HISTOPATHOLOGIC CHANGES IN EXPERIMENTAL MAGNESIUM DEFICIENCY OF CALVES

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Howard Andrew Hartman, Jr.

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HISTOPATHOLOGIC CHANGES IN EXPERIMENTAL MAGNESIUM DEFICIENCY OF CALVES

By

HOWARD ANDREW HARTMAN JR.

AN ABSTRACT

Submitted to the College of Veterinary Medicine
Michigan State University of Agriculture and
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the requirements for the degree of

MASTER OF SCIENCE
Department of Veterinary Pathology
1959

Approved by Robert F. Langham

Howard Andrew Hartman Jr.

ABSTRACT

A microscopic study was made of tissues collected from thirty-five calves in which a low serum magnesium level had been produced.

Calcification of the endocardial elastic fibers and the internal elastic membrane of blood vessels was observed. In some cases the cardiac lesions were accompanied by adjacent fibroblastic proliferation; this frequently resulted in the formation of elevated plaques. Calcified areas were also noted in the capsule of the spleen, in the thymus, and diaphragm.

An increase in frequency of these lesions was observed as the serum magnesium level was decreased below the normal limits.

Kidney lesions consisting of varying degrees of focal interstitial nephritis were observed in many animals. Previous history of diarrhea or pneumonia was common in these cases.

The effects of stress and concurrent diseases must be considered when evaluating the renal lesions.

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I. INTRODUCTION

Vascular disease continues to stimulate research by all segments of medical science. The vast wealth of information now available is but a first step toward a true appreciation of the pathogenesis of this complex disease.

In the mid-1930's, a syndrome involving cardiovascular lesions in calves was noted by Moore and others at Michigan State University. This syndrome apparently became manifest in conjunction with the experimental production of low serum magnesium levels. At that time Huffman and others of the Department of Dairy Husbandry at the same institution were studying the effects of low dietary magnesium on the serum level and behavior of the calf.

The vascular syndrome observed involved changes in the character of the yellow elastic fibers present in the sub-endothelial connective tissue layers of the heart and blood vessels. Since the reports of this condition by Moore *et al.* in 1936 and 1938 (20,21), few works of similar nature have appeared in the literature.

The studies on the effects of dietary magnesium on the serum magnesium level were continued until 1945. During this period much data was recorded concerning dietary intake, behavior, and the fluctuations of serum calcium, phosphorous, and magnesium. Although thorough necropsies were conducted and recorded for each animal, no histopathological examination of the tissues collected was undertaken.

The widespread availability of magnesium in the diets of humans and animals essentially eliminates a prolonged hypomagnesemia of dietary origin.

The true significance of magnesium in this syndrome is difficult to define. Magnesium's importance to the enzyme systems functioning in the basic metabolic processes must be remembered when considering the influence magnesium may have on the integrity of body tissue.

The objectives of this thesis are as follows:

A. To describe and illustrate the microscopic appearance of the lesions observed in thirty-five of these animals.

B. To describe an apparent correlation existing between the degree of hypomagnesemia and the occurrence of these lesions.

C. To discuss the results of this study as they may apply to the findings of Moore and other workers.

II. LITERATURE REVIEW

Extensive study concerning the effects of magnesium deficiency on animals was carried out during the 1930's. Most of these studies were carried out by Huffman on calves and by Kruse with rats and dogs.

Early reports by Huffman *et al.* noted the development of hyperexcitability and poor growth resulting from prolonged feeding of diets composed only of milk (12). These animals returned to normal with magnesium therapy or the addition of magnesium to the diet. This response did not, however, attract attention to the significance of magnesium to this syndrome. Further investigation by this group and other workers began to demonstrate the importance of magnesium.

A decrease in serum magnesium below a normal range of 1.8 to 3.8 milligrams per cent resulted in a definite syndrome. It was characterized by increasing excitability and the eventual development of convulsions and tetany. Such a change in serum magnesium has not been associated with any significant fluctuation in serum calcium or phosphorous.

Further studies by Huffman *et al.* to determine the ability of plant and mineral sources of magnesium to maintain a normal serum magnesium level were conducted (4, 10, 11).

Moore and his associates conducted the routine necropsies of these experimental calves. They noted the presence of lesions peculiar to those animals in which a prolonged low serum magnesium level had been produced. Preliminary

descriptions of these lesions were made by Moore et al. in 1936 (20). Langham described the renal pathology in 1939 (18). The entire syndrome was discussed in detail by Moore, Hallman, and Sholl in 1938 (20). Considerable variation in the occurrence of these lesions was noted.

In summary, the lesions were described as follows:

A. Macroscopic Lesions

1. Circular plaques, approximately one or two millimeters in diameter, confined to the endocardial surfaces of the heart, intima of blood vessels, pleural and peritoneal surfaces of the diaphragm, and the surface of the spleen.

2. White spots, approximately one to three millimeters in diameter, visible on the surface of the kidneys.

B. Microscopic Lesions

1. In the heart, the plaques were described as areas of calcification involving the subintimal elastic tissues and accompanied by a mild fibroblastic proliferation. Degeneration and calcification were observed in the Purkinje fibers.

2. Vascular changes noted also appeared to involve calcification of the elastic fibers in the intima, media, and adventitia of various blood vessels. Little evidence of cellular reaction accompanied these changes.

3. Degenerative changes and calcification were noted in the capsule and trabeculae of the spleen and also the covering of the diaphragm. Calcification was noted in the skeletal muscle fibers of the diaphragm.

4. Fibroblastic proliferation was noted in the periportal areas of the liver and in the interstitial areas of the renal cortex. Slight mention was made of lymphoid infiltration involving the kidney. No significant calcification was observed in the kidney.

Studies conducted by other workers on calves during this period mention few if any definite pathologic changes

(24, 25, 27). They did mention similar results concerning changes in irritability and serum magnesium fluctuations in response to changes in dietary magnesium.

Recent work has been reported by the English investigators, Blaxter and Rook (1, 2). They produced a hypomagnesemic hyperexcitability characterized by intermittent tetany and convulsions. Relationships between serum magnesium, calcium, and phosphorous were found to be similar to those previously mentioned. The lesions reported were hemorrhage and congestion of the viscera. They did not report lesions comparable to those described by Moore and co-workers.

The exhaustive studies of magnesium deficiency conducted by Kruse (14, 15, 16), Orent (27), and McCollum (19), have described a bi-phasic syndrome in rats and dogs.

The first phase was characterized by increasing excitability, dilatation of the vascular bed, convulsive seizures, and a high mortality rate. The surviving animals went into the second phase which was characterized by signs of malnutrition, cachexia, and renal damage (8, 13).

Summary comments which they made concerning this syndrome were as follows:

A. Younger animals were more susceptible to deficiency. This suggested a utilization of previously stored magnesium by older animals.

B. Differences in normal temperament of the animal species should be considered. Dogs exhibited signs of nutritional failure prior to undergoing the tetanic phase. This was considered to be the result of an inherently greater nervous stability possessed by dogs.

C. Animals fed slightly subminimal magnesium diets developed evidence of nutritional failure but did not exhibit the tetanic syndrome.

The results of these controlled works with rats and dogs offer significant data for evaluating the conflicting results reported by the British and American workers.

III. PROCEDURE

A. Materials

(1) Tissue sections were prepared by the Veterinary Pathology Department. An average of thirty sections was made from every animal. These sections represented the major organs of the body, the larger vessels, and in particular, the lesions observed. They were fixed by the use of Zenker's, Carnoy's, or formalin solutions. All were stained with hematoxylin and eosin (H & E). Complete sets of aniline-blue (A-B) and Verhoeff's (Verh.) stains were also made for seven of the thirty-five animals studied. The silver-nitrate technique was used to determine the extent of calcification.

(2) Necropsy records were obtained from the permanent file of post-mortem reports maintained by the Veterinary Pathology Department.

(3) Herd book records were studied to obtain data concerning the daily diet and behavior of the calves. This material was furnished through the courtesy of Dr. C. F. Huffman from the Department of Dairy Husbandry.

(4) Records of weekly serum determination for calcium, phosphorous, and magnesium were obtained from the Department of Agricultural Chemistry through the courtesy of Dr. C. W. Duncan.

B. Methods

(1) Prior to examining the slides from each animal,

a daily graph covering the life-span of that animal was prepared. All data relative to diet, serum fluctuations, and behavior were plotted on this graph.

