Rosenberg and Liebow,

1954). This is suggested by inhibition of vascularization to an irritated cornea when previous or subsequent administration of corticotropin. Faulty or delayed wound healing is also reported in man and animals treated with cortisone. Growth hormone and desoxycorticosterone acetate have been shown to have a stimulating effect on healing. It has also been observed that bronchial arterial collateral development following pulmonary artery ligation, was much more rapid in newborn puppies than adult dogs. Rats with the iliac artery ligated and receiving five mg. per day of cortisone showed less collateral circulation development than did the controls. Rats that received growth hormone developed more collateral circulation than did the controls. The most surprising observation was that old rats developed the best collateral circulation. This would suggest that the initial size of the potential collateral channels may be a determining factor in their ultimate size. Perhaps the final size of the animal may thus influence collateral circulation.

Sewell and Koth (1958) employed the method first used by Halsted in 1922, when he transected the thigh of the dog except for the femoral and sciatic nerves, femoral artery, vein and femur. The transected muscles were

reapposed and allowed to heal. If the femoral artery was ligated within two weeks of surgery, the limb became gangreneous. Division of the vessel after two weeks did not cause gangrene. Sewell and Koth ligated the femoral arteries between the sixteenth and twentieth day. All of the transected dogs walked within a week after surgery but following ligation two out of five limped. The other three walked normally by the third week following ligation of the femoral artery.

CHAPTER III

MATERIALS AND METHODS

In this study, 42 male dogs were used. Of this number, 36 were thyroidectomized and six served as controls. Their weights ranged from 13 to 45 pounds. They were estimated to be between one and four years of age. The dogs were housed in a central pen for some time after procurement from the pound before starting them on experimentation. Only dogs that appeared to be in good health at the end of the holding period were used.

Fecal examinations were run on all dogs and they were given vermifuges as indicated. None of the dogs were vaccinated against distemper, hepatitis or leptospirosis. Dry kibbled meal* was fed free choice until the dogs became anorexic at which time the diet was changed to canned dog food.**

After the initial holding period a total thyroidectomy was performed in all but the control dogs. The dogs were fasted twelve hours prior to surgery. Sodium

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* Fromm Complete Dog Meal, Federal Foods Inc., Thiensville, Wisconsin.

** Hill Packing Co., Topeka, Kansas.

pentobarbital was given intravenously for general anesthesia and endotracheal intubation was performed after induction. At this time the dogs were weighed and a blood sample was drawn from the jugular vein for serum protein electrophoresis, serum calcium and serum cholesterol determinations.

Electrocardiograms* were recorded with the dog placed in the right lateral position with the left fore and hind limbs slightly extended. Leads I, II, III, AVR, AVL, AVF, CR6U, CR6L, CL6U, CL6L, CF6U, and CF6L were recorded.

The surgical procedure was performed with the dog secured in the dorsal recumbent position with the head extended. The surgical area was clipped and scrubbed with liquid germicidal detergent.**

A mid-line incision was made extending from the larynx caudally for four inches. By blunt dissection the thyroid glands were exposed and the parathyroid glands were carefully dissected free with iris scissors taking care to preserve their blood supply. The thyroid vessels were then ligated and the glands removed. The right carotid

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* Edin Electronic Cardiograph Model 220, Worchester 8, Massachusetts.

** Parke, Davis and Co., Detroit, Michigan.

artery was next exposed by blunt dissection and the blood pressure was recorded by means of a direct reading mercury manometer. A one-inch 20 gauge needle was used for cannulation. After the blood pressure was recorded, bulldog clamps were placed on the carotid artery above and below the puncture and left in place until a clot formed at the puncture site. The muscle fascia was sutured and the skin incision closed with simple interrupted sutures of number 50 nylon.

On the first post-operative day, the dogs were given 100,000 units of vitamin D* orally. This was continued daily throughout the experiment.

On the forty-ninth, fiftieth or fifty-first day following thyroidectomy, the dogs were weighed and a blood sample was drawn from the jugular vein. Thiamylal sodium** was given for general anesthesia and the dogs intubated. The gastrocnemius muscle temperature was recorded from both hind limbs using a direct reading thermometer*** with a trochar electrode. This was inserted about an inch into

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* Deltalin, Eli Lilly & Co., Indianapolis, Indiana.
** Surital Sodium, Parke, Davis and Co., Detroit, Michigan.
*** Electronic Thermometer Model TMB Tri-R Instruments, Jamaica 35, New York.

the muscle. An electrocardiogram was obtained from dogs 33(B), 26(B), 2(W), BSN, and 13(Y), and a packed cell volume was determined on dogs 16(W), 37(W), 38(W), 39(W), 12(Y), and 26(B). The dogs were next placed in a lateral recumbency with the pelvic limb to be transected suspended by means of a nylon cord. The limb was clipped and scrubbed with germicidal detergent. The transection was made midway between the stifle and coxofemoral joints and was complete except for the femur, sciatic nerve, femoral artery, vein and nerve. Bleeding vessels were ligated as they were encountered. After transection was complete and hemostasis established, the muscles were carefully reapproximated and sutured with interrupted Vetafil* sutures. Skin closure was routine.

The day following transection, dogs 26(B), 2(W), 33(B), BSN, and 13(Y) were given one grain of thyroid extract per orum and this was continued every fourth day until death. The other dogs did not receive thyroid extract.

Eighteen days following transection, the dogs were again anesthetized with thiamylal sodium and intubated. Limb temperatures were taken in the same manner as

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* Bengen and Co., Hanover, Western Germany.

previously described. The dogs were then scrubbed for surgery and a skin incision was made over the femoral vessels. The femoral artery and vein were exposed by blunt dissection, double ligated with vetafil, and sectioned between the ligatures. Closure was routine.

Pelvic limb temperatures were recorded every seventh day following ligation until death of the animal.

At the time of death, the aorta, cardiac muscle, lung and kidneys were collected and fixed in ten percent formalin. The aorta was rolled so that an entire longitudinal section could be made. A piece of all tissues collected was placed in a Decal* for calcium removal. The tissues were sectioned at six microns and stained with Von Kossa's, hemotoxyln and eosin, and Weigert's-Van Gieson stains.

Control dogs were not thyroidectomized, and were not given vitamin D. At the time they were started on experiment their body weight was recorded, a jugular vein blood sample was drawn, and the right carotid blood pressure was obtained as previously described.

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* Scientific Products Division, American Hospital Supply Corp., Evanston, Illinois.

On the forty-ninth, fiftieth or fifty-first day, the body weight was recorded and the pelvic limb was transected in the same manner as the thyroidectomized group. Eighteen days following transection, limb temperatures were obtained and the femoral artery and vein were ligated and transected as previously described. Every seventh day following ligation, limb temperatures were taken as with the thyroidectomized group. On the twenty-first post-ligation day, each control dog was anesthetized, limb temperature was taken and body weight obtained. A blood sample was drawn from the jugular vein and the carotid blood pressure was determined. The dogs were then euthanized and the aortas were collected and fixed in ten percent formalin. The tissues were prepared and sectioned as previously described.

Blood samples collected during the experiment were allowed to clot. The serum was collected and frozen until serum calcium, cholesterol, and protein paper electrophoresis determinations* were made.

Calcium determinations were made by means of the

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* Model R Paper Electrophoresis System, Beckman Instruments Inc., Belmont, California.

flame photometer.* Serum cholesterol determinations were performed by the method of Ferro and Ham.**

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* Coleman Instruments, Inc., Maywood, Illinois.

** Ferro, P. V. and Ham, A. B. A simple direct method for the determination of total serum cholesterol. Publication of Dade Reagents, Inc., Miami, Florida.

CHAPTER IV

RESULTS

A. General Considerations

Dogs that were housed together in a large pen for three weeks or longer prior to experimentation were in better physical and nutritional condition and survived longer than dogs kept for shorter periods. Death occurred within eleven days in some of the dogs that were used for the experiment shortly after receipt from the pound. Many of them were asymptomatic and others showed early clinical symptoms of canine distemper prior to death. Larger dogs remained in better condition longer than smaller ones in most instances.

Two dogs died as a result of fighting. After these losses the dogs were caged individually for the remainder of the experiment.

B. Thyroidectomized Dogs

Lethargy and anorexia became evident between the fifteenth and thirtieth day following thyroidectomy. At this time the diet was changed to a mixture of dry kibbled

meal and canned dog food mixed with water. This ration was eaten better, but the appetite remained poor. By the time of death, lethargy, anorexia, dehydration, mental depression and a rough greasy hair coat were noticed in all thyroidectomized dogs. Loss of body weight was pronounced as indicated in Table I. Decubitis ulcers and eczema occurred in most of the dogs, but to a variable degree. All thyroidectomized dogs were quite debilitated by the time of limb transection. Edema following transection and ligation was minimal. Limbs that survived transection did not survive femoral artery and vein ligation as evidenced by necrosis distal to the site of transection. See Appendix V, figure 3. Figures 1 and 2 in Appendix V show the limb condition of a dog that survived limb transection.

Gross arteriosclerotic lesions in thyroidectomized dogs were quite variable as indicated in Table IV. Some dogs had minimal cardiovascular lesions at the time of death 73 days following thyroidectomy. Other dogs that died as early as the thirty-fifth post-thyroidectomized day had extensive thick plaques and intimal elevations throughout the aorta and its large branches. Gross lesions were more pronounced and seen earlier in the smaller dogs in most instances.

Microscopic lesions are described in the photomicrographs of Appendix V. Gross cardiovascular lesions are shown and described in Appendix V also.

Day of death post-transection, limb condition, and the presence of gross lesions of the thyroidectomized and transected dogs are shown in Table IV.

There was electrocardiographic evidence of myocardial damage. See Appendix IV for electrocardiographic results and interpretations.

Serum calcium and cholesterol levels increased significantly during the course of the experiment as shown in Table II. Statistical analysis showed this increase to be on the order of five standard deviations. Dogs 30(W), 813, and 955 were not included due to a possible error in labeling of the blood samples.

The albumin-globulin ratios as determined by paper electrophoresis were within normal limits.

Limb temperatures are compared in Table III. However they were not statistically significant.

Dogs 26(b), 2(W), BSN, B(Y), and 33(B) received one grain of desiccated thyroid orally starting with the first post-transection day, and given every fourth day until death. Thyroid administration caused no visible change

in the behavior or general physical condition of the dogs. The effect that thyroid supplement had on gross, and microscopic lesions is not known.

C. Control Dogs

Control dogs evidenced no appreciable loss of body weight. They remained alert and active throughout the experiment. Growth rate of hair following clipping was normal and the hair coat was unaffected. The appetite remained good throughout the experiment. Neither decubitis ulcers nor eczema was a problem. All dogs survived limb transection and vessel ligations. There was considerable edema distal to the transection for about five days following surgery. A slight reoccurrence of edema was noted following vessel ligation.

Body weights at the time the dogs were started on the experiment are compared with the weights at termination in Table I. Serum calcium and cholesterol levels were not altered appreciably during the course of the experiment as shown in Table II. The transection data and gross lesions are shown in Table V. Paper electrophoresis revealed no appreciable changes in the albumin-globulin ratios.

At necropsy, one dog was found to have minimal gross arteriosclerotic lesions at the aortic valves.

TABLE I

Body Weights of the Thyroidectomized Dogs

Dog Numbers	Weight in Pounds Following Thyroidectomy		
3 (W)	Day 1	Day 50*	Day 68**
3 (W)	22	15	--
16 (W)	37	21	--
37 (W)	30	20	16
38 (W)	35	25	20
39 (W)	32	17	--
11 (Y)	17	--	--
12 (Y)	27	25	--
13 (Y)	31	20	15
3 (Y)	17	--	--
33 (B)	39	27	21
BSN	40	30	--
2 (W)	25	13	--
26 (B)	30	20	--
24 (W)	30	20	--

Body Weights of the Control Dogs

Dog Numbers	Body Weight in Pounds		
	Day 1	Day 50	Day 89
15 (B)	24	23	23
955	17	19	17
24 (B)	20	24	22
32 (B)	30	35	32
22 (W)	35	50	37
35 (W)	31	32	27

* Day of limb transection
** Day of vessel ligation

TABLE II

Serum Calcium and Serum Cholesterol Levels in Thyroidectomized Dogs

Dog Number	Day Post-operative	Calcium Mg %	Cholesterol Mg %	Day of Death Post-Thyroidectomy
26(B)	1	12.1	148.6	
	51	20.0*	300.0	
	69	20.0	238.0	72
813	1	----	323.0	
	37	----	340.4	
	63	20.0*	212.8	66
37(W)	1	13.0	166.0	
	49	18.6	----	73
13(Y)	1	13.4	166.0	
	51	13.2	190.0	72
21(B)	1	12.2	160.0	
	32	17.7	----	35
8 (W)	1	15.0	132.2	
	33	----	256.0	51
958	1	10.0	181.8	
	36	----	298.8	57
39(W)	1	9.9	152.0	
	51	----	200.0	51
3 (Y)	1	10.5	----	50
2 (W)	1	10.5	----	
	50	20.0*	----	55
38(W)	1	11.2	165.0	
	49	19.8	162.6	73

* Maximum capacity of flame photometer was 20 mg.%.

TABLE II (Continued)

Dog Number	Day Post-operative	Calcium Mg %	Cholesterol Mg %	Day of Death Post-thyroidectomy
33(B)	1	11.0	----	
	49	18.4	----	
	67	20.0*	190.0	68
36(B)	1	14.4	122.0	
	32	15.2	----	45
8 (B)	1	----	144.0	
	35	9.6	----	Euthanized
	63	14.8	----	63
16(W)	1	12.3	----	
	31	12.2	----	59
3 (W)	1	16.0	86.2	
	32	----	258.0	55
24(W)	1	14.5	153.8	
	34	20.0*	----	51
11(Y)	1	15.7	78.0	35
12(Y)	1	----	230.0	
	50	----	238.0	56
30(W)	1	17.7	206.0	
	30	12.8	----	34

* Maximum capacity of flame photometer was 20.0 mg. %.

TABLE II (Continued)

Serum Calcium and Serum Cholesterol Levels in Control Dogs

Dog Number	Day Post-operative	Calcium Mg. %	Cholesterol Mg. %	Day Euthanized
955	1	12.9	----	
	30	11.0	----	
	61	8.8	248.0	
	89	14.4	182.0	89
15(B)	1	----	----	
	30	9.8	----	
	61	11.3	160.0	
	89	10.3	130.0	89
35(W)	1	13.8	144.7	
	30	10.2	144.7	
	61	12.8	153.1	
	89	17.4	153.1	89
24(B)	1	13.6	153.1	
	30	10.2	170.0	
	61	15.1	148.4	
	89	12.0	182.9	89
32(B)	1	13.2	126.0	
	30	10.9	170.0	
	61	12.8	152.0	89
22(W)	61	9.6	180.0	89

TABLE III

Limb Temperatures of Thyroidectomized Dogs

Dog Number	Limb Transected	Limb Measured	Post-operative Temperatures in °C.	
			Day 50*	Day 68**
13(Y)	Right	Right	35.75	27.00
		Left	34.50	28.25
33(B)	Right	Right	37.25	35.90
		Left	36.00	35.90
BSN	Left	Left	35.00	
		Right	34.50	
2 (W)	Right	Right	33.00	
		Left	34.50	
26(B)	Left	Left	35.50	35.50
		Right	35.50	37.00
37(W)	Right	Right	33.50	34.00
		Left	32.75	36.00
38(W)	Left	Left	36.00	32.00
		Right	36.50	31.00
39(W)	Right	Right	28.00	
		Left	30.50	
12(Y)	Left	Left	35.00	
		Right	36.00	

* Day of limb transection
** Day of femoral artery and vein ligation

TABLE III (Continued)

Limb Temperatures of Control Dogs

Dog Number	Limb Transected	Limb Measured	Post-operative Temperatures in °C.			
			Day 68**	Day 75	Day 82	Day 89
15(B)	Right	Right	37.00	34.80	34.00	36.50
		Left	37.50	37.00	36.00	38.00
955	Left	Left		35.50		36.25
		Right		37.50		37.10
24(B)	Right	Right		34.00		37.00
		Left		36.50		36.60
32(B)	Left	Left		34.50	35.80	36.90
		Right		38.50	37.75	37.25
22(W)	Right	Right	36.90	38.10	35.50	36.00
		Left	37.25	38.50	36.50	37.75
35(W)	Left	Left	36.00	36.00	35.50	36.00
		Right	36.00	37.00	36.50	38.00

* Day of femoral artery and vein ligation.

TABLE IV

Post-transection Data on Thyroidectomized Dogs

Dog Number	Day of Death Post-transection	Limb Condition at Death	Gross Lesions of Blood Vessels. Degree of Plaque Formation
3(W)	6th	Necrotic distal to transection	Marked
16(W)	10th	Early necrosis	Moderate
37(W)	wnd	Early necrosis	Moderate
38(W)	24th	Early necrosis	Moderate
39(W)	Same day	----	Marked
12(Y)	6th	Viable	Minimal
13(Y)	20th	Viable	Marked
33(B)	19th	Functional and healed	Moderate
BSN	7th	Viable	None
26(B)	21st	Necrotic	Moderate
2(W)	5th	Viable	Minimal
813	3rd	Viable	Marked
8(W)	1st	Viable	Moderate
24(W)	Same day	Viable	Marked

* See Appendix I for more detailed information

TABLE V

Transection Data and Gross Lesions for Control Dogs

Dog Number	Condition of Limb at Time of Vessel Ligation	Condition of Limb at Time of Experiment Termination	Gross Lesions
955	Healed and functional	Healed and functional	None
24(B)	Healed and functional	Not functional	None
32(B)	Healed and functional	Foot ulcerated limping	Moderate plaques at aortic valves
22(W)	Healing incomplete	No healing on anterior surface. Not functional	None
35(W)	Healed and functional	Healed and functional	None
15(B)	Healed and functional	Healed and functional	None

Additional data in Appendix II

CHAPTER V

DISCUSSION

A. General Considerations

Pound dogs that are used for experimental purposes present numerous problems. The age of the animals is a rough estimation. Shipments usually contain whatever sex, breed and size of dogs are available when the order is filled. The stress that these dogs have recently been subjected to prior to receipt predispose them to outbreaks of distemper, hepatitis, leptospirosis and other diseases. Parasitism, both external and internal, also debilitate the dogs. In this study these factors lead to numerous early death losses which were not encountered in dogs that were maintained on a good nutritional and health plan three weeks prior to use as an experimental animal.