(2) Slides from each animal were assembled, sorted, and indentified. A description of any pathologic change was then noted as each slide was examined.

(3) The serum data were studied to determine the degree and duration of hypomagnesemia which existed in each animal. The animals were grouped according to the prolonged low level of serum magnesium achieved with the diet.

(4) These groups were then studied to determine any correlation which might exist between the degree of hypomagnesemia and the lesions observed.

IV. RESULTS

A. Summary of Available Serum Data

The calves were grouped according to decreasing serum magnesium levels. The average serum calcium and phosphorous levels for each group were studied and compared with the levels defined as normal by other investigators (Chart I). As the animals were studied individually, no significant fluctuations in serum calcium or phosphorous were observed in conjunction with the development of prolonged hypomagnesemia. General fluctuation of the phosphorous level was noted throughout the life-span of every animal.

CHART I

COMPARISON OF THE AVERAGE SERUM LEVELS OF CALCIUM AND PHOSPHOROUS WITH THAT OF MAGNESIUM

	Magnesium	Calcium	Phosphorous	Animals
Group I	2.0 or higher	11.4	7.8	6
Group II	1.6-2.0	11.4	7.6	8
Group III	1.2-1.6	11.6	7.1	10
Group IV	1.2 or less	11.4	7.1	11
Normal levels	1.8-3.8	10-13	6-8	--

Values expressed in milligrams per cent

Chart references (2, 4, 5, 6)

B. Summary of Group Observations

(1) Group One consisted of six animals averaging 158

days of age. The serum magnesium level had been maintained at or above a level of 2 milligrams per cent for an average of 121 days.

The diet consisted of milk supplemented with magnesium in mineral or plant form. Viosterol was fed to prevent rickets.

The lesions observed in Group One were as follows:

a. Cardiac lesions

One animal in this group displayed a few plaques 1 to 2 millimeters in diameter in the endocordium of the left ventricle. This animal died of uremia resulting from an extensive interstitial nephritis. No lesions were observed in the other animals.

b. Renal lesions

The only other lesions observed in this group were white foci, 1 to 3 millimeters in diameter, on the surface of the kidney. These foci were areas of connective tissue proliferation. Three of the four animals exhibiting these lesions had suffered from a diarrhea at some time prior to death. The remaining animal had an interstitial nephritis of undetermined cause. Foci of lymphocytes were frequently observed in this case.

(2) Group Two consisted of eight animals with an average age of 190 days. The serum magnesium level had been maintained within a range of 1.6 to 2.0 milligrams per cent for an average period of 93 days.

The diet of these animals consisted of milk and various supplements such as magnesium oxide, rice krispies, a mineral mix (Fe, Cu, Co, Mn,) designed to prevent anemia, and viosterol.

The lesions observed in Group Two were as follows:

a. Cardiac lesions

Five animals exhibited plaques in the endocardium of the left ventricle and in the left atria. No plaques were noted in the right side of the heart.

b. Vascular lesions

Six of the eight animals displayed gross lesions involving the larger arteries and veins. These lesions were in the form of raised roughened areas of the intimal surfaces which sometimes felt granular or abrasive. These vessels were the aorta, pulmonary, carotid, and renal arteries, and the jugular veins. There was much variation in the number of vessels involved in each animal.

c. Renal lesions

White foci were noted on the kidney surface of four animals. Microscopically, these foci were made up of lymphocytes and proliferating fibroblasts. In each case, the animal had suffered from a diarrhea or bronchopneumonia at some time prior to, or at the time of death.

d. Diaphragmatic lesions

Two animals displayed whitish roughened areas

in the pleural and peritoneal surfaces of the diaphragm. These areas were approximately 2 to 3 millimeters in diameter.

e. Splenic lesions

Three animals showed firm roughened areas in the capsule of the spleen. Microscopically these were found to be areas of calcification. A fourth animal displayed similar microscopic lesions but no macroscopic lesions.

(3) Group Three consisted of ten animals with an average age of 178 days. The serum magnesium level had been maintained within a range of 1.2 to 1.6 milligrams per cent for an average of 75 days.

The diet of these animals was composed chiefly of milk. Rice krispies, mineral supplements, and viosterol were also fed.

The lesions observed in Group Three were as follows:

a. Cardiac lesions

Six animals displayed plaques in the endocardium of the left ventricle. One animal had plaques present in the right ventricle and in both atria.

b. Vascular lesions

Five animals had plaques varying from definite intimal elevations to roughened areas likened

to the surface of sandpaper. The aorta, pulmonary and carotid arteries, and pulmonary veins were involved.

c. Renal lesions

Seven animals had white foci on the surface of the kidney. Microscopic examination revealed three general types of lesions. These were, lymphocytic foci, groups of lymphocytes and fibroblasts, or mature fibrous connective tissue. The animals with foci of lymphocytes were those which had serum magnesium levels below 1.4 milligrams per cent and no history of diarrhea or pneumonia. The animals with a history of diarrhea or pneumonia possessed foci of mixed lymphocytes and fibroblasts, or of mature fibrous connective tissue.

d. Diaphragmatic lesions

Two animals displayed rough wrinkled areas on the surface of the diaphragm.

e. Splenic lesions

Two animals had roughened areas in the capsule of the spleen.

f. Thymic lesions

One animal revealed a few areas of grayish coloration in the capsule of the thymus. Microscopically they were calcified areas.

(4) Group Four consisted of eleven animals with an average age of 193 days. The serum magnesium level had been maintained at or below 1.2 milligrams per cent for an average of 158 days.

The diet of these calves consisted chiefly of milk. Rice krispies were fed for varying intervals. Mineral supplements and viosterol were also fed.

The lesions observed in group four were as follows:

a. Cardiac lesions

Eight of the eleven animals had extensive plaque formation in the endocardium of the left ventricle.

b. Vascular lesions

Definite intimal roughness and plaque formation were noted in many vessels. The distribution of lesions in Group Four was more extensive than in the other groups. The aorta, carotid, pulmonary, and renal arteries, jugular, pulmonary, portal, and renal veins, and the anterior vena cava were affected.

c. Renal lesions

Seven animals displayed white foci on the surface of the kidneys. One animal had an interstitial nephritis composed of fibroblasts and lymphocytes. This animal exhibited a severe diarrhea for one week prior to death. Foci of lymphocytes were observed to be the primary renal lesion in five

animals. These foci seemed to arise in the region of the renal corpuscle. Three animals exhibited foci of fibrous connective tissue at the periphery of the cortex.

d. Splenic lesions

Eight animals exhibited roughened areas and definite plaque formation in the capsule of the spleen.

e. Diaphragmatic lesions

Changes ranging from grayish foci to definite plaque formation were noted in eight animals.

f. Thymic lesions

No definite macroscopic lesions were noted in the thymus. Microscopic examination revealed irreguarly shaped hyaline bodies in three animals. These bodies measured about 100 microns by 300 microns. They appeared calcified. Evidence of a nucleus similar to those seen in Hassall's bodies was noted in these bodies. One other animal possessed what appeared to be spicules of bone in the thymus. They were dark blue staining and about the same size as the other bodies mentioned.

C. Serum Magnesium Levels in Calves Which Displayed Evidence of Hyperexcitability

Eight of the 35 calves in this study were noticed to

exhibit definite symptoms of hyperexcitability.

An animal described as being irritable prior to death had a serum magnesium level of 1.7 milligrams per cent for 60 days.

The other animals were described as being in tetany or convulsions prior to death. Five of these animals had serum magnesium levels of 1.2 milligrams per cent for approximately 100 days. The remaining two had serum magnesium levels of about 1.5 milligrams per cent for 100 days.

D. Description of Gross and Microscopic Lesions

Cardiac Lesions

The lesions described in the endocardium varied from irregular grayish discolorations in the sub-intimal tissue to definite plaques elevating the intimal surface. The size of these lesions was approximately 1 to 3 millimeters in diameter. The consistency of the elevated plaque was noted to be very granular in the lower serum magnesium groups. These animals displayed greater numbers of plaques than those with higher serum levels.