B. Thyrodectomized Dogs

The method of Crisp et al. (1958) was employed in this study to produce experimental arteriosclerosis. Problems of anorexia, early death losses and debilitation were encountered by the author in this study, however, Crisp et al. (1958) do not mention any of these problems.

Perhaps the reason for this would be that thyroidectomy was not complete in their study and thus metabolism was not as greatly disrupted. If smaller doses of vitamin D were administered over a longer period, or if the interval of time between administration were greater, it might be possible to produce lesions of arteriosclerosis without such severe vitamin D toxicity. If this is possible it would make collateral circulation studies more feasible.

The lack of uniformity in the size of the dogs used in this experiment made it difficult to evaluate the time required to produce arteriosclerotic lesions since all dogs received the same amount of vitamin D irrespective of size. There was, as a rule, earlier development of lesions in the smaller dogs and death occurred sooner in this group.

The lesions seen by producing hypervitaminosis D in these dogs were in agreement with those described by Crisp et al. (1958), Smith and Jones (1957), and similar to those described in rabbits by Follis (1955) and by Sodeman (1956) in rats.

The elevations of serum calcium levels obtained in this experimental study agree with the reports in dogs by Detweiler (1958), Smith and Jones (1957) and Hueper (1944).

Similar elevations were reported in rats subjected to massive doses of vitamin D by Follis (1955). Hass et al. (1960) reported no persistent or consistent elevations in serum calcium levels in animals subjected to hypervitaminosis D. No reports were found as to changes in the serum cholesterol levels in animals on a hypervitaminosis D regime.

The albumin-globulin ratio of this group of dogs remained within normal limits. No change was expected and no literature was found in relation to this.

Variations in limb temperatures were not statistically significant. If more sensitive recording equipment had been used and if the temperature had been recorded shortly after limb transection, there might have been significant differences. This, however, is an assumption. Hueper (1944) reported hypertension due to renal arteriosclerosis, and vasoconstriction due to overdose with vitamin D. This could influence the development of collateral circulation and limb temperatures. If the vasoconstriction was mild, the hypertension would aid in the development of collateral circulation. The dogs used in this experiment were too debilitated to risk direct blood pressure recordings,

therefore it was not known if they were hypotensive, normotensive or hypertensive. More modern electronic recording equipment such as a transducer would alleviate the problem of blood pressure recordings.

Of the four dogs which had an electrocardiogram prior to and following transection, two showed definite signs of myocardial disease, one had changes suggestive of myocardial disease, and one showed no significant changes. The other dogs were started on experimentation sooner and at that time the early death loses were not anticipated so that death occurred before a second electrocardiogram was recorded.

C. Control Dogs

The control group of dogs were not thyroidectomized and received no vitamin D. They did have a limb transected in a manner identical to that of the thyroidectomized group.

Serum calcium and cholesterol levels were not altered from the normal range and the albumin-globulin ratios remained within normal limits as was expected.

One control dog, 32(B), exhibited minimal gross arteriosclerotic lesions at the semilunar valves. Microscopic examination revealed the area to be calcified from the

intima to the adventitia. This dog was estimated to be about four years old and the lesions were thought to be spontaneous in origin. This finding is in accord with the reports of spontaneous arteriosclerosis by Morehead and Little (1945), Hueper (1945), Detweiler (1958) and other investigators.

Limb survival following transection agrees with the work of Sewell (1958), however, the dogs in this study did not use the transected limb as well following vessel ligation as did the dogs reported by Sewell (1958).

D. General Discussion

It would seem that much valuable information could be gained from a more thorough study of domestic animals in the age range that spontaneous cardiovascular disease becomes quite common. Detweiler *et al.* (1960) states that in dogs over eleven years of age, 415 in every 1,000 would be expected to have some form of cardiovascular disease.

Kountz (1950) compared the incidence of vascular disease in a group of people with low basal metabolic rates, a number of years prior to death, to those having a normal basal metabolic rate, a number of years prior to death, and found a considerably higher incidence in the

former group. This might well be an important aid in detecting or avoiding vascular disease in man. The ease with which cardiovascular disease can be produced in animals by depressing thyroid function or by thyroidectomy would lend greater weight to a more extensive use of basal metabolic rate studies.

A low basal metabolic rate in conjunction with cardiovascular lesions would help to substantiate the anoxemia theory of arteriosclerosis.

CHAPTER VI

SUMMARY AND CONCLUSIONS

Forty-two male dogs were used in this study. Thirty-six were thyroidectomized taking care to preserve the parathyroid glands. The thyroidectomized dogs were given 100,000 units of vitamin D orally daily throughout the course of the experiment. Six animals served as controls. The age of the dogs was estimated to be between one and four years. Body weight ranged from 13 to 45 pounds. Dogs housed a considerable period prior to starting on experimentation survived longer and remained in better health as a rule. All thyroidectomized dogs started showing signs of vitamin D toxicity between the fifteenth and thirtieth day. This was generally more pronounced in the smaller dogs.

Of the thirty-six dogs which were thyroidectomized, 14 survived to the time of pelvic limb transection on the forty-ninth, fiftieth or fifty-first day following thyroidectomy. Twenty-eight thyroidectomized dogs died prior to the time of limb transection. The femoral artery and vein were ligated on the eighteenth day post-transection. Five dogs lived to ligation date and nine died prior to

this procedure. Limb temperatures were taken at regular intervals from the gastrocnemius muscle.

Dogs receiving thyroid supplement following limb transection did not survive long enough to determine the effects of this procedure. Gross and microscopic lesions were variable in this group.

Gross lesions of arteriosclerosis varied from none, to heavy plaques throughout the aorta and its branches in the thyroidectomized group. The histopathologic findings consisted of disruption of the internal elastic lamellae, intimal plaques and medial and adventitial calcification.

Myocardial damage was evidenced by the electrocardiographic changes.

Thyroidectomy and oral administration of 100,000 units of vitamin D daily proved to be a rapid means of producing arteriosclerotic lesions. However the toxic effect of the vitamin D was too debilitating to permit a good collateral circulation study.

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APPENDIX I

DATA ON THE INDIVIDUAL THYROIDECTOMIZED DOGS

Dog 3(W) was anemic at the time of transection. Two hundred cc. of whole blood were administered intravenously following surgery. Death occurred the sixth post-operative day. The transected limb was necrotic distal to the transection.

Dog 16(W) died ten days after the transection. A massive pocket of purulent exudate was found at the site of transection. There was no evidence of healing. The limb distal to the transection was becoming necrotic. Lung abcesses comparable to those of chronic distemper were seen at necropsy.

Dog 37(W) died the second post-transection day. The transected limb was becoming gangrenous distal to the transection.

Dog 38(W) died the sixth post-ligation day. The limb was becoming necrotic distal to the transection.

Dog 39(W) was anemic at the time of transection. Two hundred cc. of whole blood were administered following surgery, however, death ensued shortly.

Dog 12(Y) was fairly active and alert at the time of transection, however, he died six days later. The site of transection was purulent and there was no evidence of healing. Lung abscesses were evidenced as seen in chronic distemper.

Dog 13(Y) was too weak to stand by the time of vessel ligation. A purulent exudate was coming from the transection site. Death occurred two days following ligation.

Dogs 33(B) died on the first post-ligation day. The limb was functional and well healed prior to death.

Dog (BSN) developed generalized sarcoptic mange. Death occurred the seventh post-transection day. There was no evidence of healing at the site of transection.

Dog 26(B) died the third day following ligation. The line of transection was open on the anterior surface of the limb. The limb was sloughing distal to the site of transection.

Dog 2(W) developed a generalized eczema by the time of limb transection. Death was on the fifth post-transection day. There was very little evidence of healing at the transection. Vascular lesions were confined to the region of the aortic valves. These were minimal.

Dog 813 was transected on day 63; sodium pentobarbital was the anesthetic used. The dog died the third post-transection day. Anesthetic recovery was not complete at the time of death. Necropsy revealed well defined vascular lesions throughout the aorta and pulmonary artery.

Dog 8(W) died on the first post-transection day. Vascular lesions were well defined in the pulmonary artery and aorta.

Dog 24(W) died at the time of limb transection, probably due to anesthesia. Well defined vascular lesions were found in the aorta, pulmonary and carotid arteries. Minimal lesions were noted in the femoral arteries.

APPENDIX II

DATA ON THE INDIVIDUAL CONTROL DOGS AT TIME OF EUTHANASIA

Dog 955. The transected limb was healed and functional. No gross lesions were seen at necropsy.

Dog 24(B). The dog did not use the transected limb. The site of transection was healed.

Dog 32(B). The dorsal surface of the second and third digits started to necrose seven days post-ligation. At euthanasia the lesion was to the bone. The dog used the limb but limped.

Dog 22(W). This dog licked out the sutures on the anterior surface of the transection. These were replaced twice but were gone at the time of euthanasia. There was an open pocket exuding serosanguineous fluid where licking had occurred. Limb function due to contraction of the muscles on the posterior aspect of the limb was poor. This was pronounced so that the limb was somewhat flexed. Necropsy revealed a hypertrophic periosteal reaction of the femur at the site of transection.

Dog 35(W). Function of the transected limb approached normal.

APPENDIX III

DOGS THAT DIED PRIOR TO TRANSECTION

Dog 3(Y). Emaciation and icterus were pronounced at the time of death on the fiftieth day. Moderate lesions were evident from the aortic valves to the renal arteries.

Dog 11(Y). By the time of death on the thirty-fifth day, moderate lesions were evident in the pulmonary, femoral, anterior mesenteric, splenic, internal and external thoracic arteries, and from the aortic valves to the renal arteries.

Dog 958. At necropsy on the fifty-seventh day, vascular lesions were observed in the region of the aortic valves and were minimal.

Dog 8(B). Due to the severe generalized moist eczema and debilitation this dog was euthanized on the sixty-third day. Marked gross lesions were observed in the pulmonary, carotid, external iliac and femoral arteries, and the aorta.

Dog 7(B). At death, on the forty-third day, necropsy revealed well defined lesions of the aorta.

Dog 6(B). Death was on the sixty-first day. Minimal lesions were found at the aortic valves, and from the renal to the external iliac arteries.

Dog 21(B). At death on the thirty-fifth day, minimal lesions were evident at the aortic valves.

Dog 36(B). Death occurred on the forty-fifth day. Moderate lesions were observed from the aortic valves to the femoral arteries.

Dog 18(B). This dog died following a fight on the twenty-fifth day. No vascular lesions were present.

Dog 21(W). Death resulted from a fight on the thirtieth day. There were moderate lesions throughout the aorta at the origin of the intercostal ascending branches.

Dog 30(W). Death was on the thirty-fourth day at which time vascular lesions were minimal and confined to the pulmonary artery.

Dog 22(B). At death on day 33, no vascular lesions were observed.

Dogs 3(B) and 7(W). These dogs died on the twenty-second and twenty-third days respectively. They were not necropsied.

Dog 5(R). This dog went into tetany the third day following thyroidectomy. Twenty cc. of a calcium

solution* was administered intravenously and tetany abated. Death ensured two days following treatment; however, no further signs of tetany were observed prior to death. At the time of thyroidectomy two parathyroid glands were located. The blood supply of one was interrupted during the course of surgery.

Dog 7(B). This dog exhibited tonic-clonic convulsions the first day post-thyroidectomy. The seizures occurred at about 15 minute intervals. Three such seizures were observed. No treatment was administered and no further convulsions were evidenced during the course of the experiment.

Dog 25. This dog was found comatose five days after it was started on the experiment. A radiograph revealed a prostatic calculus. This was removed surgically; however, death occurred the second post-operative day. Bilateral hydronephrosis was seen at necropsy.

Dogs 36(W), 21(W), 25(W), 7(B), 22(W), 16(W), 5(R), 18(W), and 25(B) died in eleven days or less following thyroidectomy. None of these dogs were necropsied.

---

* Calsem, S. E. Massengill Co., Fifth St.,Bristol, Tenn.

APPENDIX IV

ELECTROCARDIOGRAPHIC DATA AND INTERPRETATIONS

Dog 33(B)

5-5-60

This tracing appears within normal limits, except for lead AVR which has apparently been mislabelled.

6-23-60

There is a bigeminal rhythm produced by coupled ventricular premature beats which occur every other beat. There is depression of the S-T segment in leads facing the left ventricle (II,III,AVF) and reciprocal elevation in lead AVR. Precordial leads over the free wall of the left ventricle also show S-T segment depression.

7-11-60

The bigeminal rhythm is still present, the extra-systolic QRS complexes being of similar configuration to those seen on 6-23-60. S-T segment depressions seen on that date (6-23-60) are more prominent. CF6U and AVL may have been switched in the labelling in this dog. The tracings labelled CF6U appear more characteristic of AVL in the dog, and AVL looks more like CF6U should look.

Interpretations:

The changes in the latter two tracings are definite evidence of myocardial disease.

Dog 2(W)

5-5-60

This tracing is within normal limits. The QRS axis is approximately 80°.

6-24-60

The QRS has shifted to 90°, a slightly more vertical axis. The P waves are higher in amplitude. These changes might well be due to positional changes of the dog during taking of the tracings and probably are not significant.

Interpretations:

The EKG changes in this dog are not significant.

Dog 20(B)

5-21-60

This tracing is within normal limits. The QRS axis is approximately 60°.

7-11-60

There is a marked sinus tachycardia. The QRS axis is slightly more vertical, but no other significant changes have occurred.

7-29-60

The T waves have broadened and deepened, and the S-T segments sag in I, II, III, AVF, and the left precardial leads.

Interpretations:

The T wave changes, although suggestive of myocardial disease, are not conclusive evidence.

Dog 13(Y)

4-25-60

Within normal limits.

7-5-60

There has been a slight shift in the T Vector, resulting in inversion of T in II, III and AVF. T was previously upright in these leads on 4-25-60.

7-24-60

There are numerous ventricular premature beats, at times giving a bigeminal rhythm. Runs of paroxysmal ventricular tachycardia occur in several places.

Interpretations:

The last tracing is indicative of definite myocardial disease.

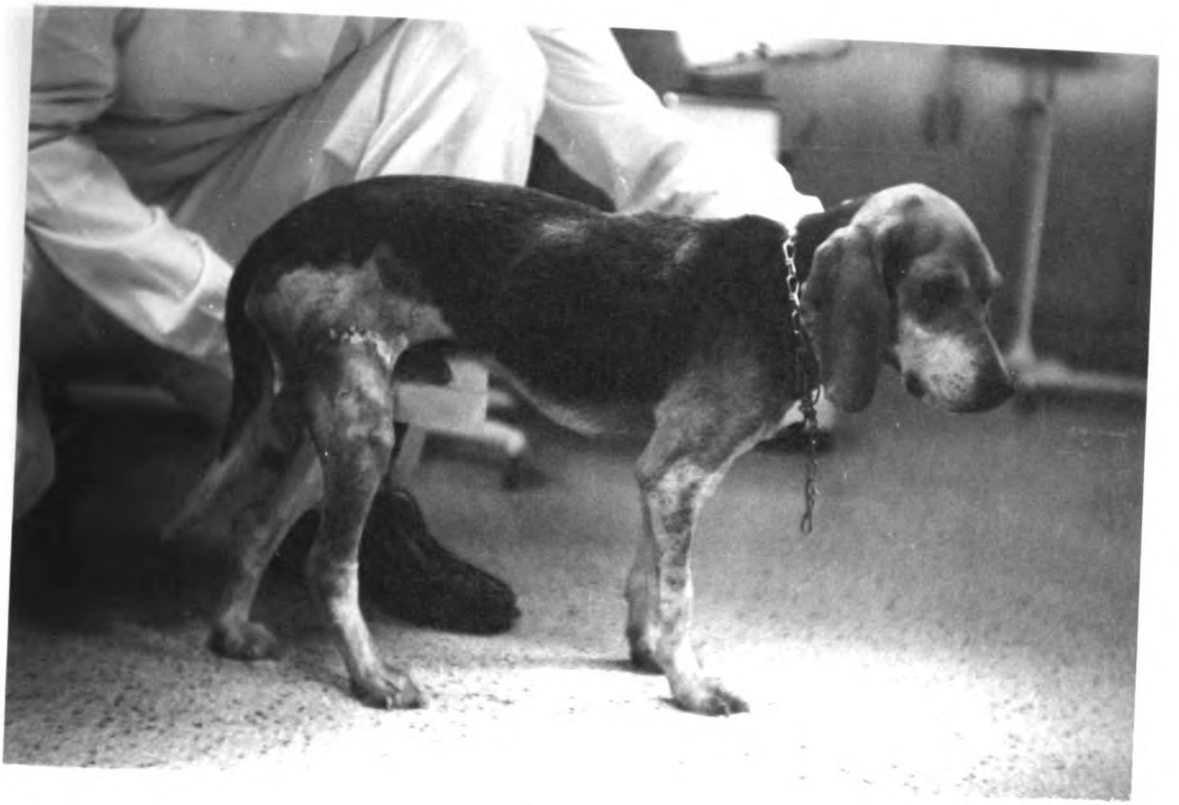
The T wave changes observed in the second tracing may be indicative of myocardial abnormality, but are not clear-cut enough to be reliable evidence.

APPENDIX V

Gross Pictures and Photomicrographs of Thyroidectomized Dogs

Figure 1. Condition of transected limb of thyroidectomized dog prior to vessel ligation.

Figure 2. Close-up of the same limb.



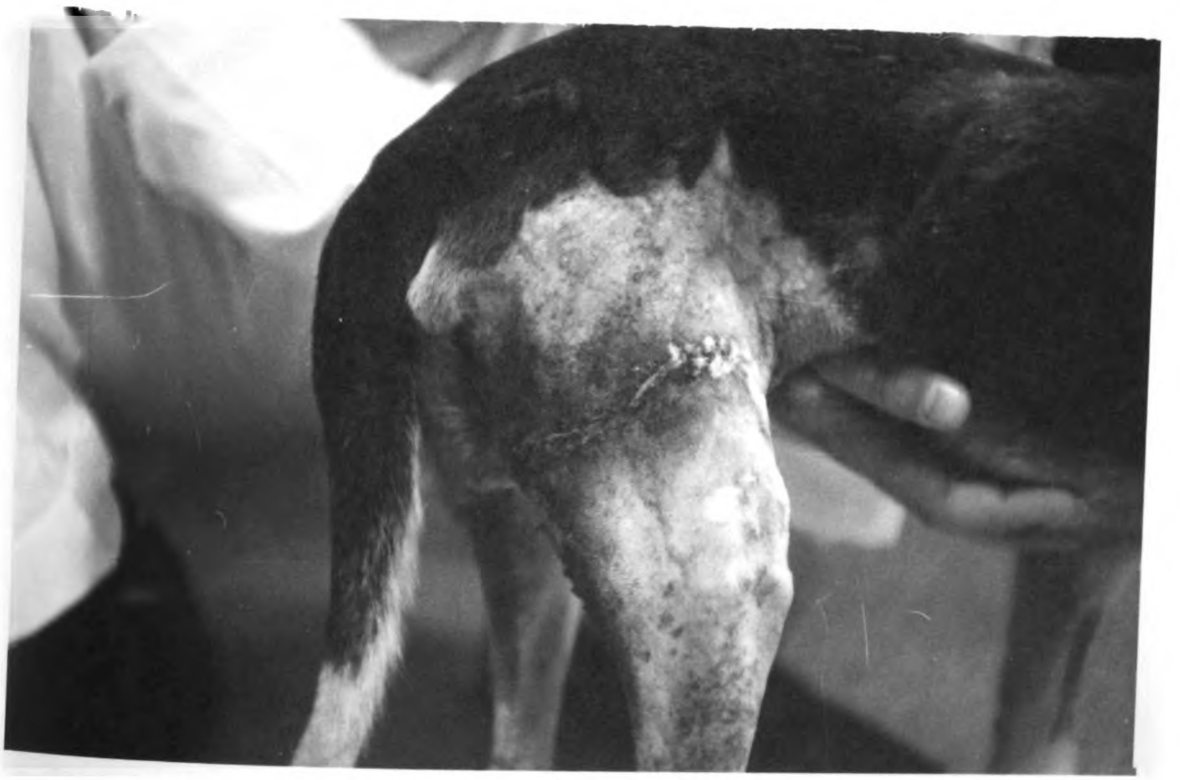


Figure 3. Illustration of transection technique showing femoral artery, vein and nerve in left foreground and sciatic nerve in right background.

Figure 4. Transected limb of thyroidectomized dog on the fifth post surgical day. Necrosis well evidenced distal to site of transection.

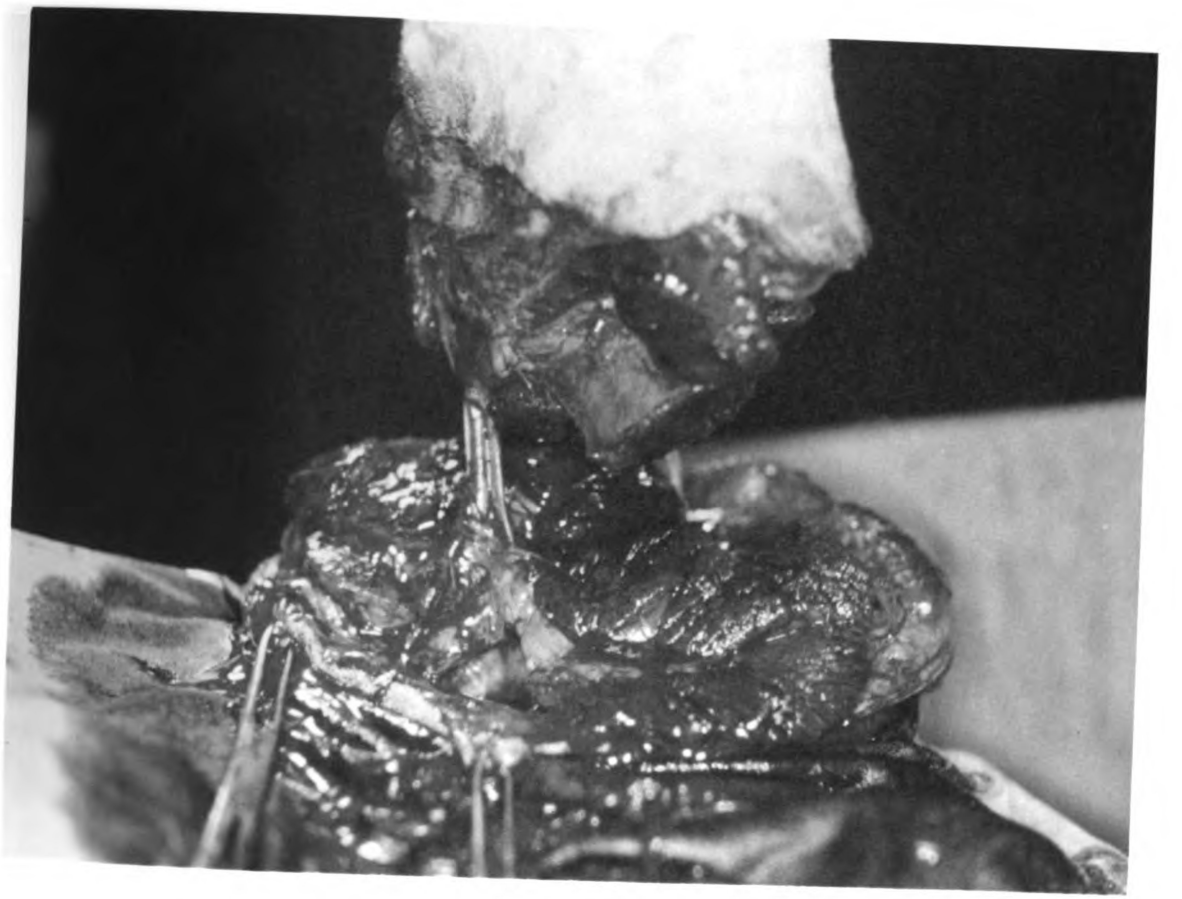
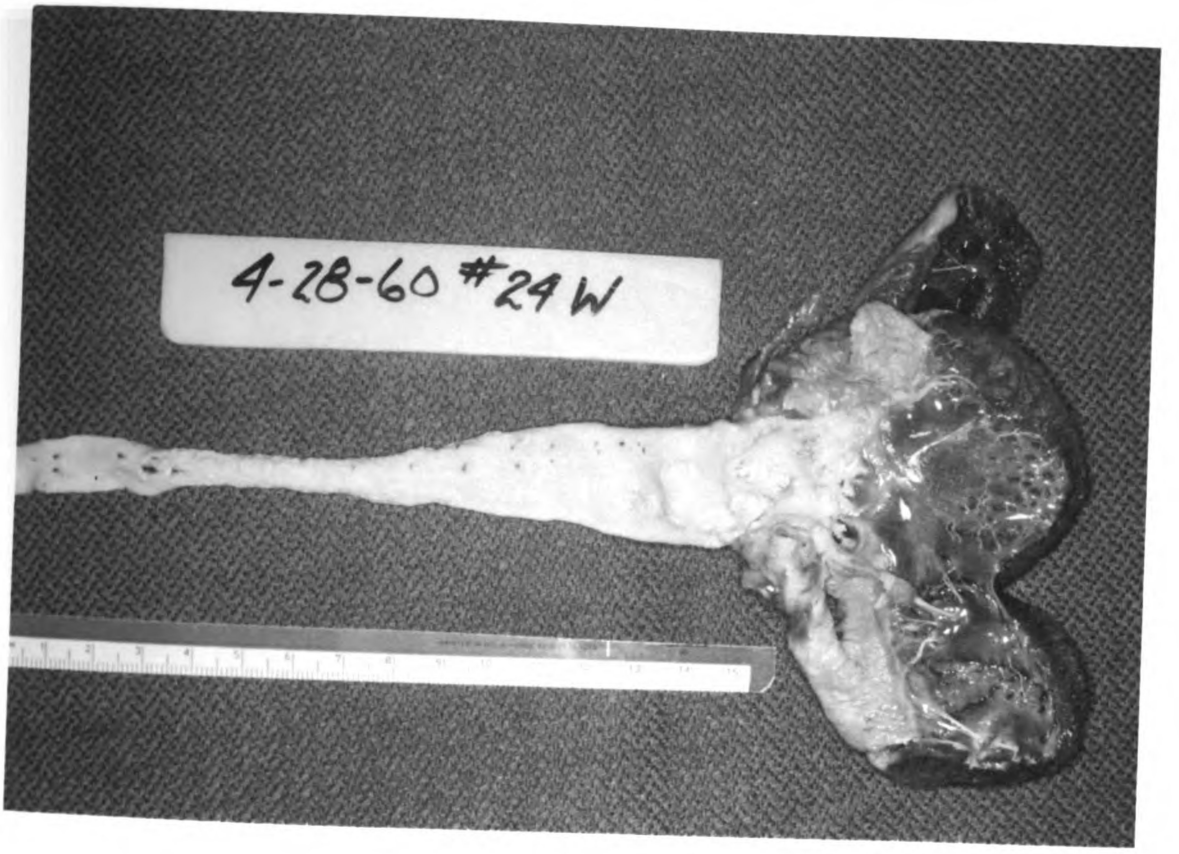


Figure 5. Gross arteriosclerotic plaques from experimental dog.

Figure 6. Close-up view of the above picture.


4-28-60 #24 W

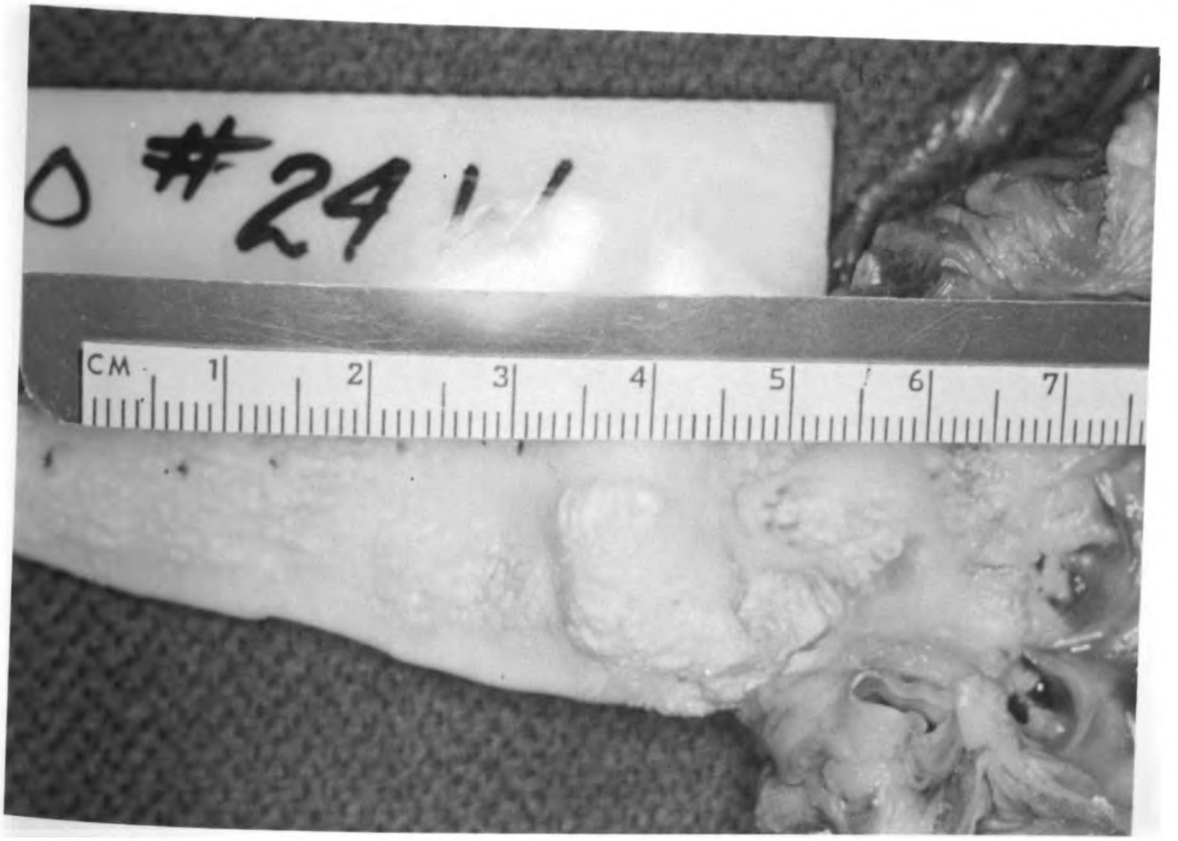
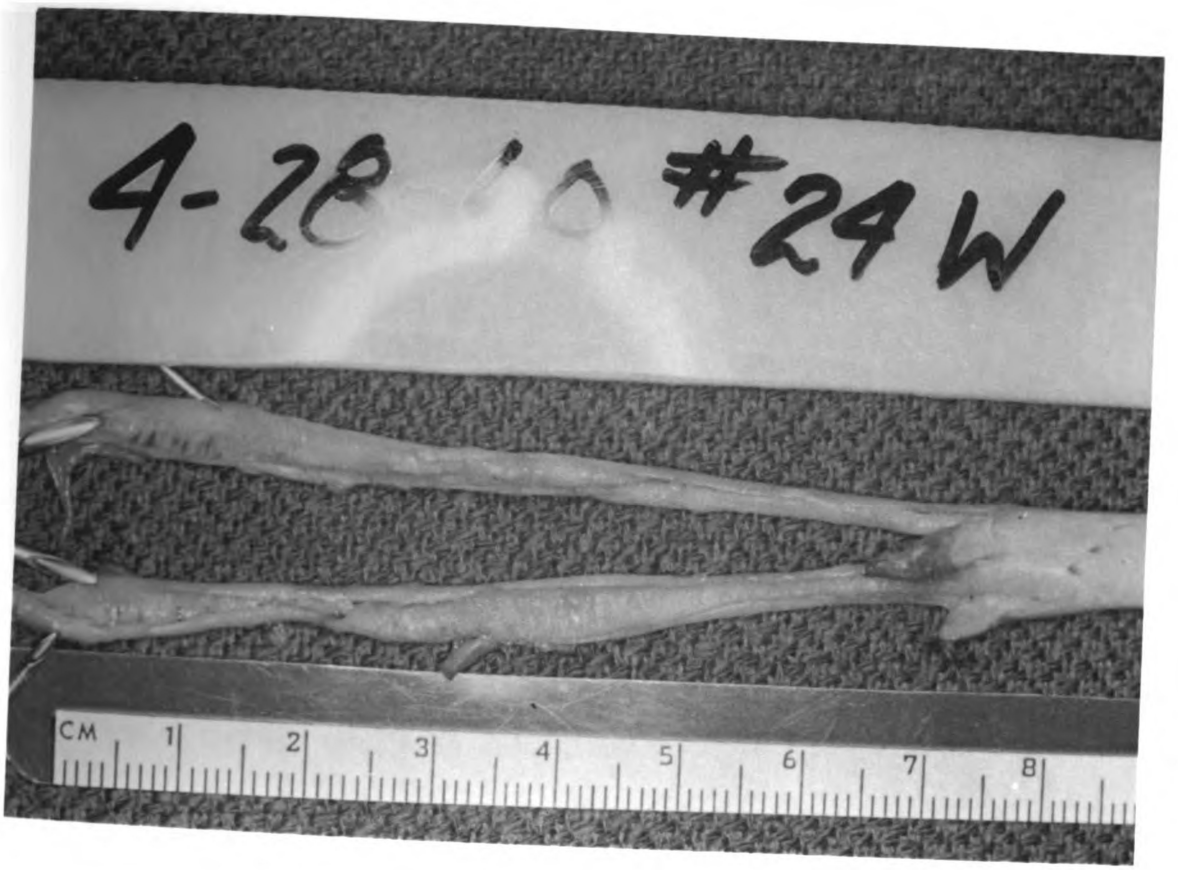

#24
CM
1
2
3
4
5
6
7

Figure 7. Gross arteriosclerotic plaques of the external iliac arteries extending through the femoral arteries.

Figure 8. Gross arteriosclerotic plaques of the common carotid artery and a portion of the aorta.