One of the most subtle changes was the deposition of calcium in the deeper layers of the endocardium and subendocardial tissues. No definite alteration in tissue pattern was observed (Figure 1). Another slight but definite change in these tissue layers was frequently observed (Figure 2). It appeared to be a definite disruption of the elastic tissue

pattern and slightly edematous. The fibers appeared distinct yet did not possess the compact nature of the adjacent tissue (Figure 2B). The fibers did not appear calcified. More obvious changes involved calcification of the elastic fibers and the development of raised areas on the intimal surface. With Zenker's fixation and hemotoxylin and eosin stain, evidence of calcification was observed in two ways. The calcified areas appear homogenious, bluish pink, and hyaline to granular in appearance (Figures 3A, 4A, 4C). The other indication of calcification was noted as an apparent stiffness in the calcified elastic fibers (Figure 4A). This stiffness becomes more apparent when noted in the calcified internal elastic membrane of the blood vessels. Surrounding areas of calcification (Figures 3A, 4C) an increased cellularity is noted (Figures 3B, 4B). Aniline-blue stains of these areas revealed this to be a fibroblastic proliferation. This proliferation was often noted to involve the subendocardial tissues (3C) and extend to the cardiac muscle. Verhoeff's elastic tissue stain was valuable in the examination of the elastic tissues in the plaque. This technique revealed much disruption (Figures 5B, 6) and fragmentation (Figures 5B, 6B) of the elastic fibers. The areas of proliferation were also observed with this technique (Figures 5A, 6A). Examination of the large endocardial plaque revealed areas of necrosis and calcification (Figures 7A, 9A). Connective tissue proliferation was noted to surround the entire plaque (Figures 7B, 8B, 9B, 10B).

The large empty spaces in the centers of these plaques indicate the degree of calcification prior to fixation (Figures 7, 8, 9, 10). Fragmentation was clearly demonstrated in these areas (Figures 8C, 10C). It was noted that calcification didn't appear to interfere with the staining quality of the elastic fibers. Fragments of these fibers are noted in the calcified areas (Figures 8A, 10A).

Vascular Lesions

Gross lesions observed in the aorta consisted of elevated calcified plaques, and wrinkled areas of the intimal surface. These lesions were noted in all parts of the aorta, but were more frequently found in the thoracic portion.

Microscopic examination revealed calcification of the sub-intimal elastic tissue (Figures 11A, 12, 13). These lesions were not accompanied by an adjacent proliferation of connective tissue. Further examination of the media (Figure 14) and adventitia (Figures 15, 16) of the aorta, revealed calcification of the elastic fibers. Here again, the character of stiffness in calcified fibers was noted. Adjacent fibers which do not appear calcified have been disrupted (Figure 15A). The typical lesion observed in the arterial vessels of smaller caliber was calcification of the internal elastic membrane. The stiffness of the calcified membrane produced apparent distortion in the intimal surface as the caliber of the vessel decreased after death (Figures 20, 21, 23, 24, 25, 26, 27).

In the calcified areas, fragmentation of the elastic fibers was frequent (Figures 18, 19, 22, 23, 25, 26). The fibers sometimes appeared stretched or torn apart (Figure 25A). Evidence of intimal injury resulting from these changes was occasionally noted to include thrombus formation (Figure 26A). Gross lesions in the form of elevated plaques were observed in some of the larger veins (Figure 28). Their occurence was not frequent, and no lesions were noted in the smaller veins during microscopic examination.

Splenic Lesions

The gross lesions of the spleen were described as grayish discolorations of the capsule which were often roughened and firm. These areas varied much in size and shape.

Microscopic examination of the capsule and trabeculae of the spleen disclosed areas of calcification often almost the thickness of the capsule (Figure 29). Stains varied in their ability to differentiate the calcified areas. Hematoxylin and eosin revealed only faint bluish hyaline areas. These were difficult to differentiate from non-calcified areas in the capsular tissue. Silver nitrate stain for calcium revealed large areas of calcification (Figure 29). Verhoeff's elastic tissue stain revealed little if any change in the character of the elastic fiber pattern in the calcified areas. In a few cases, calcification was detected only by microscopic examination.

Thymic Lesions

Gross lesions were seldom observed in the thymus. Occasionally animals with the lowest serum magnesium levels displayed grayish discolorations of the capsule. These were areas of calcification.

Microscopic examination of the thymic tissue from this group of animals revealed large hyaline calcified bodies. They measured approximately 100 microns by 300 microns in size and were irregular in shape (Figure 30). One animal displayed many such bodies which had the character of bone trabeculae. Evidence of nuclear remnants was observed (Figure 31) and concentric ring formation around the remnants was noted. The size of these bodies was larger than the average Hassall's body.

Diaphragmatic Lesions

The lesions in the diaphragm varied from linear streaks to elevated plaques occurring on both surfaces of the diaphragm.

During microscopic examination of the diaphragm it was difficult to ascertain the nature of the gross lesion. Seldom did elastic fibers show any alteration of pattern. Alterations which were seen usually corresponded to areas of reddish discoloration noted with aniline-blue stains. This reddish discoloration occurred in the blue staining elastic and connective

tissue areas. Discolorations of this type were noted in the calcified internal elastic membranes of blood vessels. Degenerative changes in the skeletal muscle which Moore et al. discribed were not present.

Renal Lesions

Gross lesions on the surface of the kidney were white foci approximately 3 millimeters in diameter, or more diffuse mottled areas witn no distinct outline. Similar lesions were noted on cut section.

Microscopically, three types of lesions were observed. The white foci were frequently found to be accumulations of mature fibrous connective tissue. Also noted were proliferating fibroblasts with variable numbers of lymphocytes. Lesions of this type were frequently found in animals which had suffered from a diarrhea or pneumonia. A third type of lesion was a focal accumulation of lymphocytes. These were usually seen around the renal corpuscles or in the adjacent interstitial area (Figures 32-35). Bowman's capsules were thickened occasionally, and surrounded by concentric rings of fibrous connective tissue (Figure 35).

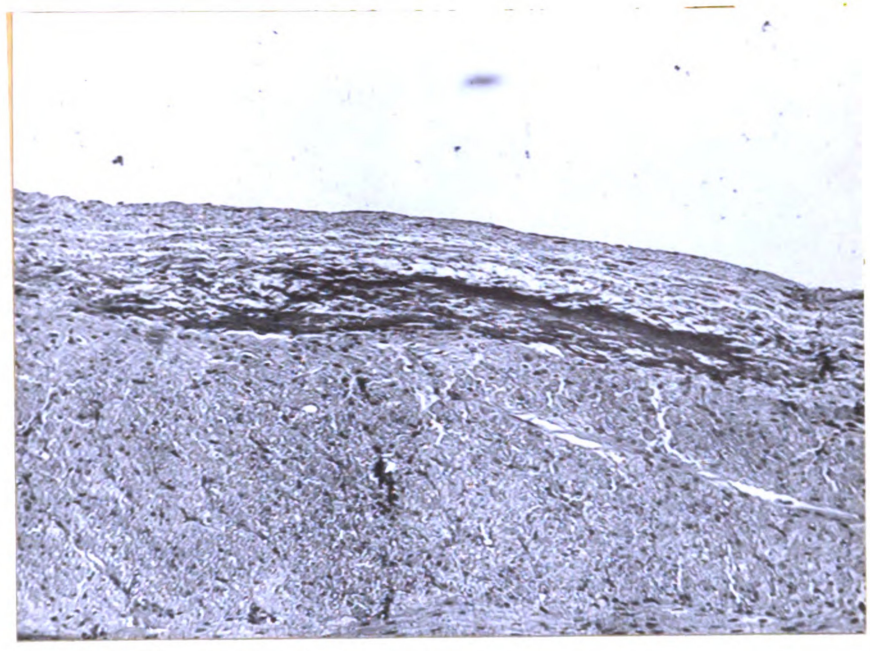

Figure 1 Early deposition of calcium in the endocardial and subendocardial tissues.
(MSU-6365 H&E x140)