4-28 #24 W
CM 1 2 3 4 5 6 7 8


4-28- #24 W
CM 1 2 6 7

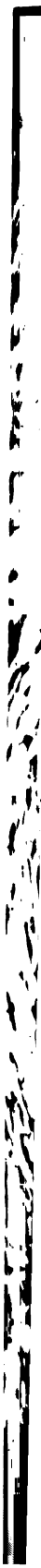


Figure 9. Coronary artery showing calcification and fragmentation of the internal elastic membrane. (H&E x149) 2(W)

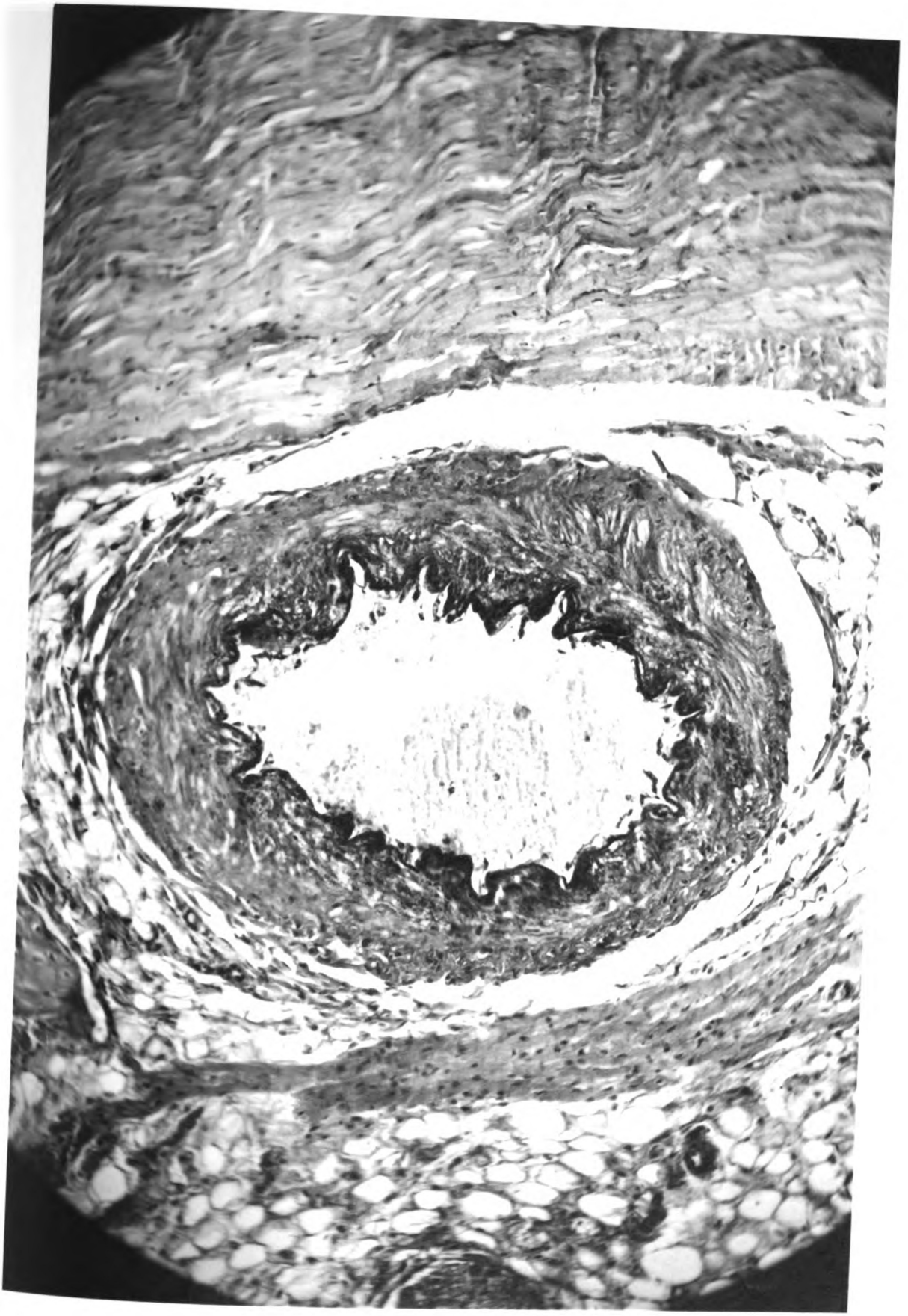


Figure 10. Intimal proliferation and calcification of the aorta. (H&E x150) 39(W)

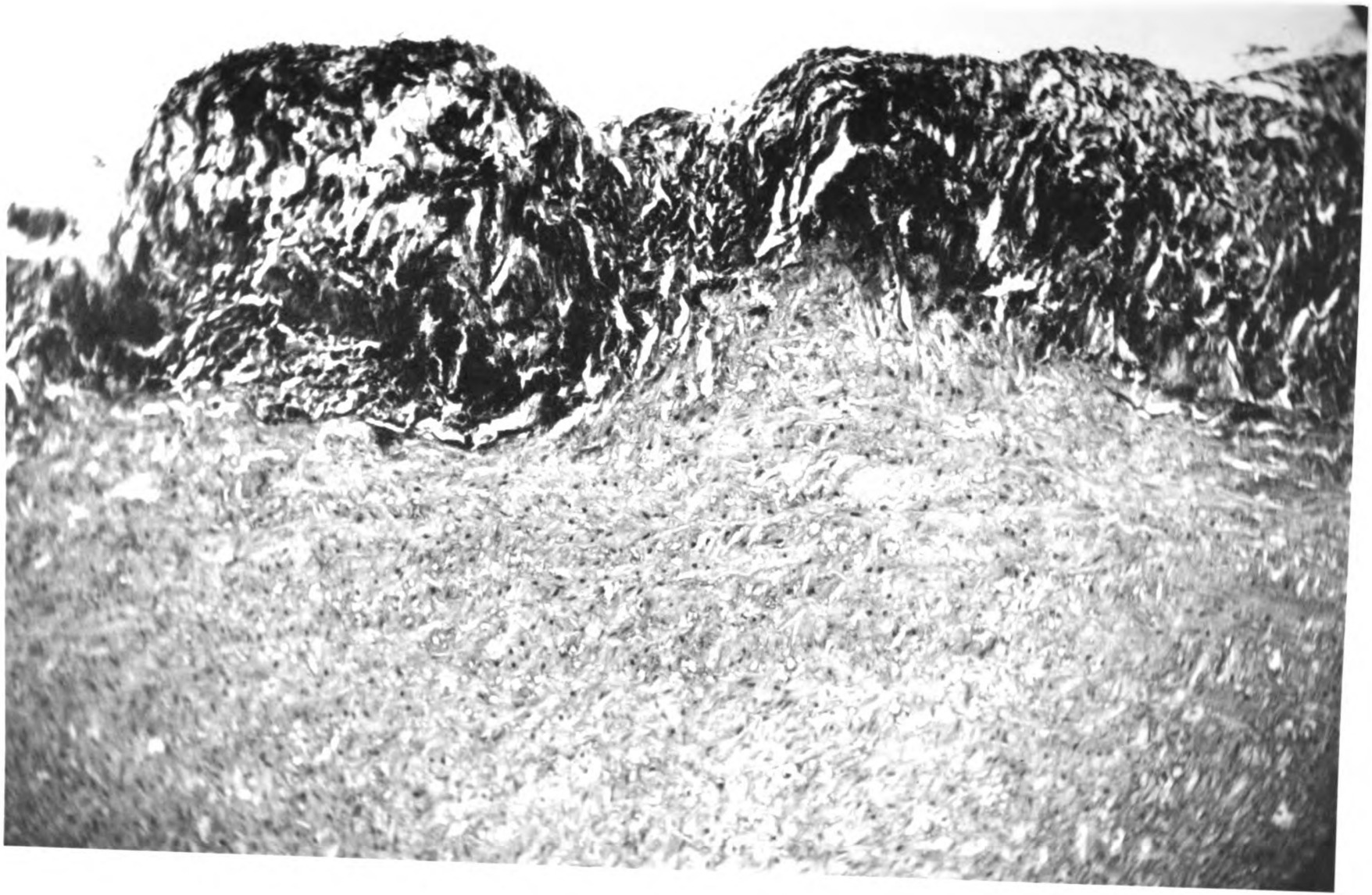
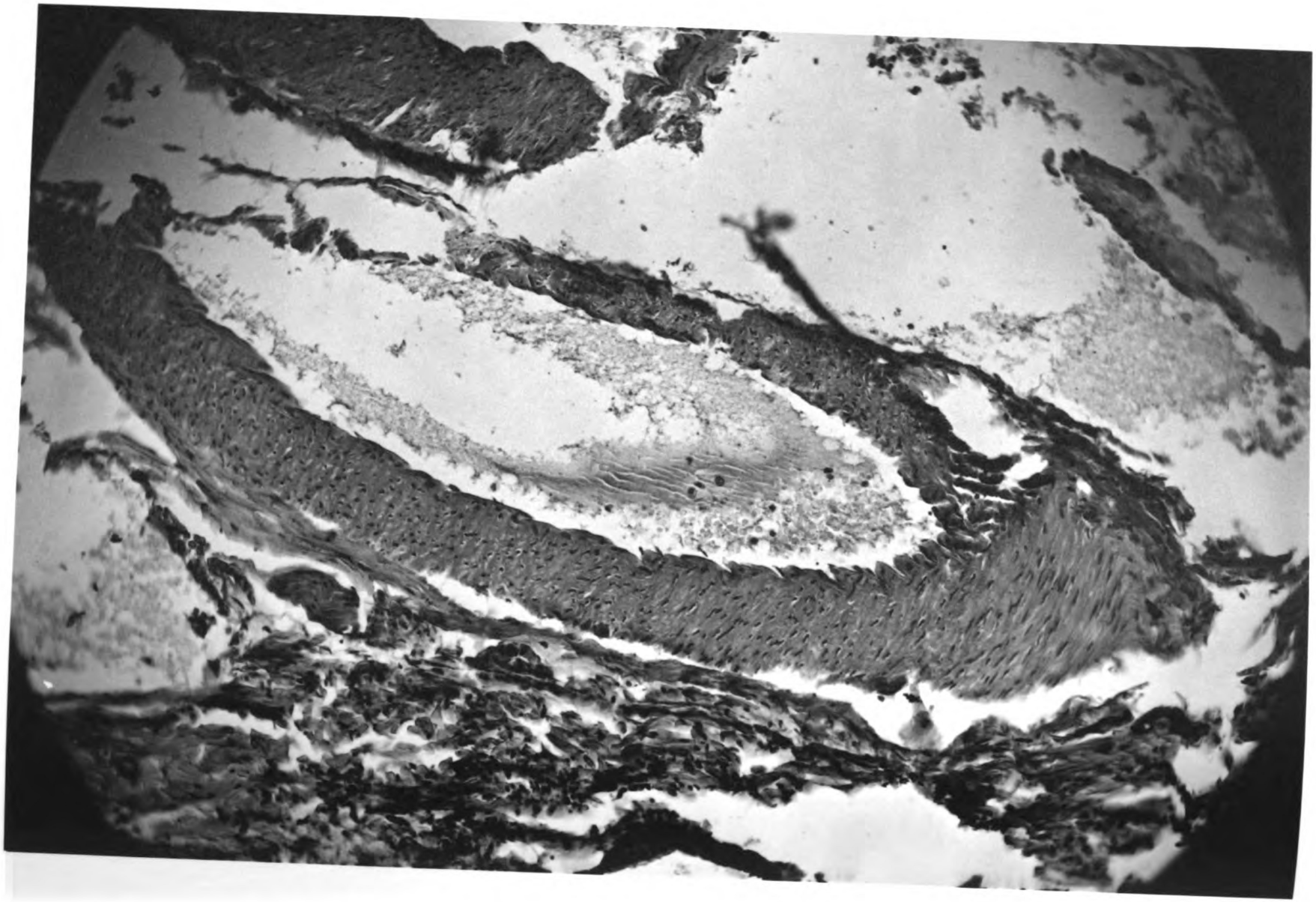


Figure 11. Medial degeneration and calcification of renal artery.
(H&E x154) 8(B)



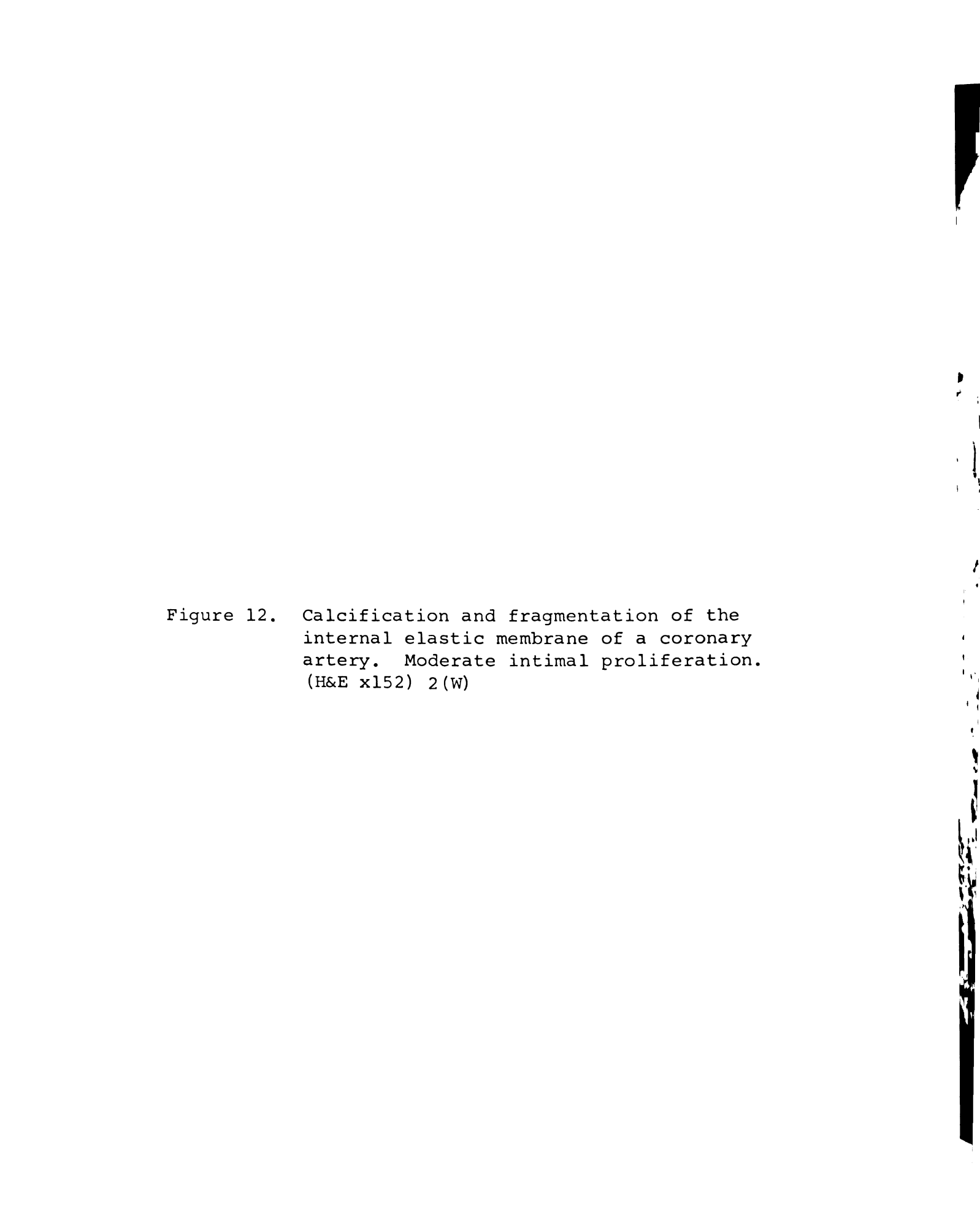


Figure 12. Calcification and fragmentation of the internal elastic membrane of a coronary artery. Moderate intimal proliferation. (H&E x152) 2(W)

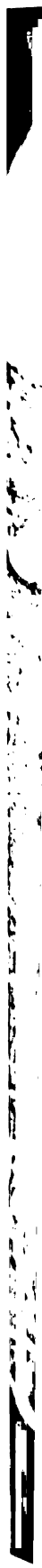
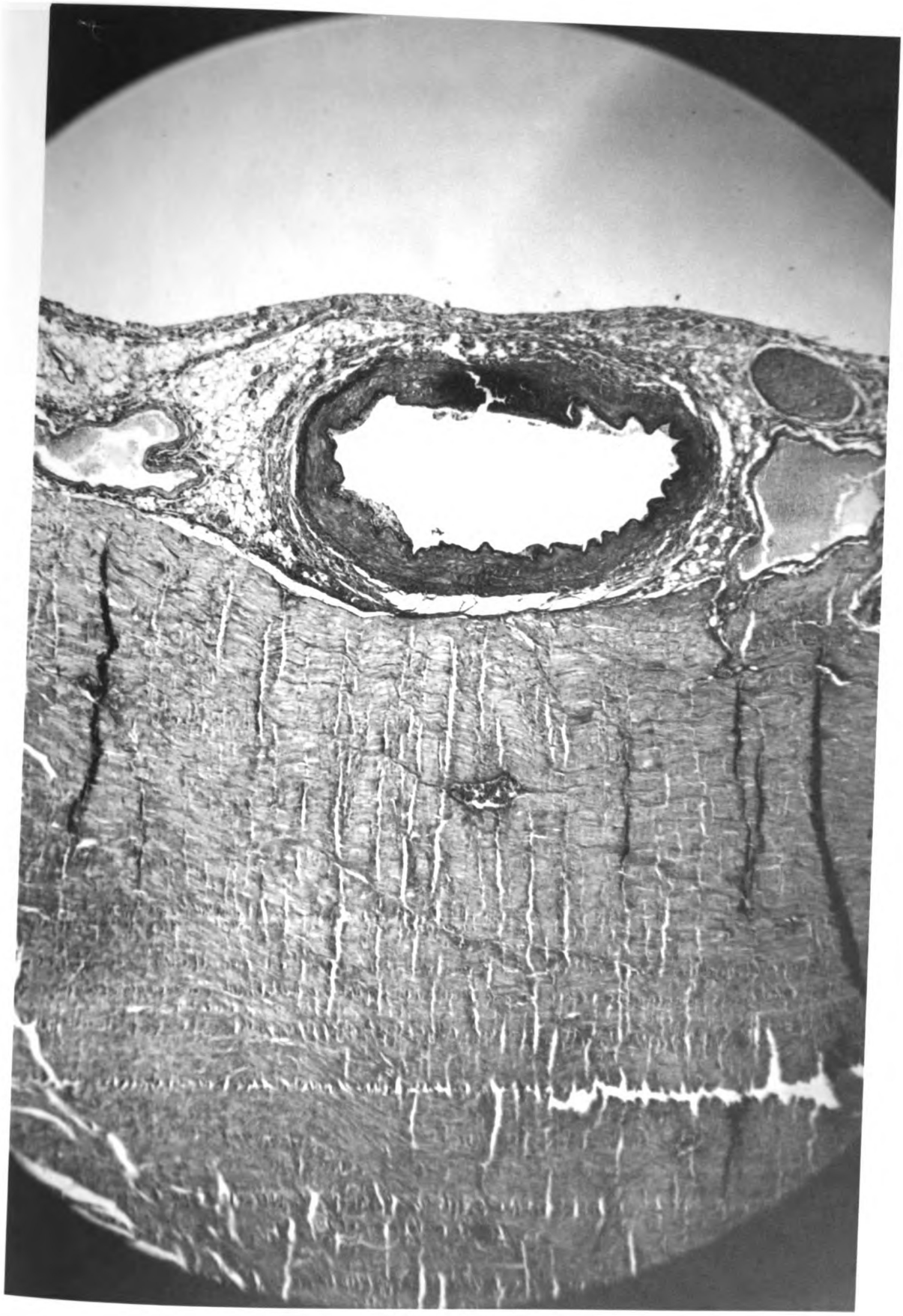


Figure 13. Coronary artery with medial calcification, calcification of the internal elastic membrane, and intimal proliferation. (H&E x151.25) 11(Y)