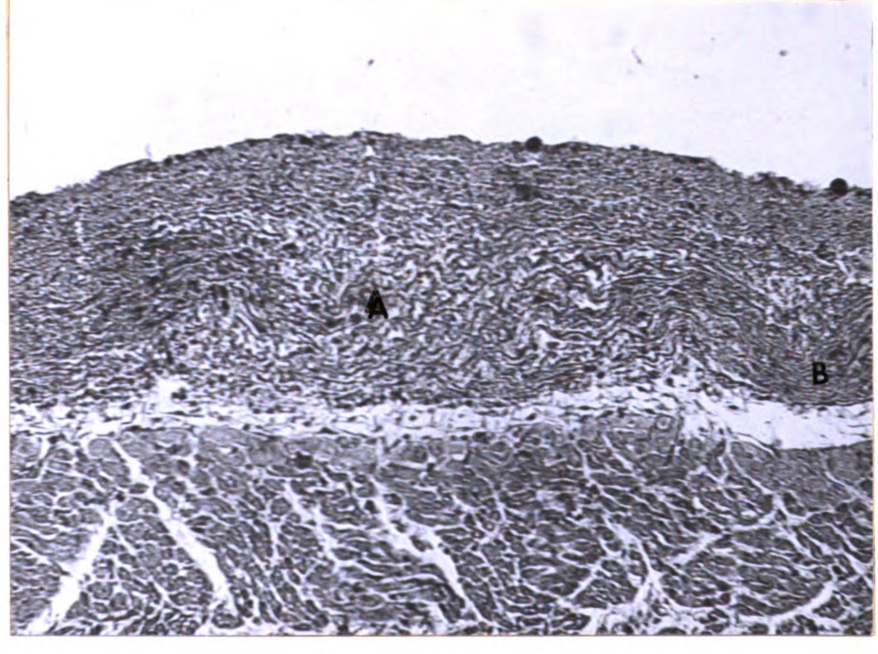

Figure 2 Disruption of elastic tissues (A), and normal compact elastic tissues (B).
(MSU-6909 H&E x140)

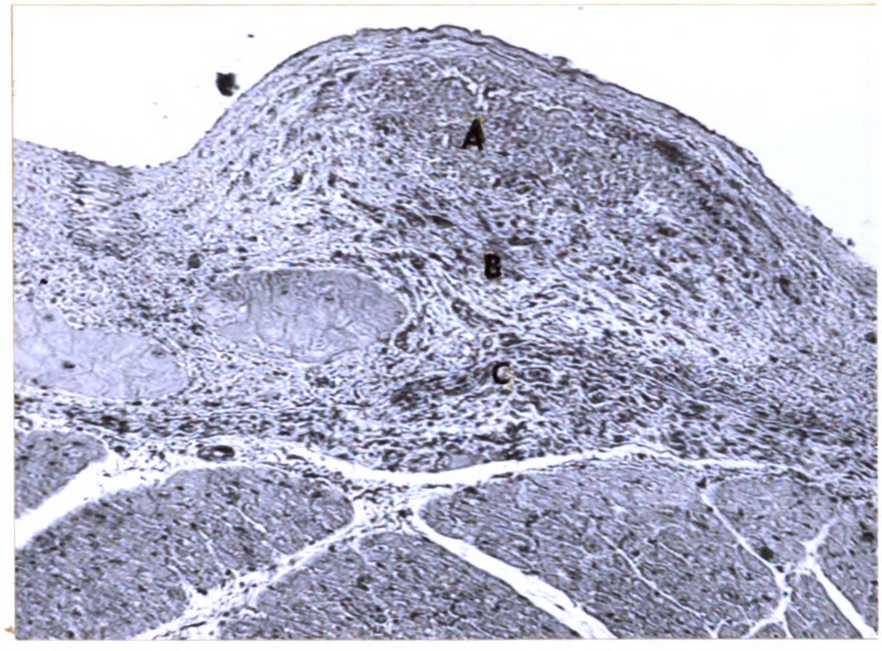

Figure 3 Calcification (A) and fibroblastic proliferation (B, C) resulting in definite elevation of the intima.
(MSU-6909 H&E x140)

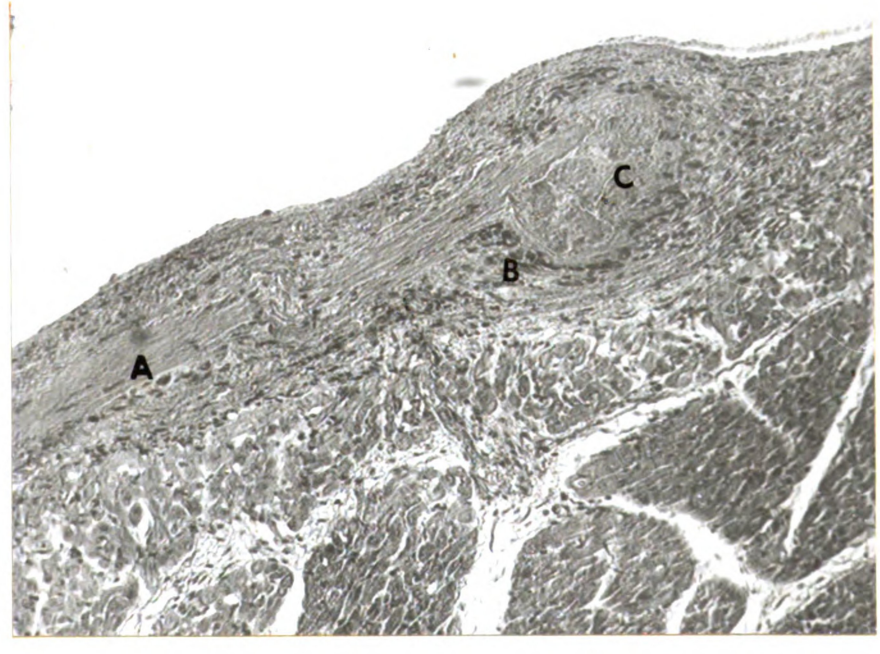

Figure 4 Calcification of elastic fibers (A) and proliferating fibroblasts (B) adjacent to a small calcified plaque (C).
(MSU-5641 H&E x140)

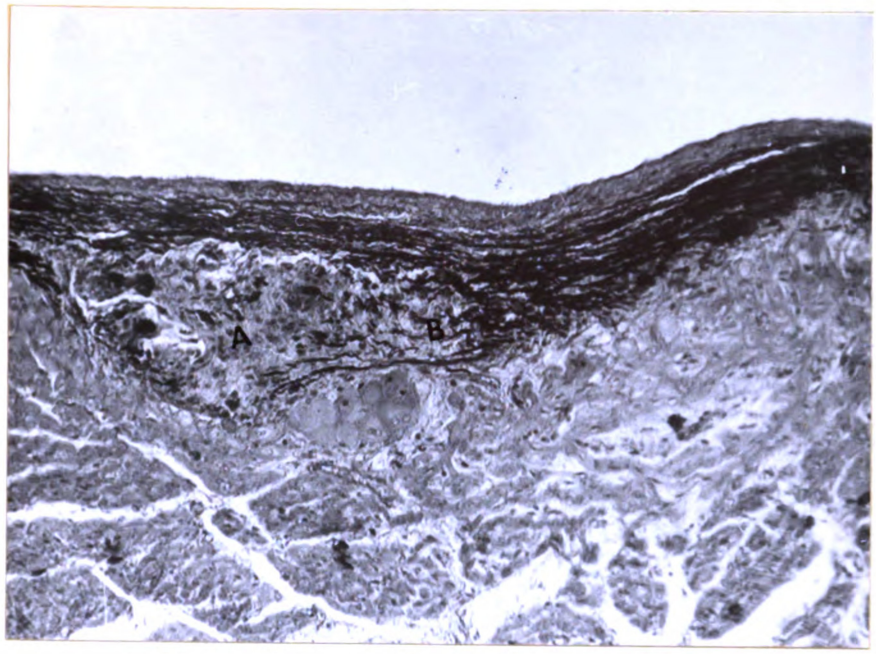

Figure 5 Increased connective tissue (A) and disruption and fragmentation of elastic fibers (B). (MSU-6966 Verh. x140)