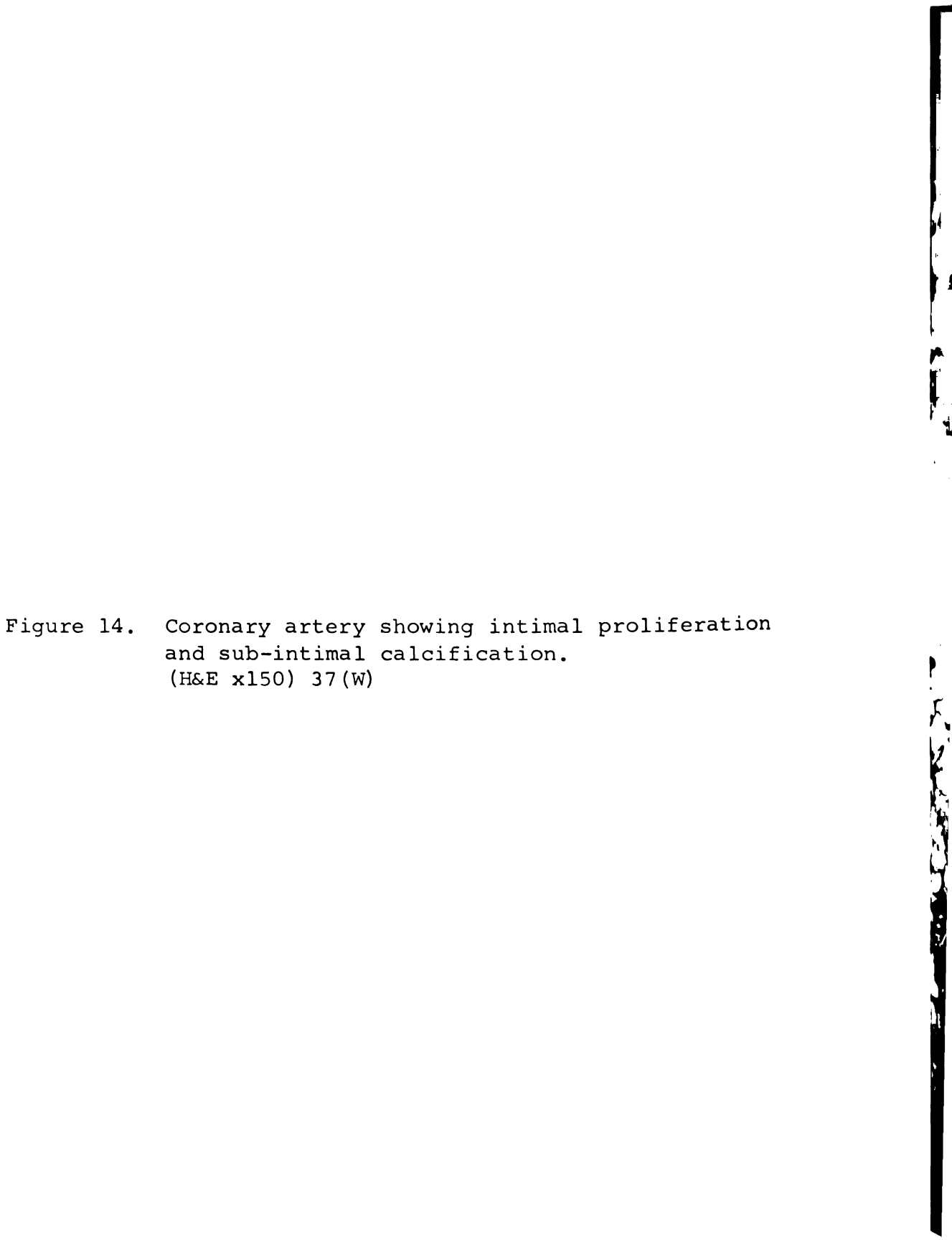


Figure 14. Coronary artery showing intimal proliferation and sub-intimal calcification.
(H&E x150) 37(W)

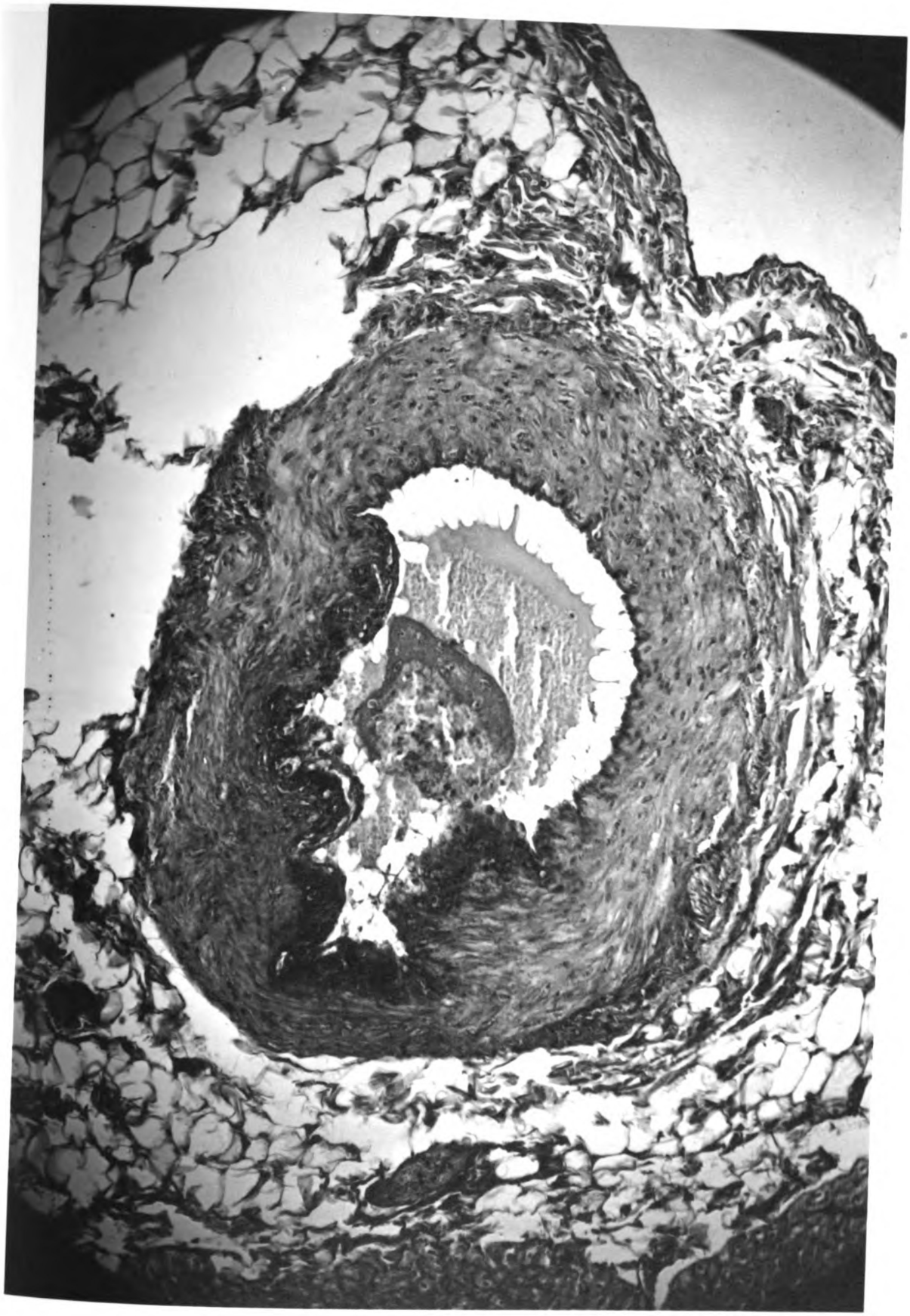


Figure 15. Myocardial degeneration and hemorrhage. (H&E x157) 35(W)

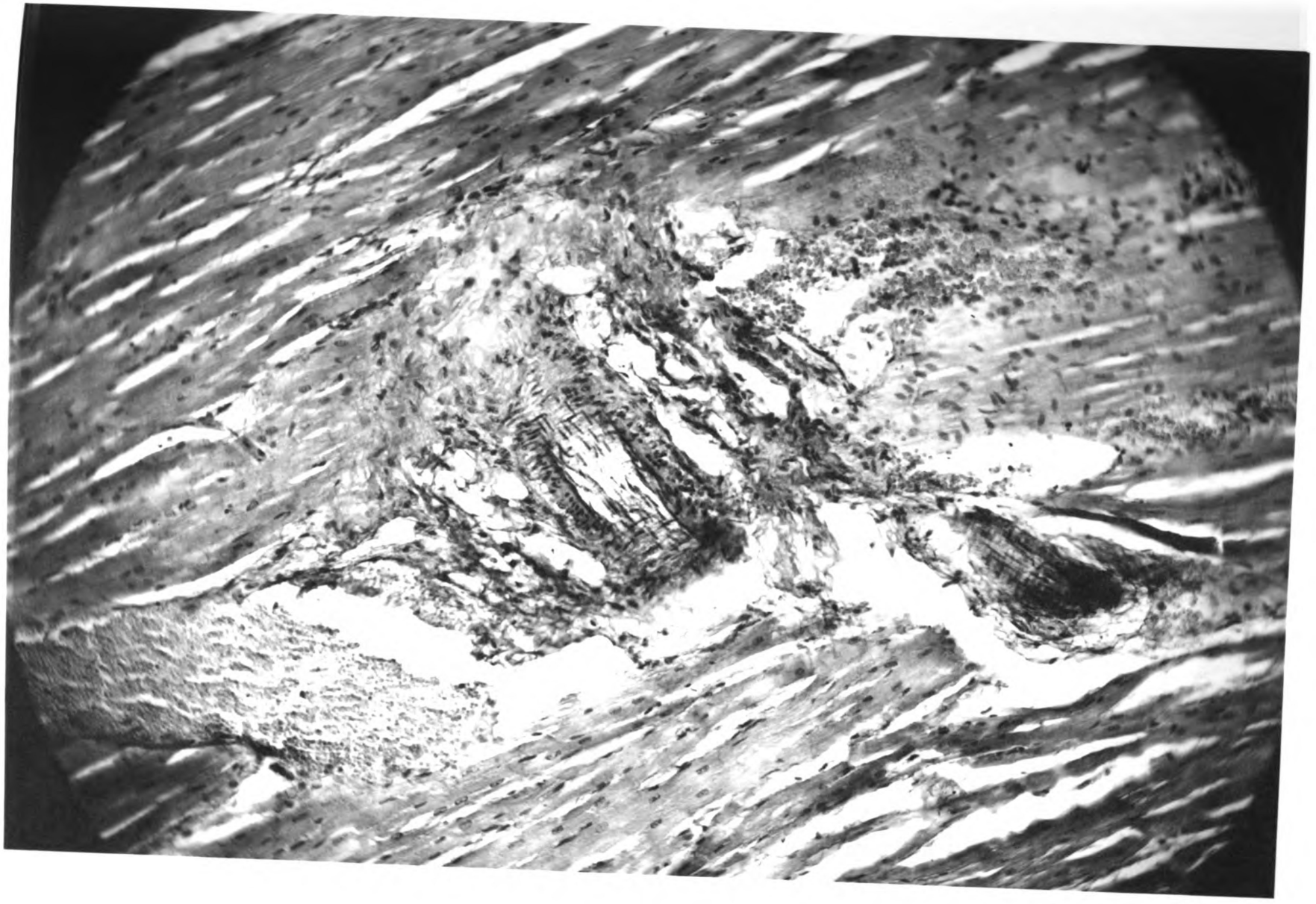


Figure 16. Calcification of the myocardium. Intimal calcification and degeneration of the internal elastic membrane of the coronary artery. (H&E x157) 38(W)

Figure 17. Calcification of the semilunar valve. (H&E x154) 32(B)

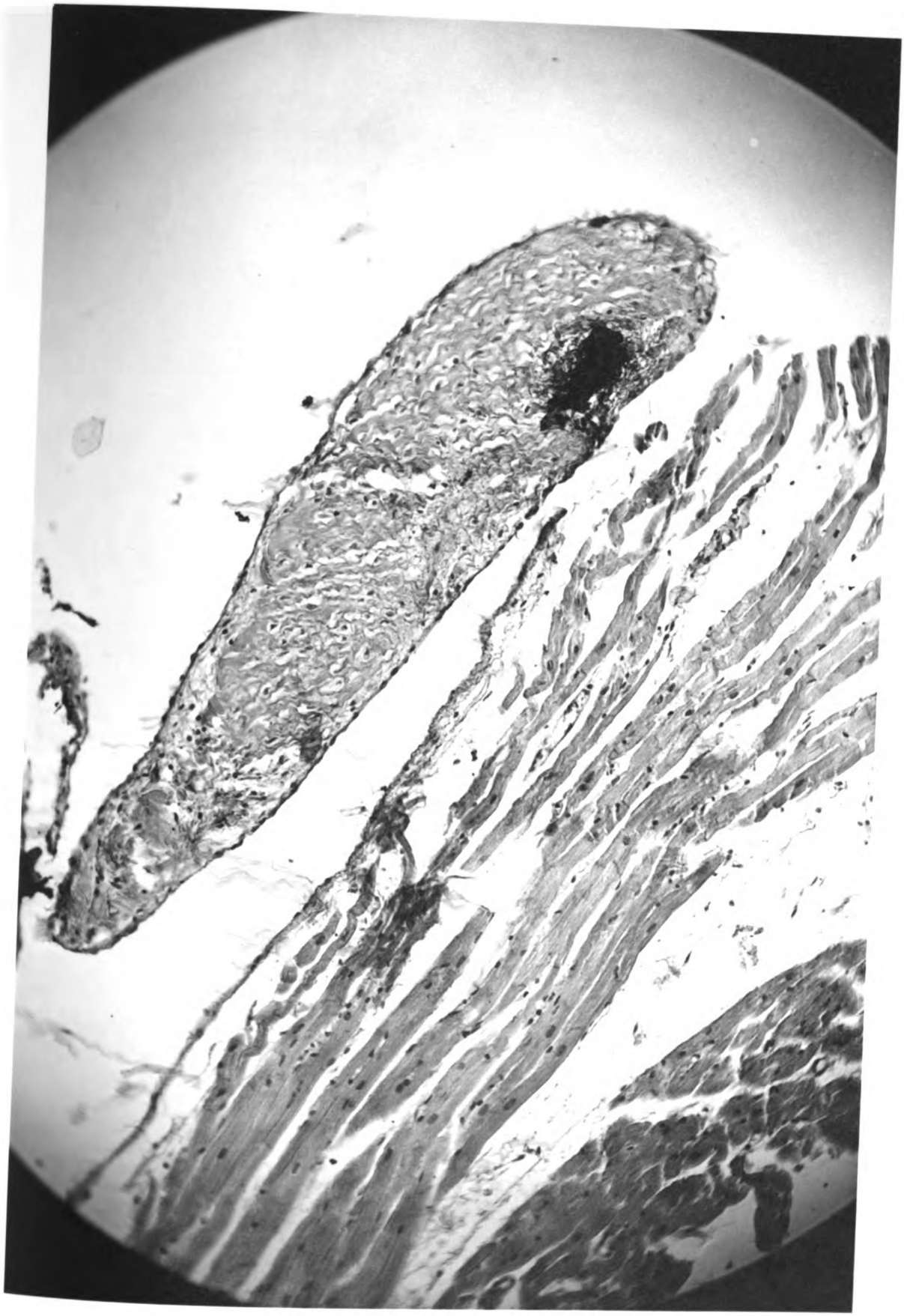


Figure 18. Sub-intimal calcification and intimal proliferation of a coronary artery. (H&E x154) 813

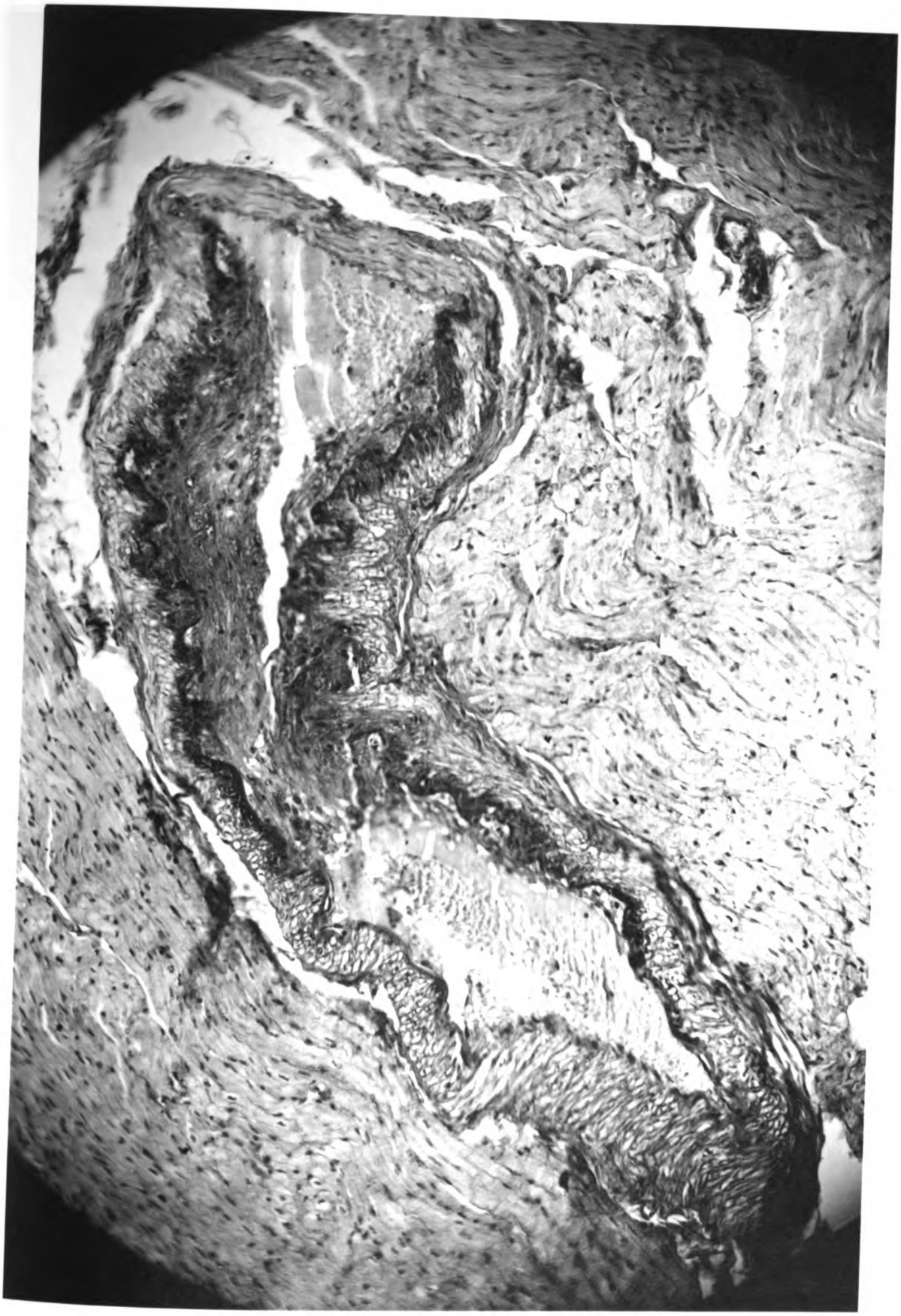


Figure 19. Endocardial calcification.
(H&E x170) 8(B)

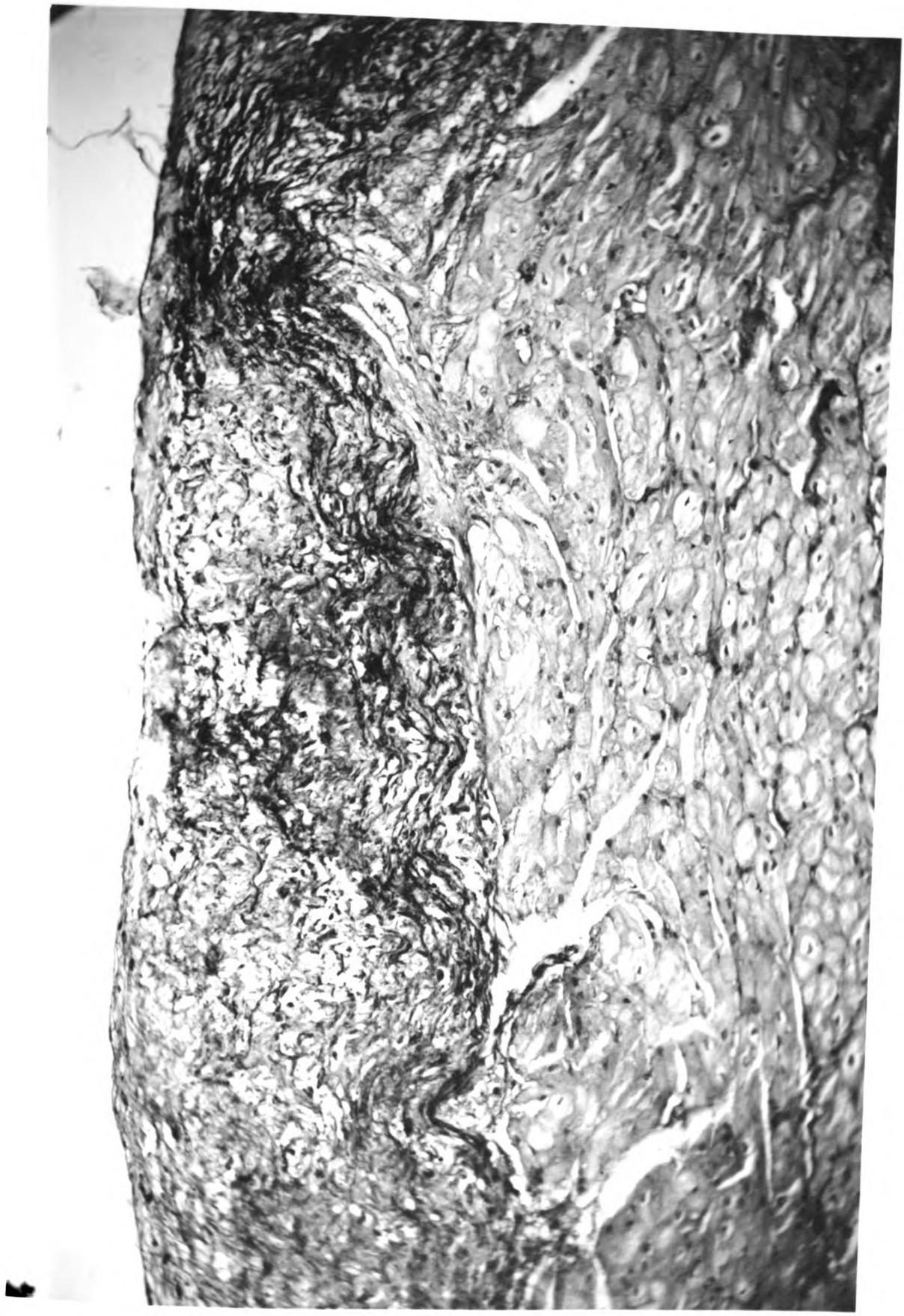


Figure 20. Calcification and fragmentation of the internal elastic membrane of a coronary artery.
(H&E x152) 2(W)

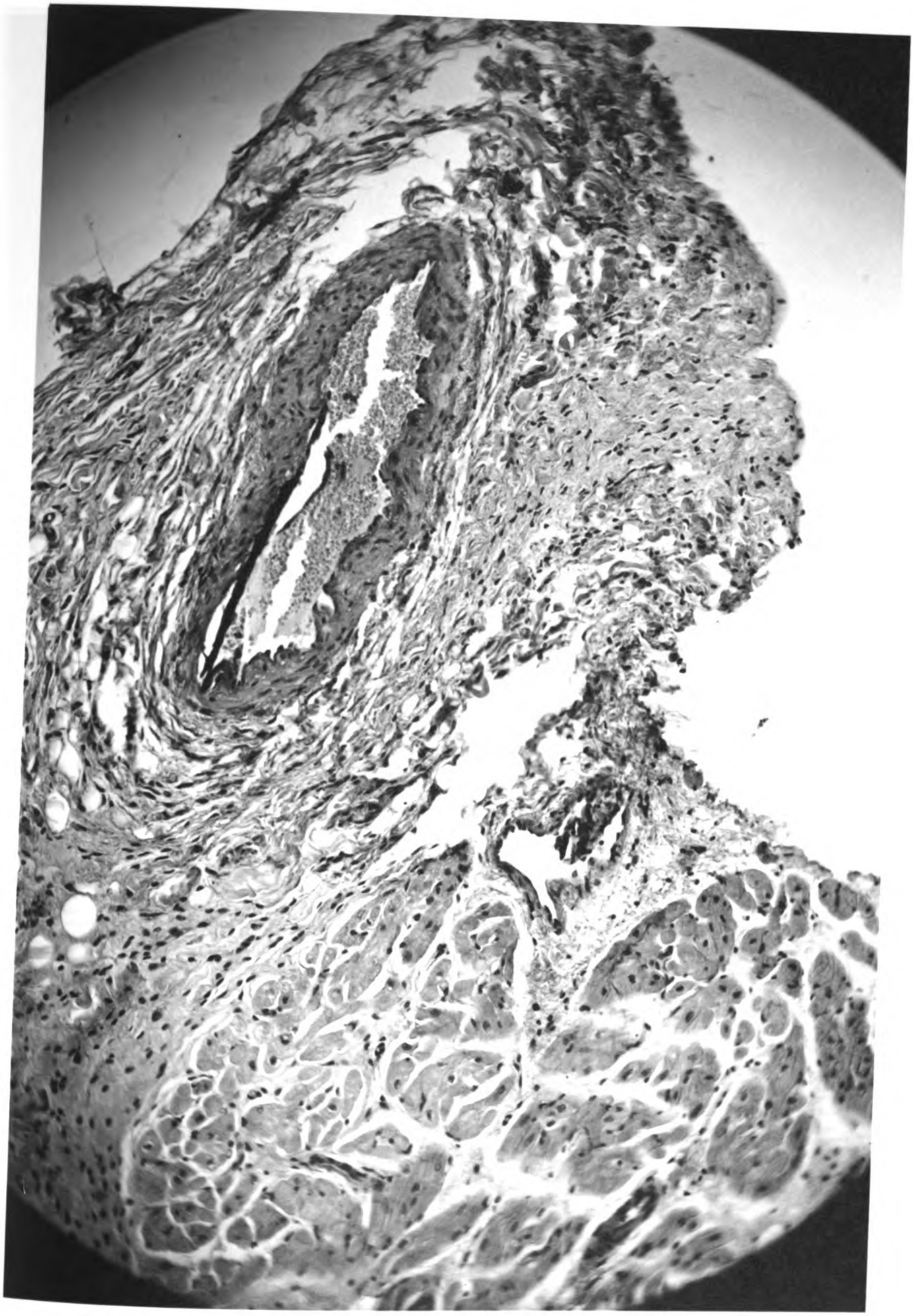


Figure 21. Decalcified aorta showing voids from which calcium was removed from the media. (H&E x54) 11(Y)

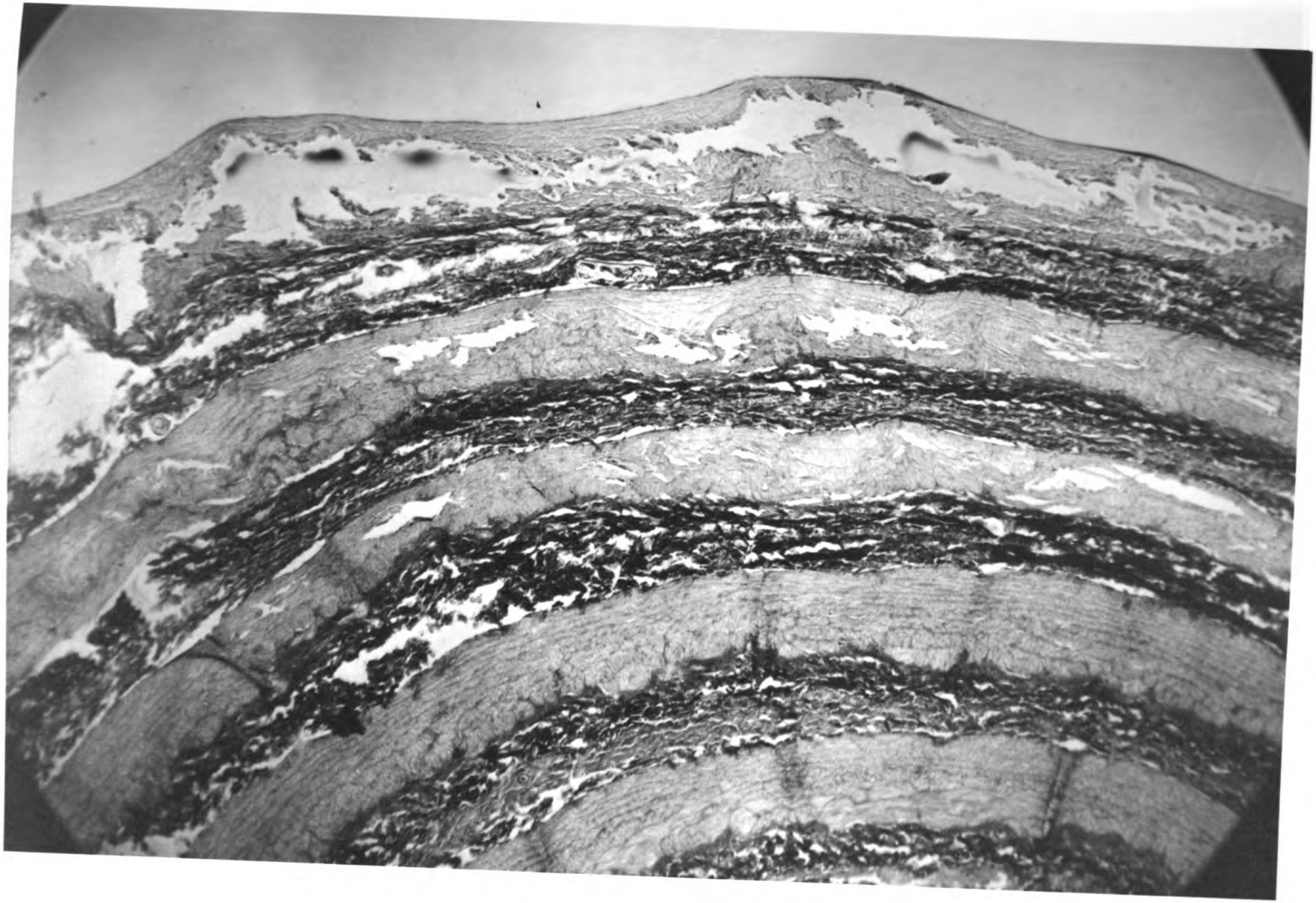


Figure 22. Diffuse medial calcification of the aorta. (H&E x50) 24(W)

Figure 23. Aortic medial calcification with disruption of the intima.
(Von Kossa's x53) 11(Y)

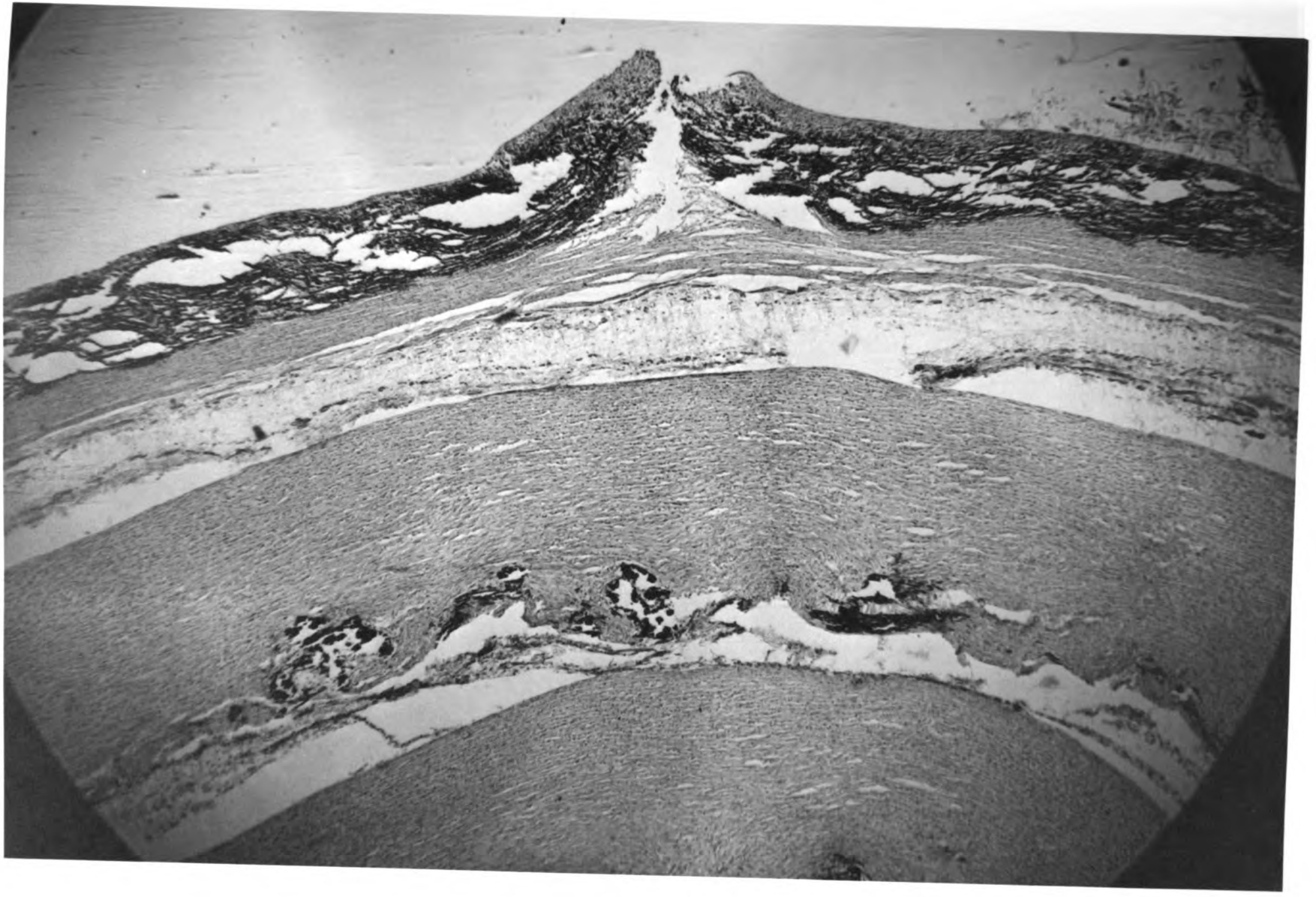


Figure 24. Intimal proliferation, sub-intimal calcification and deep medial calcification of an aorta. (H&E x50) 39(W)

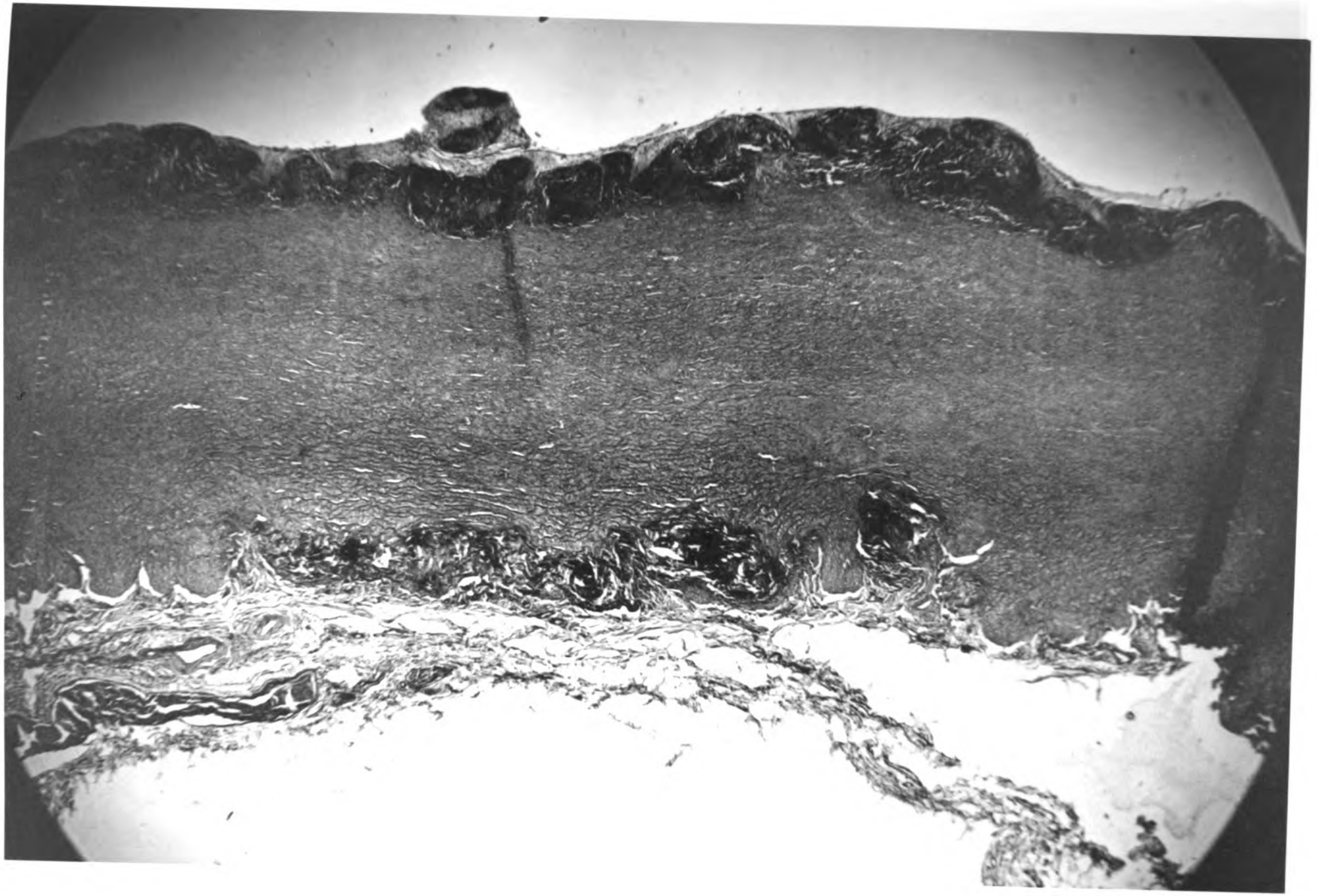


Figure 25. Calcification of the endocardium.
(H&E x154) 813

Figure 26. Sub-intimal and medial calcification of the myocardium.
(H&E x174)