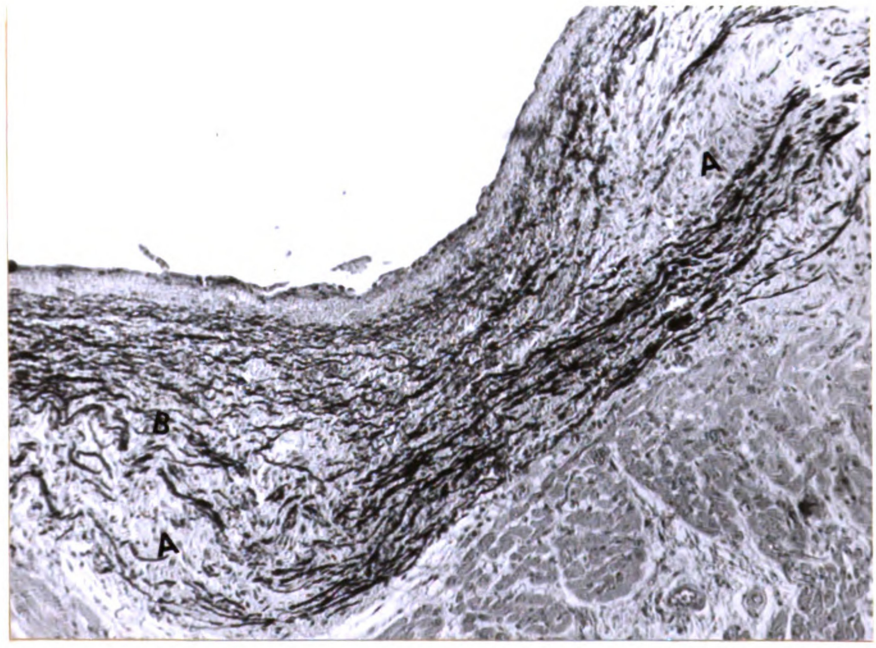

Figure 6 Increased connective tissue (A) and fragmentation of the elastic fibers (B). (MSU-6909 Verh. x140)

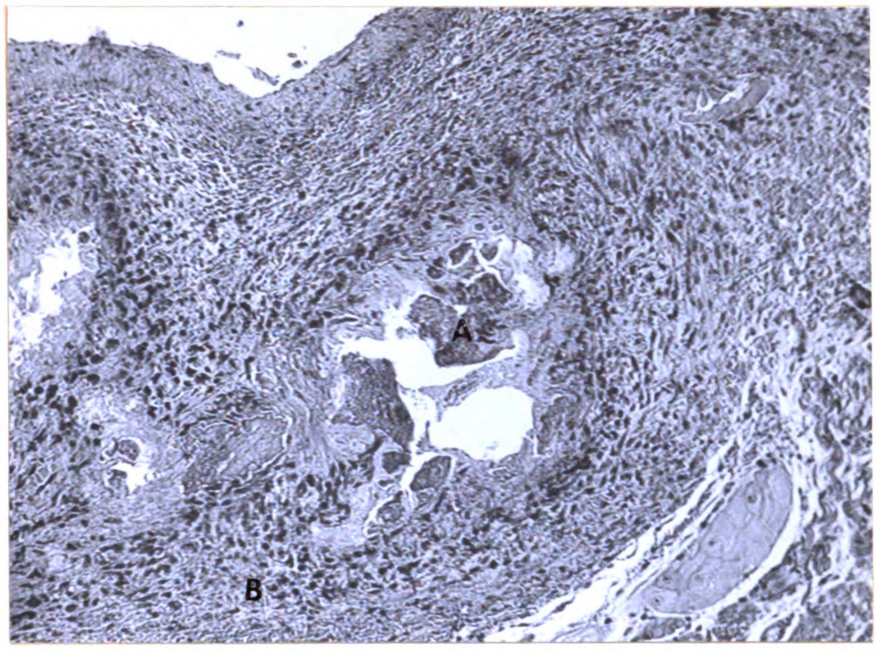

Figure 7 Endocardial plaque with degenerate calcified areas (A), and connective tissue proliferation (B).
(MSU-6909 H&E x140)

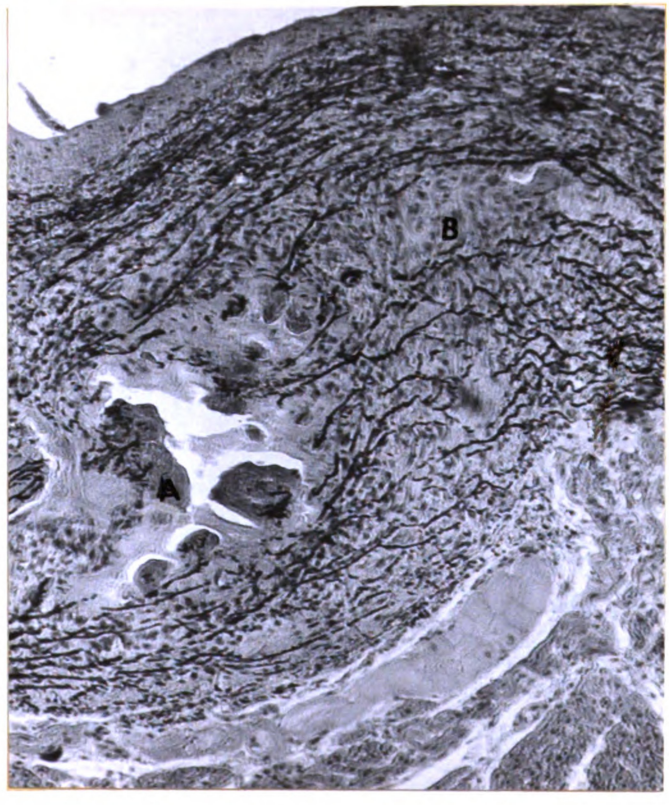

Figure 8 Demonstration of elastic fibers present in necrotic calcified area (A), areas of connective tissue proliferation (B) and fragmentation of elastic fibers (C).
(MSU-6909 Verh. x140)

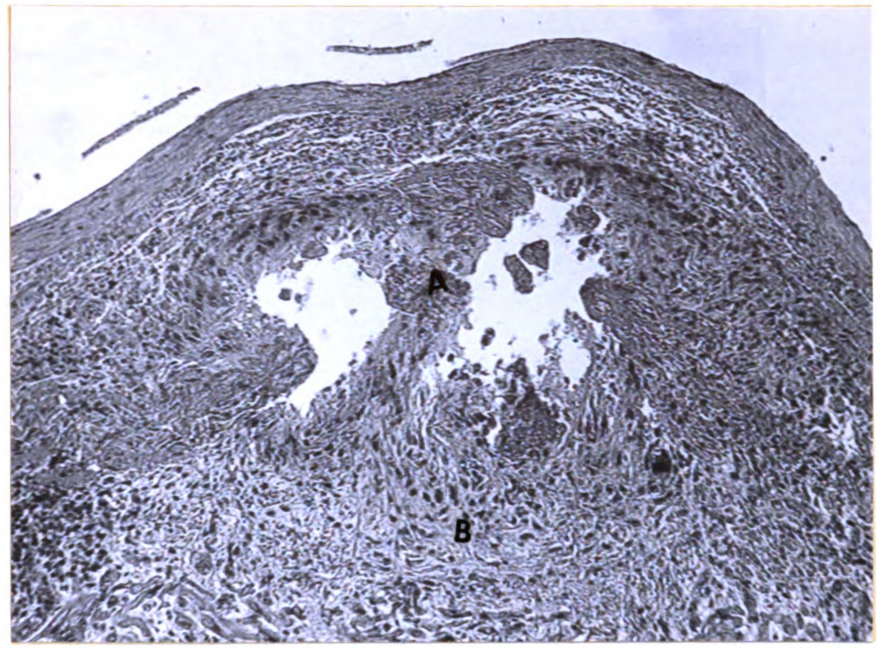

Figure 9 Endocardial plaque with degenerate calcified areas (A), and connective tissue proliferation (B).
(MSU-6909 H&E x140)

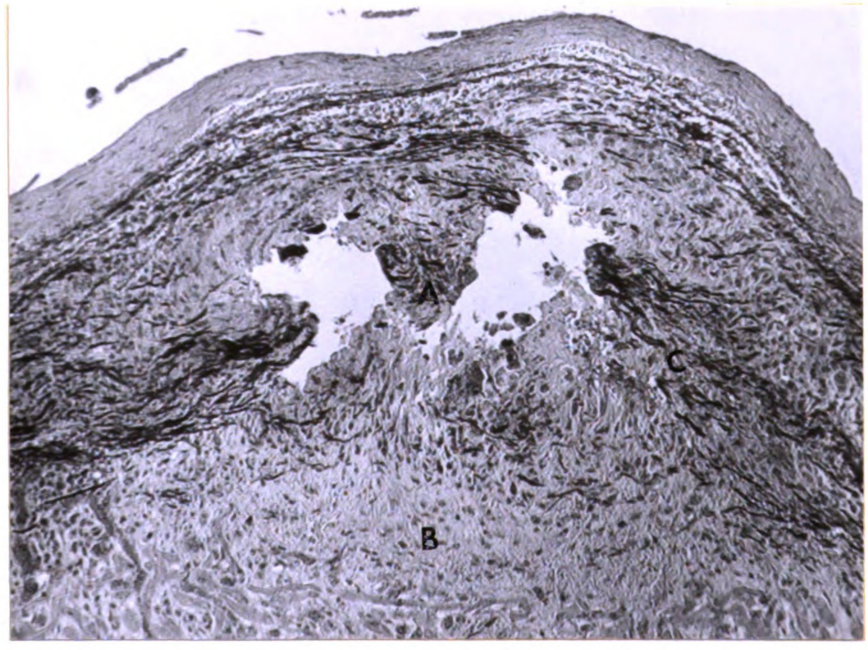

Figure 10 Elastic tissue fragments present in calcified area (A), zone of connective tissue proliferation (B), and fragmentation of the elastic fibers (C).
(MSU-6909 Verh. x140)

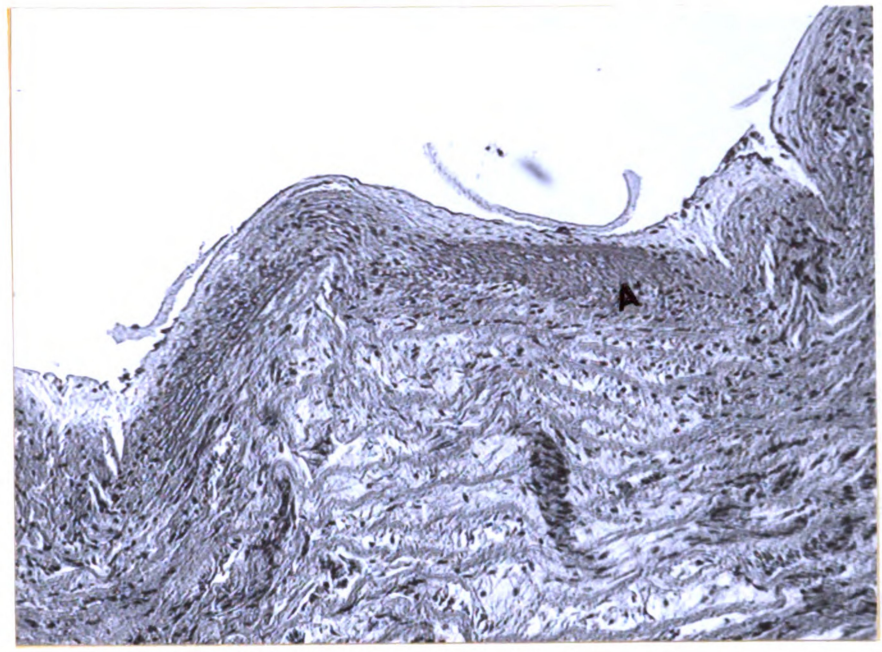

Figure 11 Calcification of sub-intimal elastic fibers (A).
Aorta (MSU-6785 H&E x140)

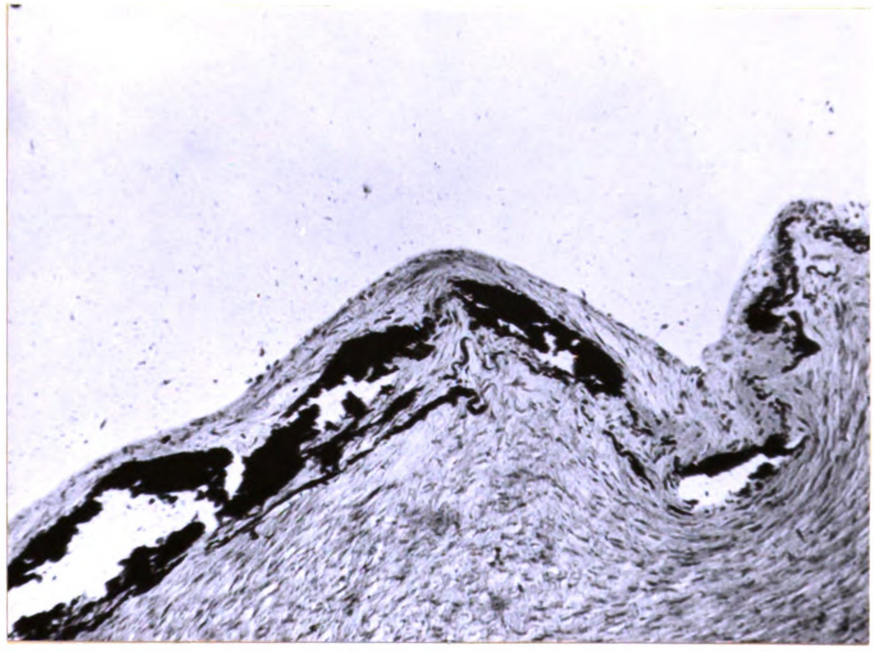

Figure 12 Calcification of sub-intimal elastic tissues.
Aorta (MSU-6785 $AgNO_3$ x140)

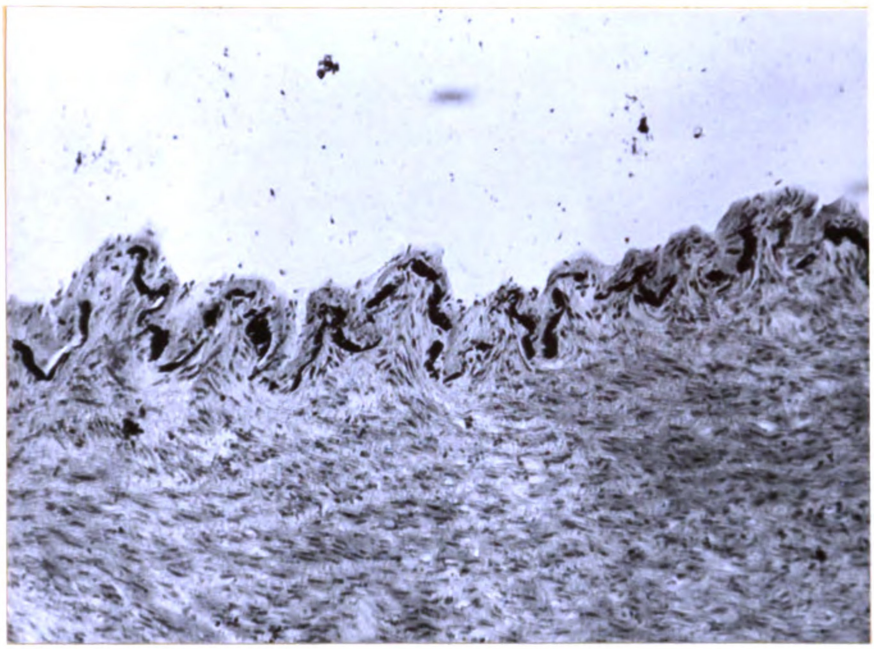

Figure 13 Calcification of subintimal elastic fibers. Aorta (MSU-6785 $AgNO_3$ x140)

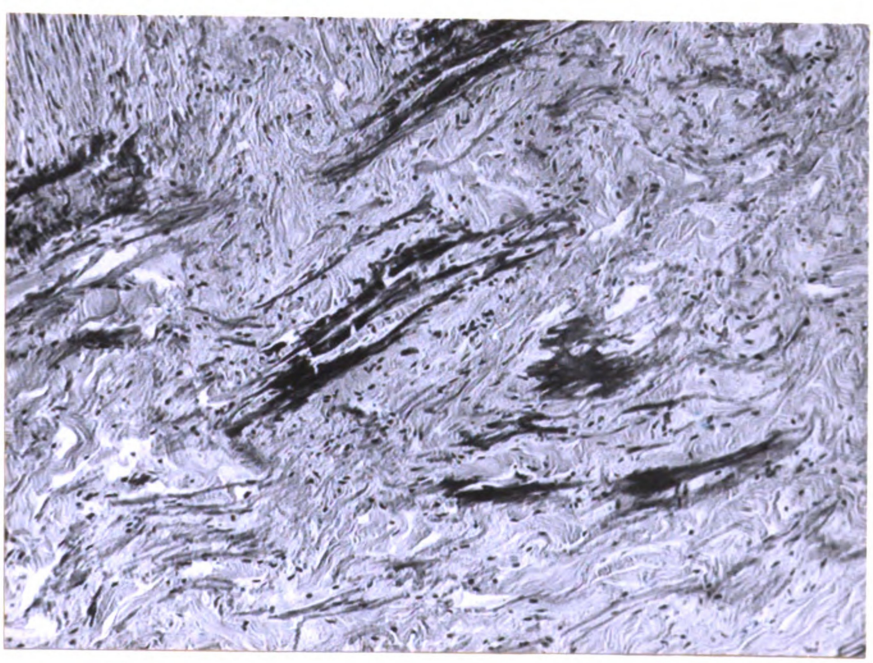

Figure 14 Calcification of elastic fibers in the media of the Aorta.
(MSU-6785 H&E x140)