Figure 27. Calcification of the intima and internal elastic membrane of the renal artery. (H&E x58) 11(Y)

Figure 28. Calcification of Bowman's membrane and the basement membrane of the tubules. (H&E x146) 813

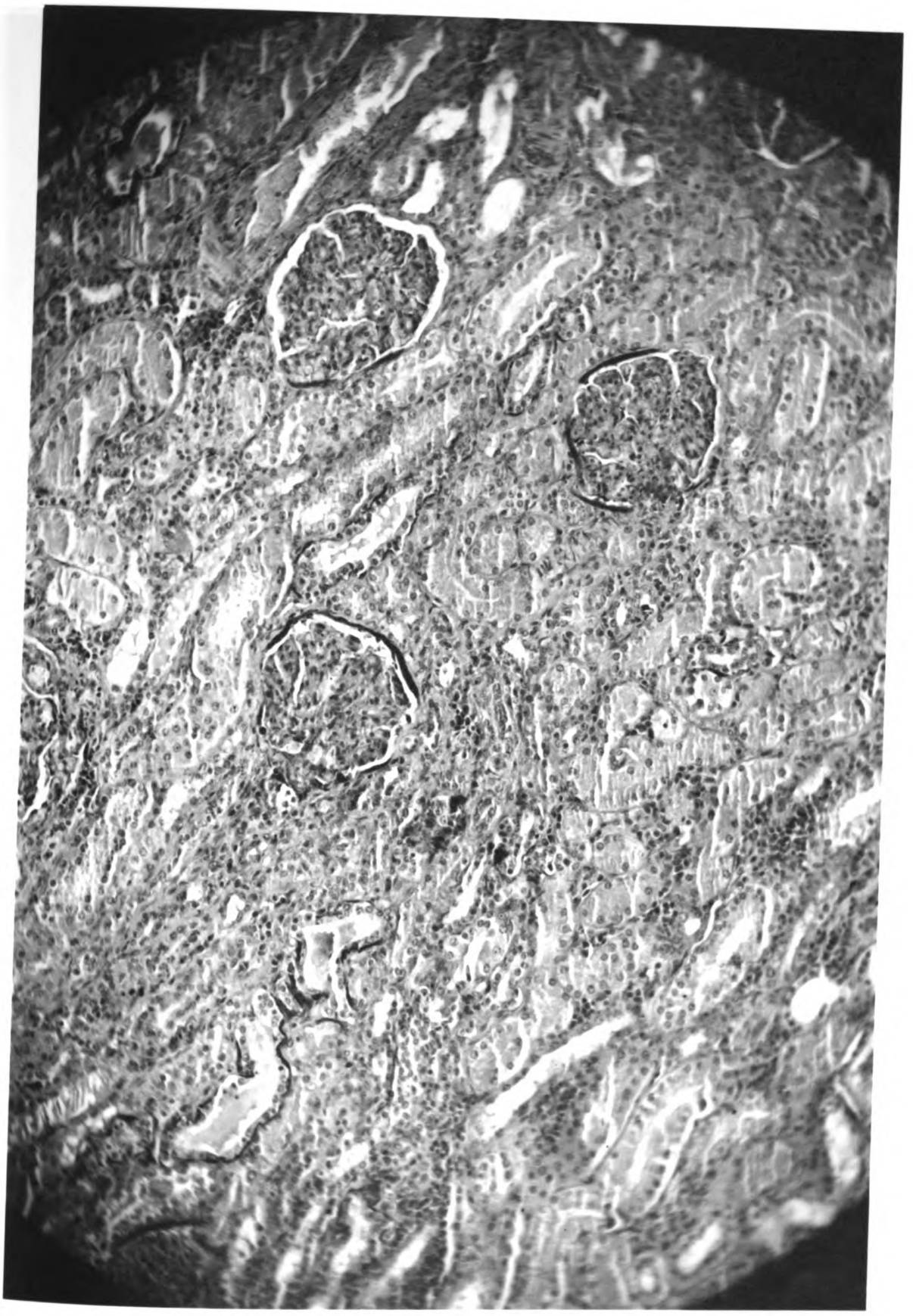


Figure 29. Renal tubular calcification of the medulla. (H&E x157) 8(B)

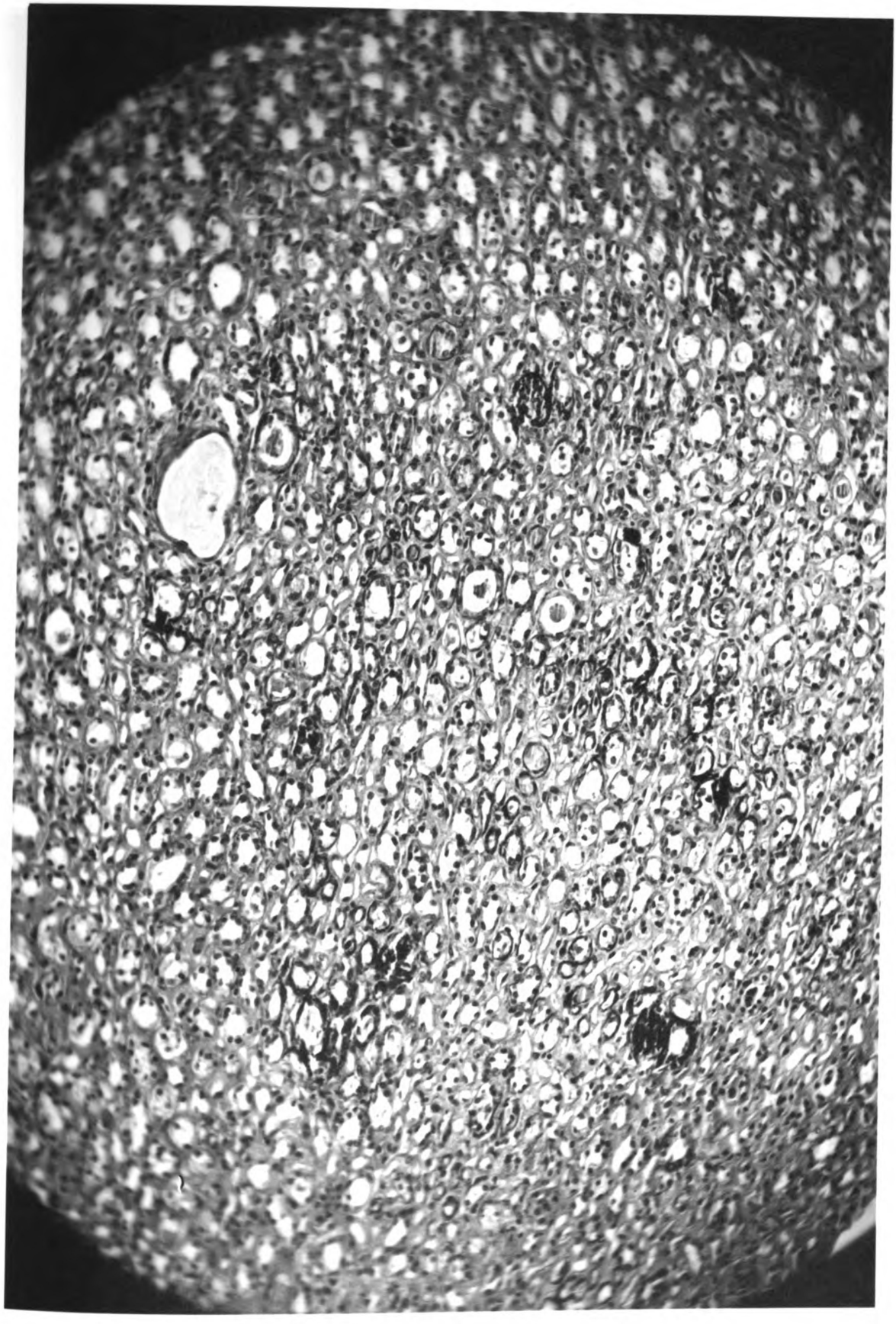


Figure 30. Calcific deposits in the renal medulla. (H&E x154) 8(B)

Figure 31. Calcific deposits within the renal tubules. (H&E x149) 2(W)

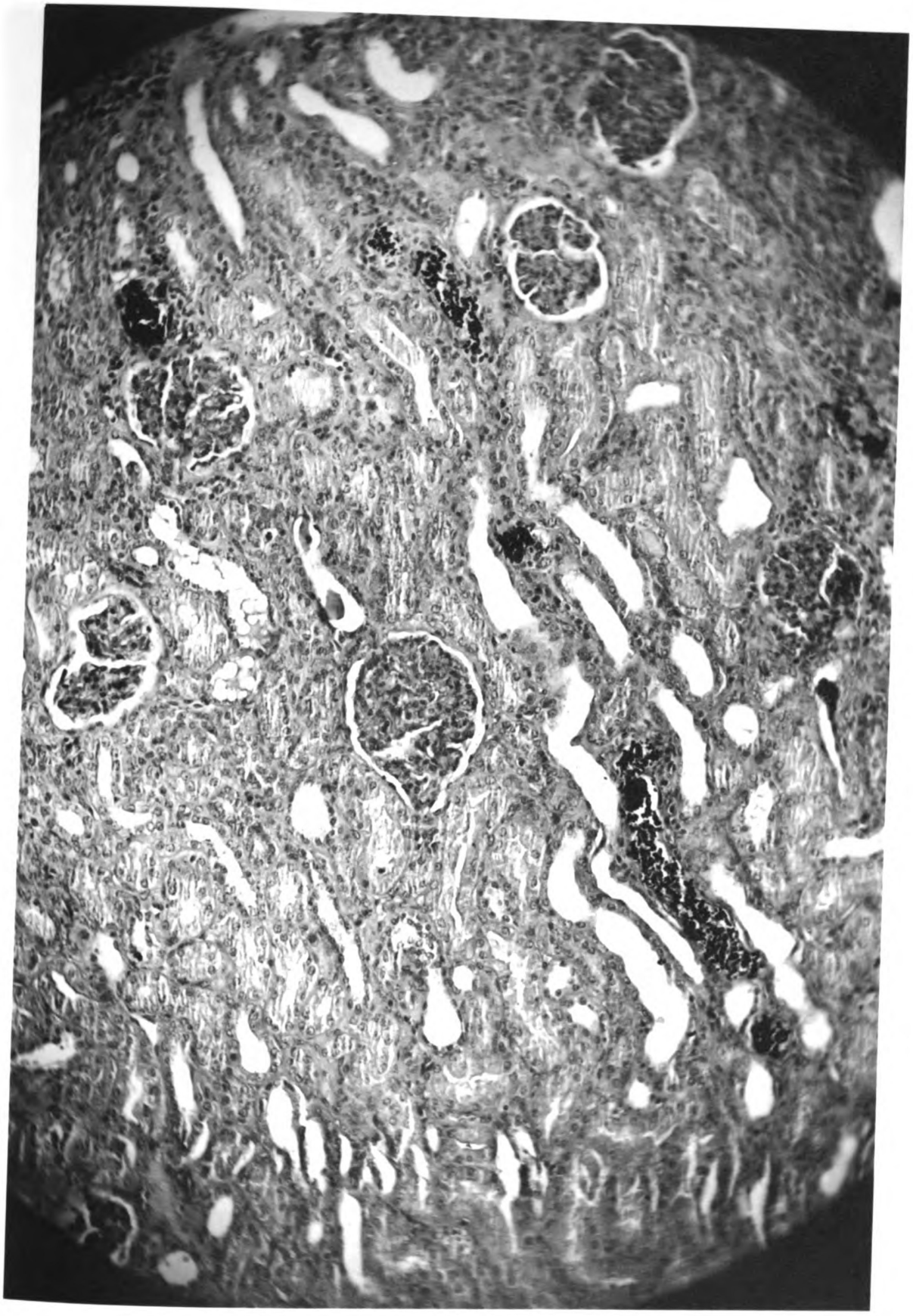


Figure 32. Hyaline casts in the renal tubules.
(H&E x149) 8(B)

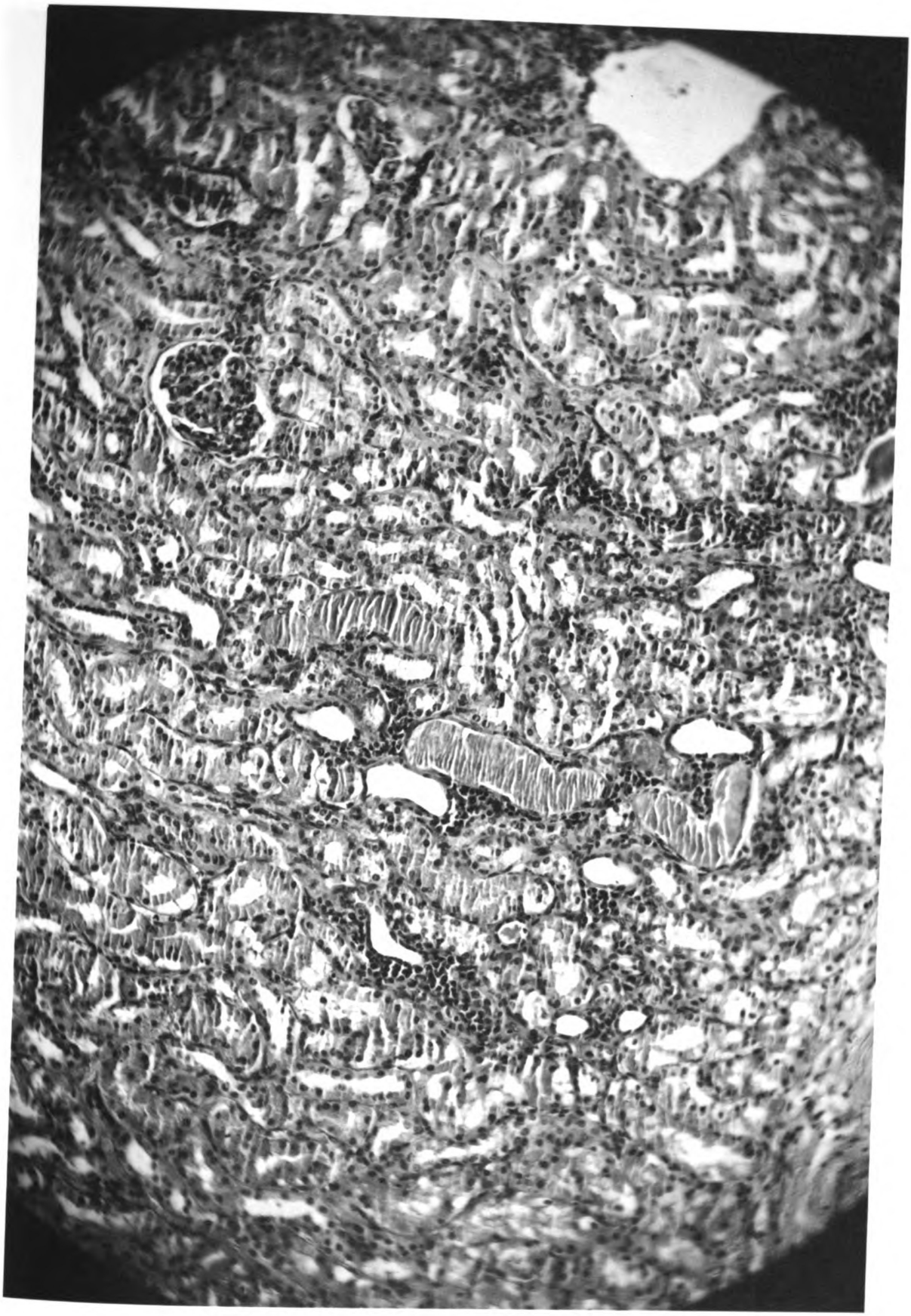


Figure 33. Calcium deposits in the renal tubules. (H&E x152) 2(W)

Figure 34. Calcification of the pulmonary veins. (H&E x53) 13(Y)