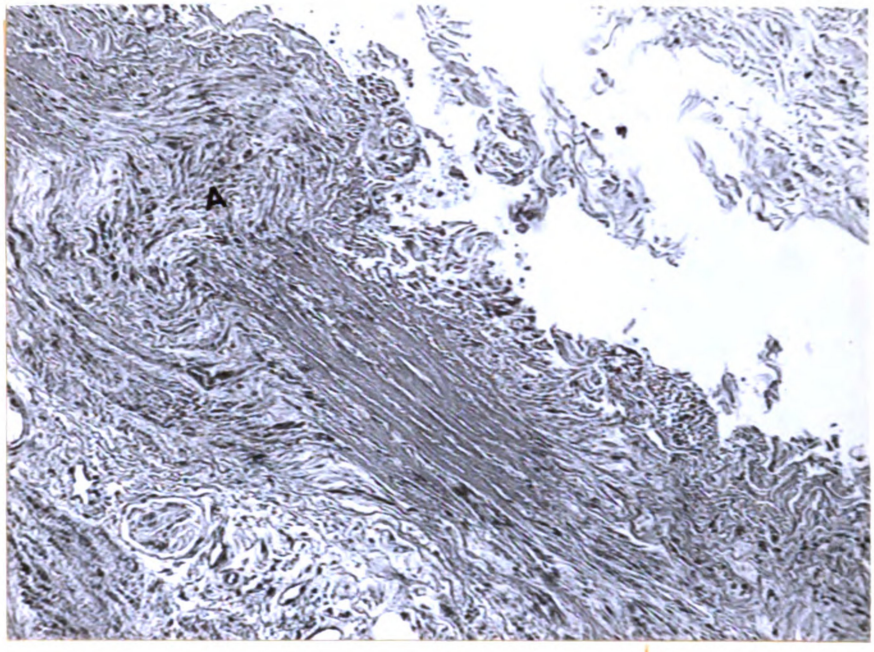

Figure 15 Calcification of adventitial elastic fibers with disruption of the non-calcified fibers (A).
(MSU-6785 H&E x140)

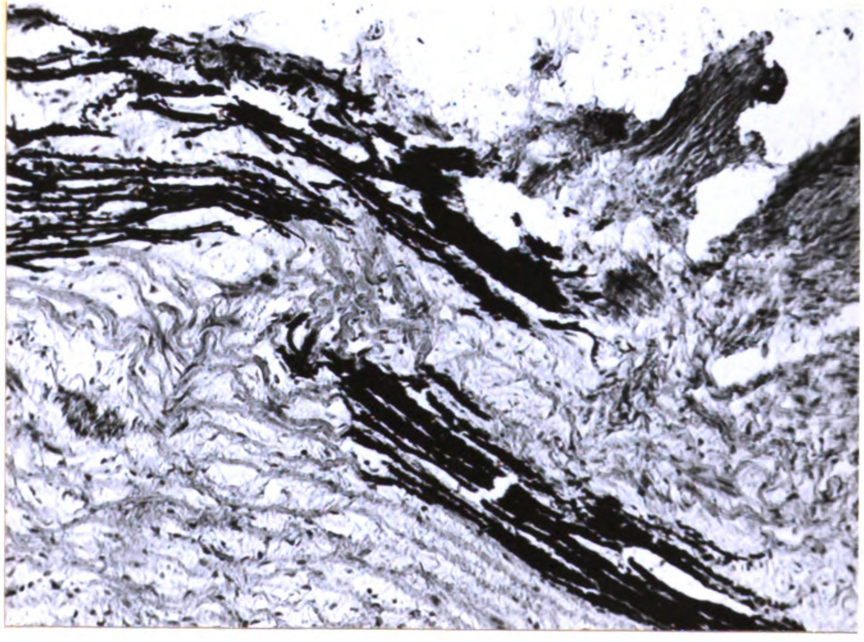

Figure 16 Calcification of the adventitial elastic fibers.
(MSU-6785 $AgNO_3$ x560)

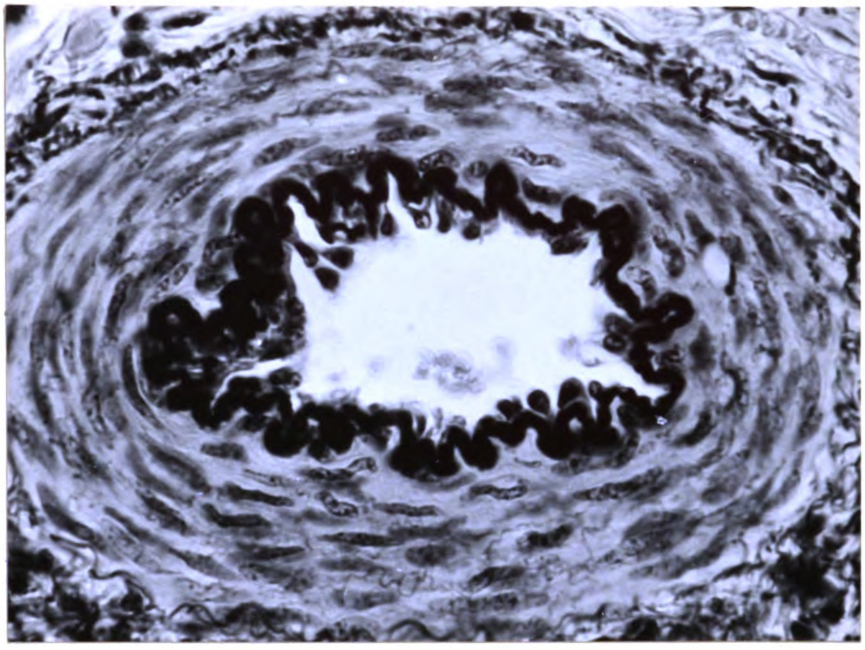

Figure 17 Normal vessel illustrating the staining character of the internal elastic membrane. (MSU-6944 Verh. x700)

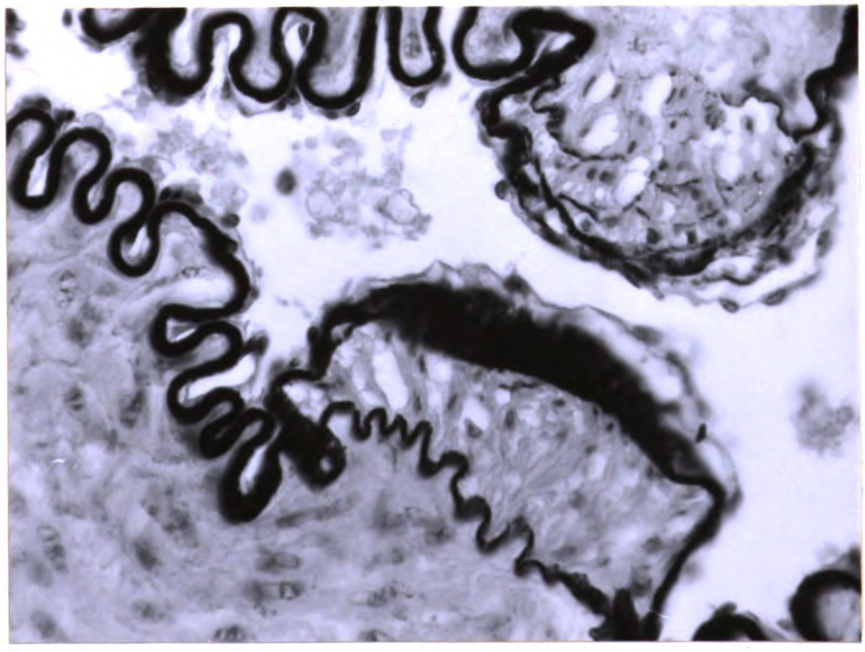

Figure 18 Renal vessel with fragmentation of the internal elastic membrane. (MSU-6909 Verh. x560)