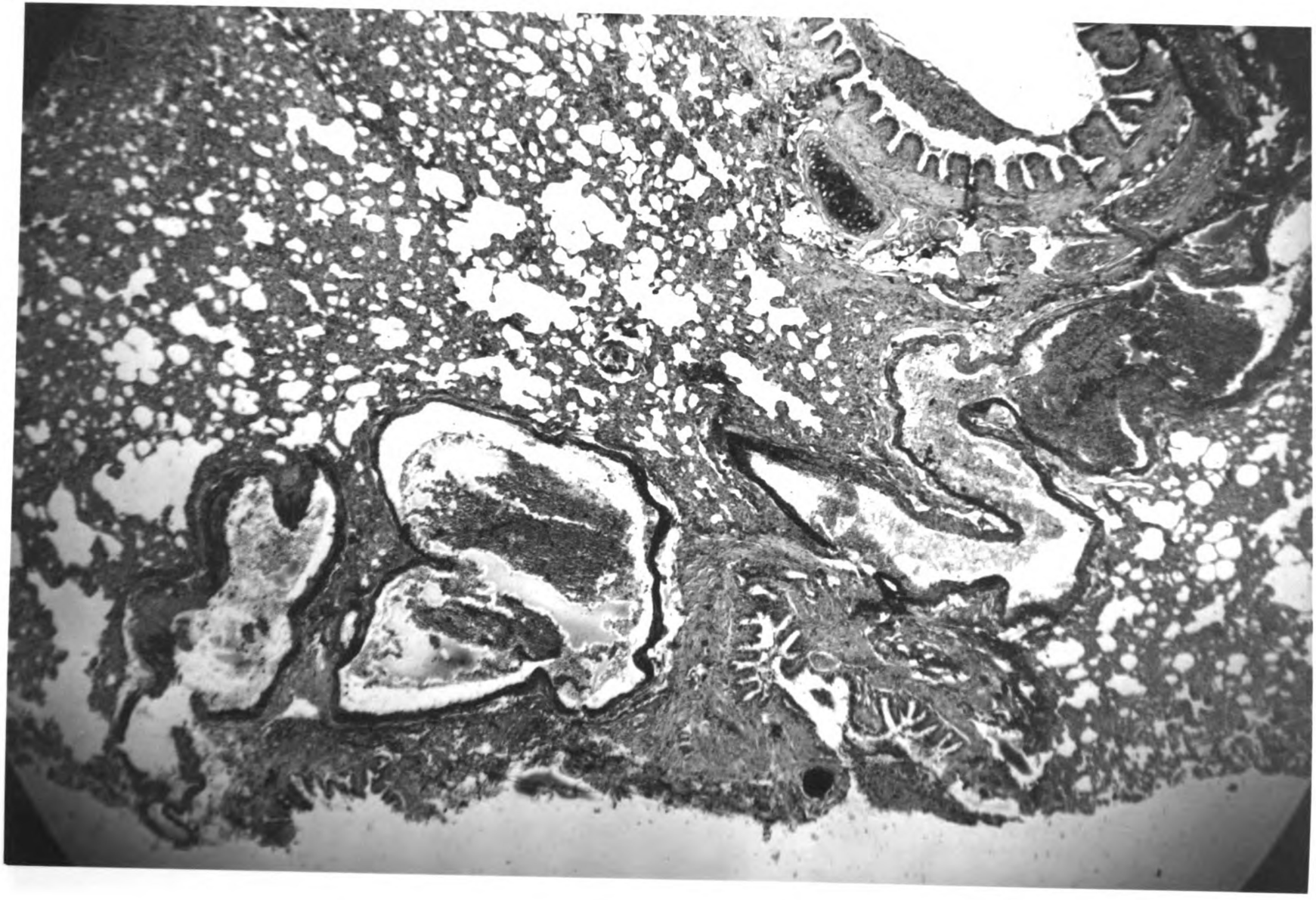


Figure 35. Pulmonary alveolar calcification and fragmentation.
(H&E x154) 2(W)

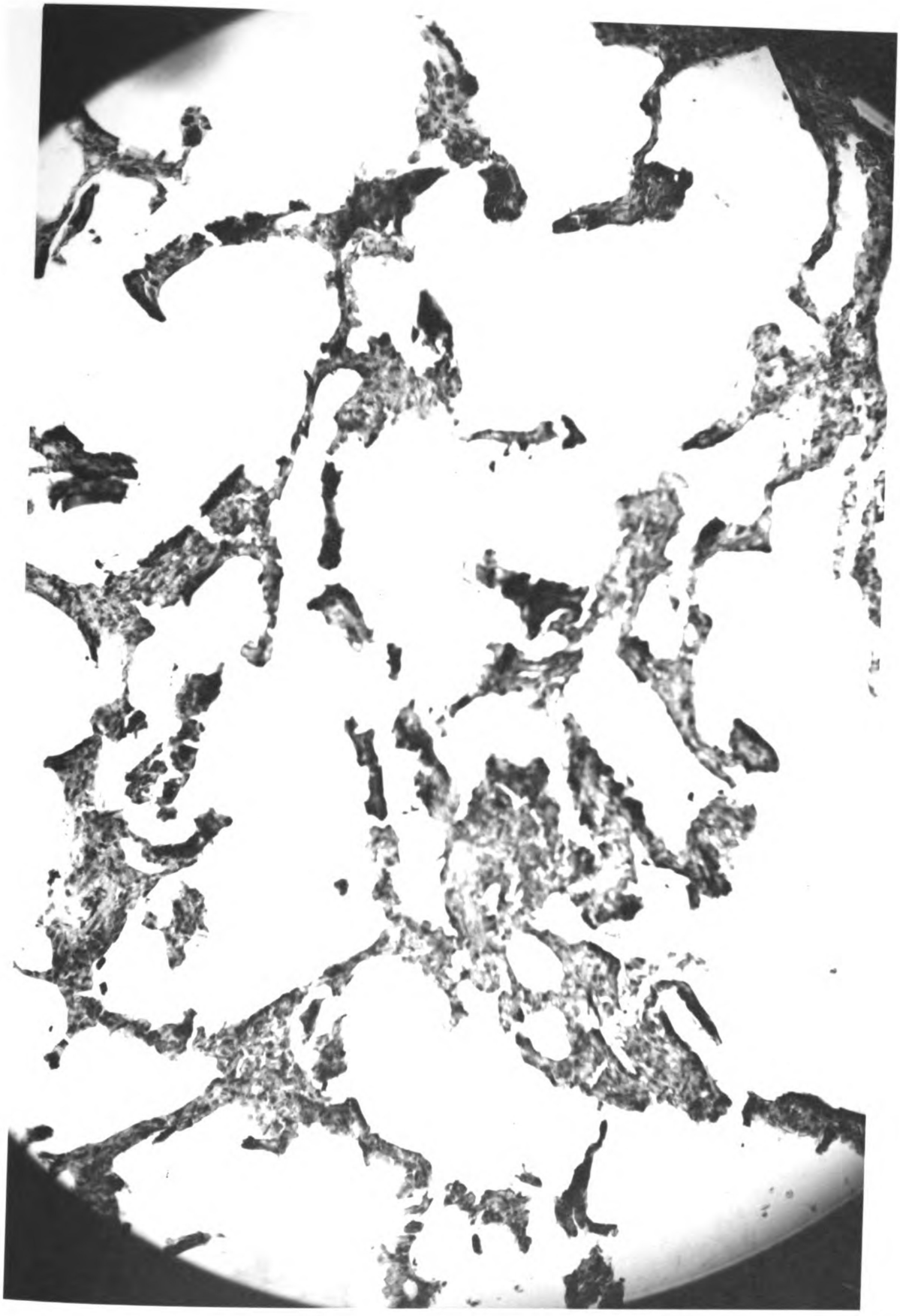


Figure 36. Calcification of the elastic fibers of a pulmonary vein and intimal proliferation. (H&E x154) 13(Y)

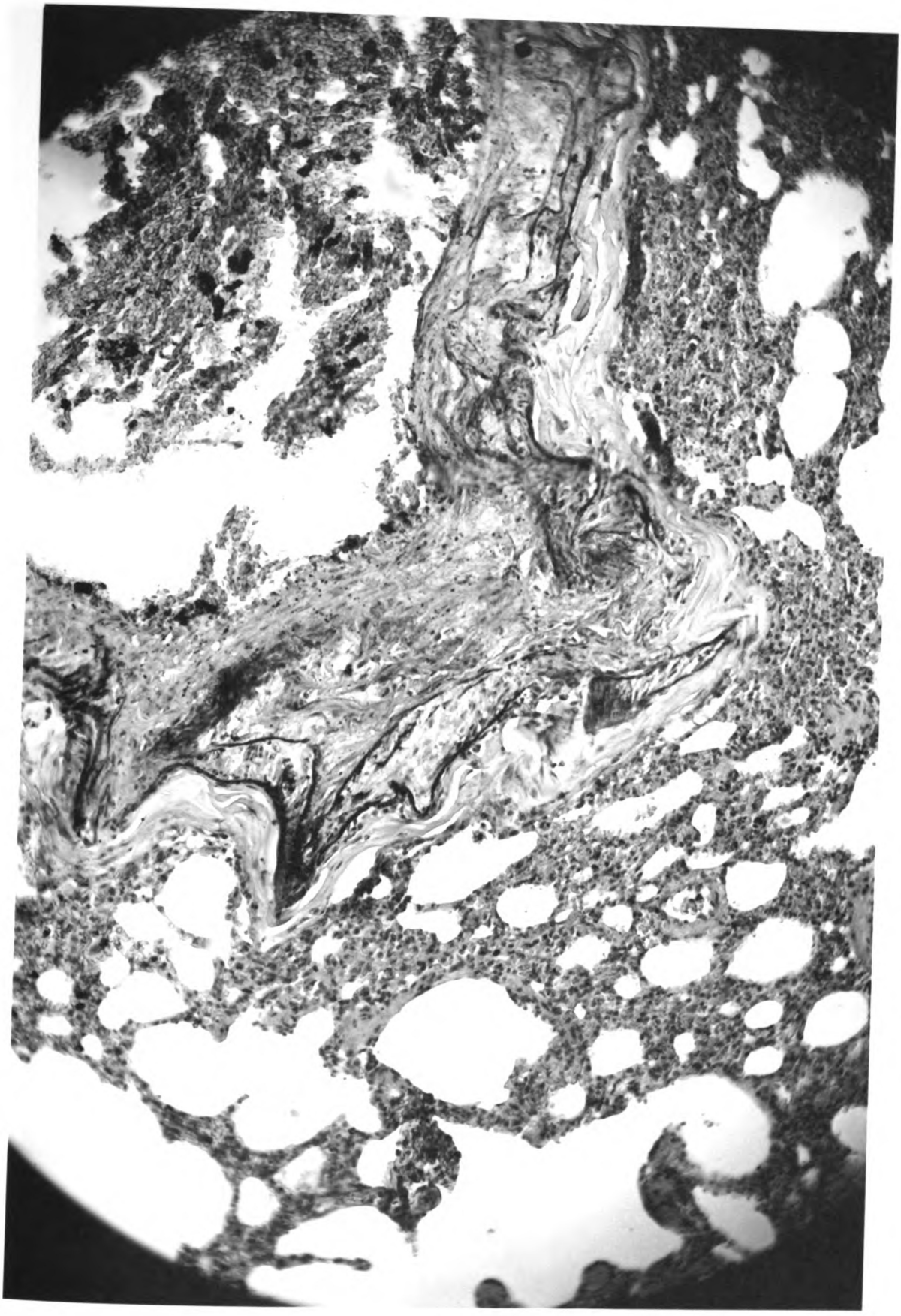


Figure 37. Calcification of the elastic fibers of a pulmonary vein.
(H&E x150) 37(W)

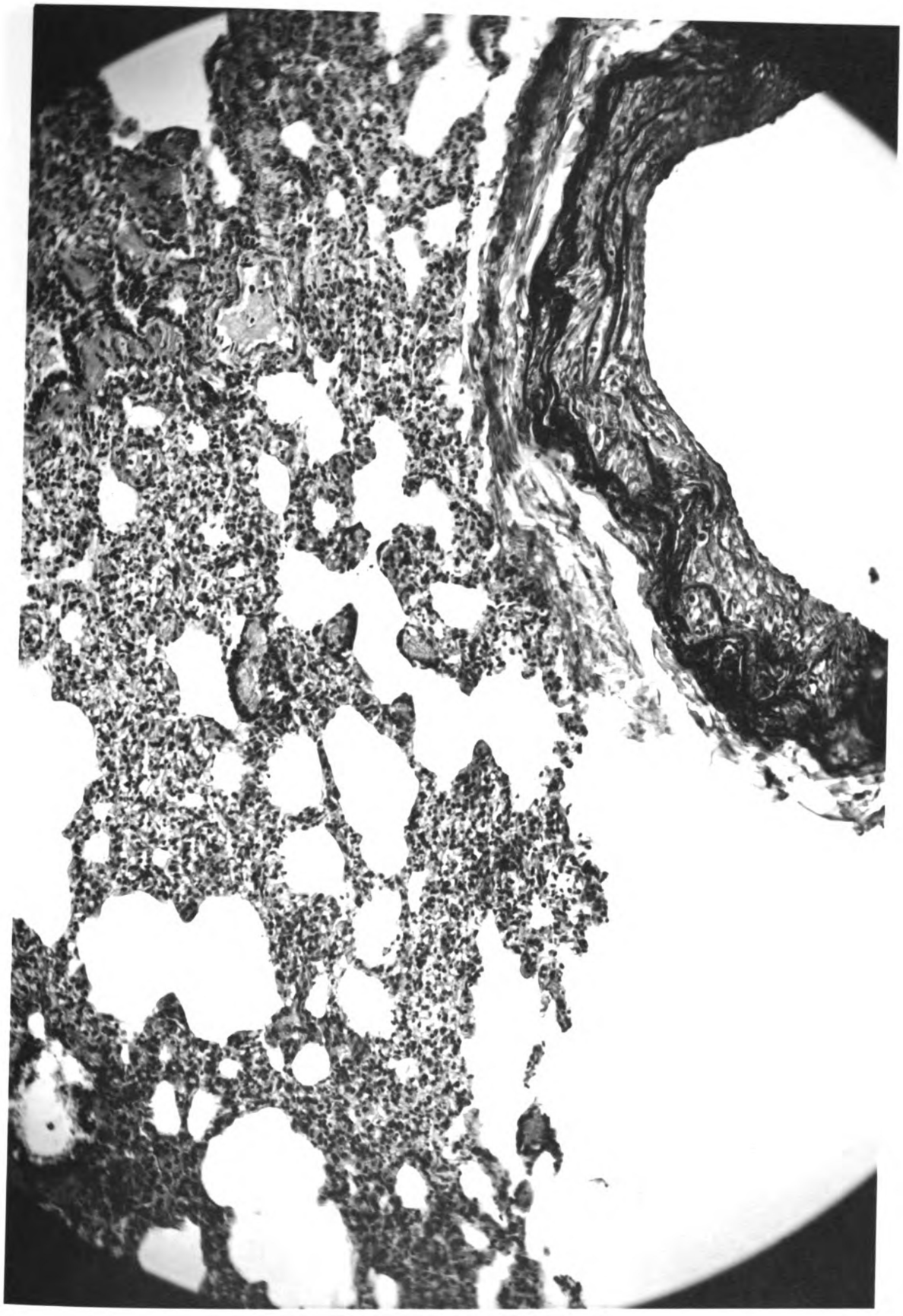


Figure 38. Trabecular artery calcification and fragmentation of the spleen. (H&E x150) 8(B)

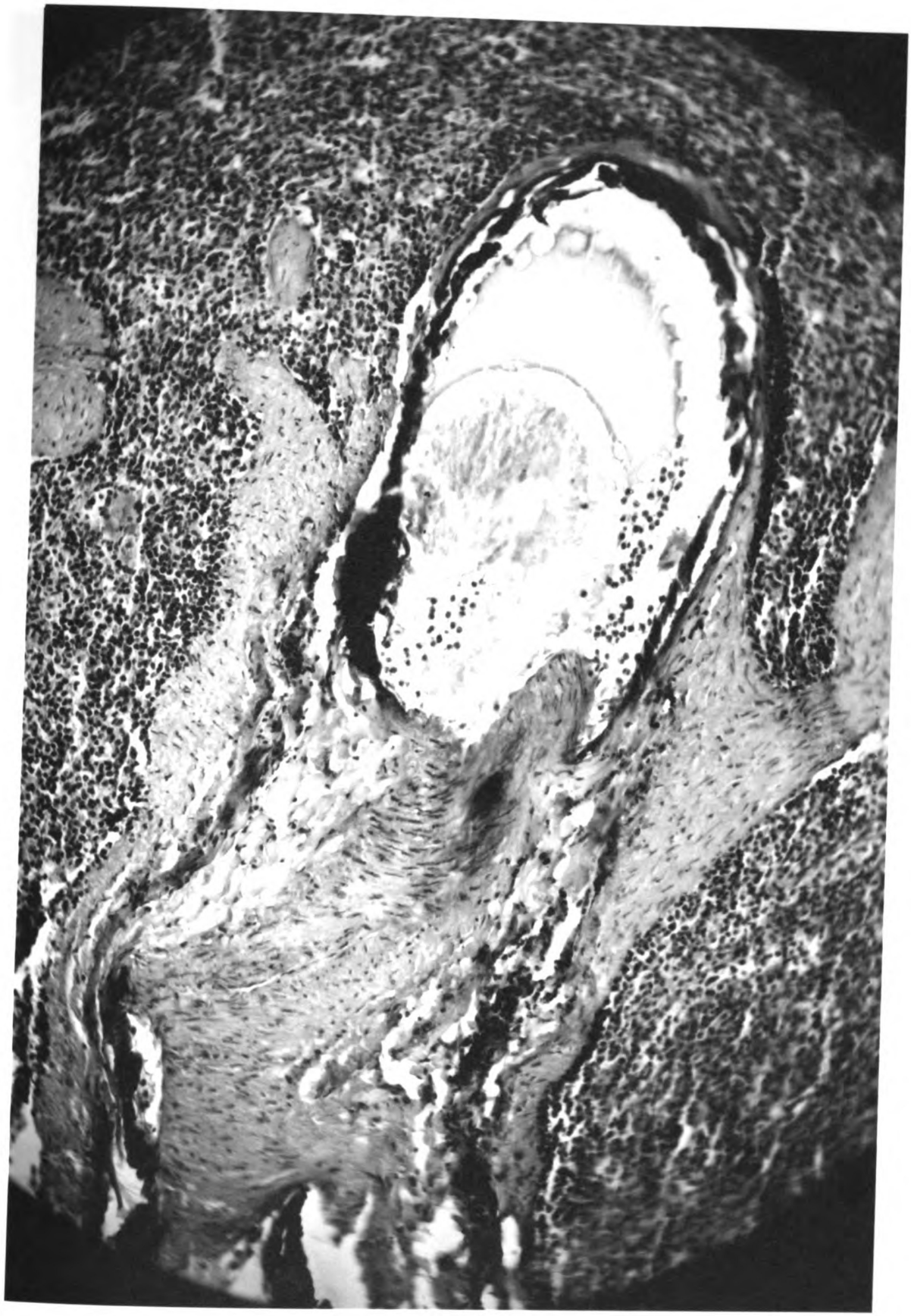


Figure 39. Medial necrosis and calcification of a trabecular artery in the spleen. (H&E x152) 8(B)