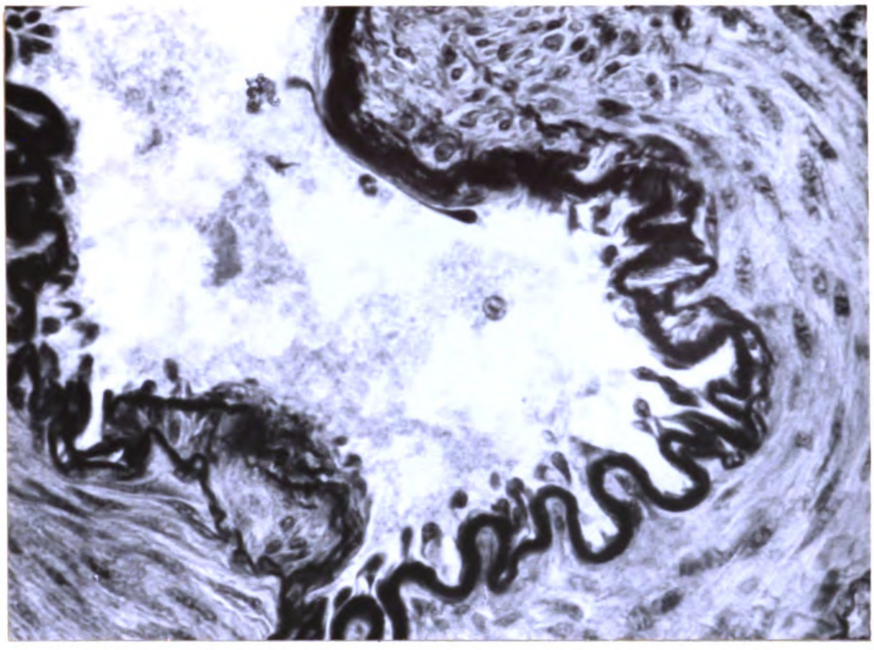

Figure 19 Calcification and fragmentation of the internal elastic membrane in a small renal vessel.
(MSU-6966 Verh. x700)

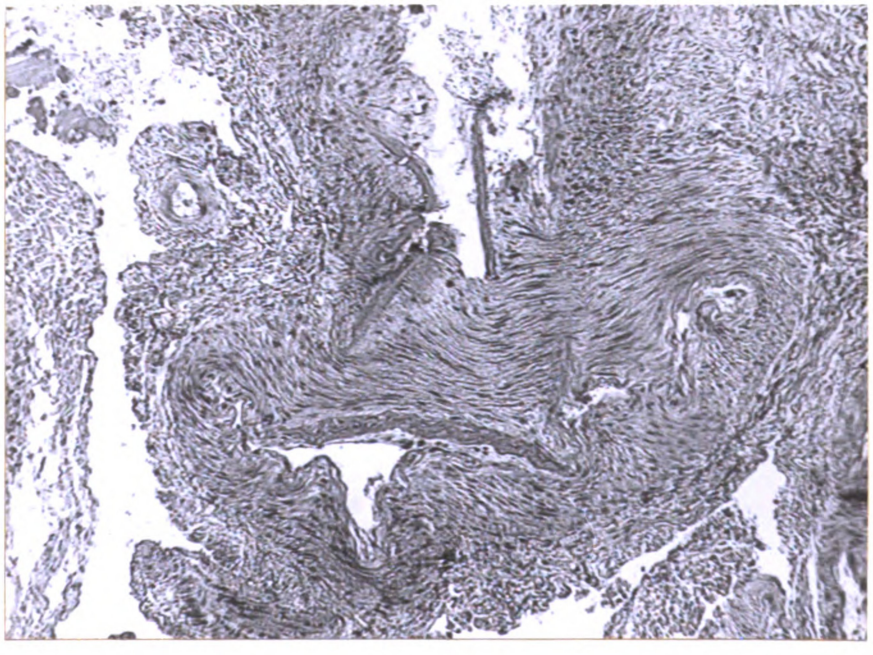

Figure 20 Calcification of internal elastic membrane present in small arteries at the base of the pituitary.
(MSU-7042 H&E x140)

Figure 21 Calcification of the internal elastic membrane in a coronary artery.
(MSU-6320 H&E x140)

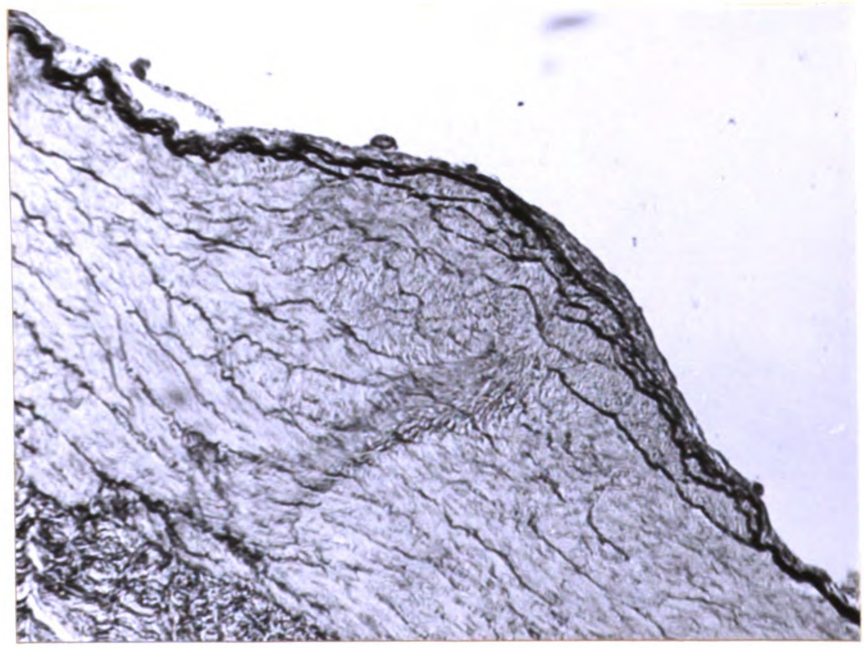

Figure 22 Disruption of the elastic fibers in a coronary artery.
(MSU-6909 Verh. x140)

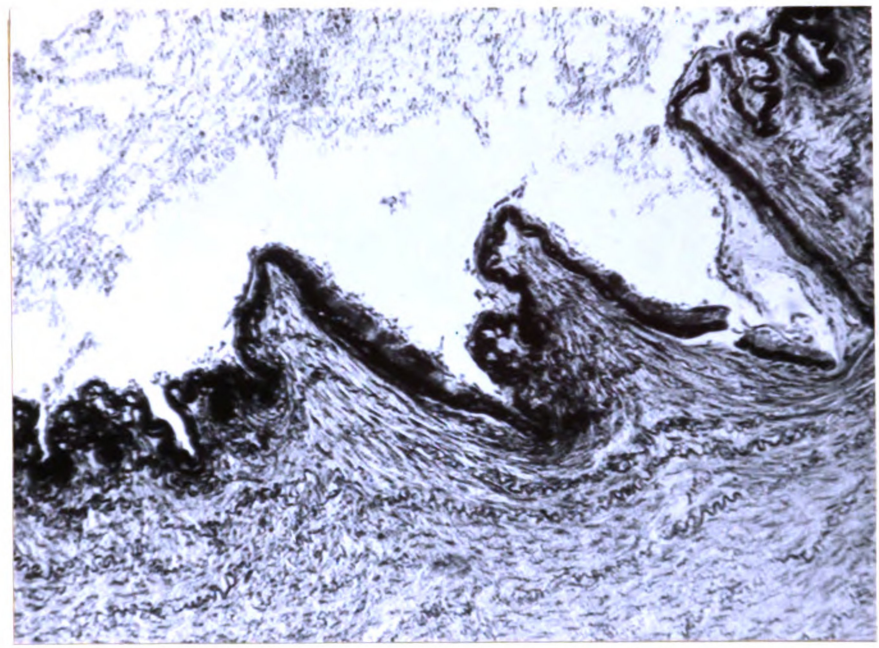

Figure 23 Calcification and fragmentation of elastic fibers in the carotid artery. (MSU-6365 Verh. x140)

Figure 24 Disruption and calcification of elastic tissues in the carotid artery. (MSU-6365 Verh. x140)

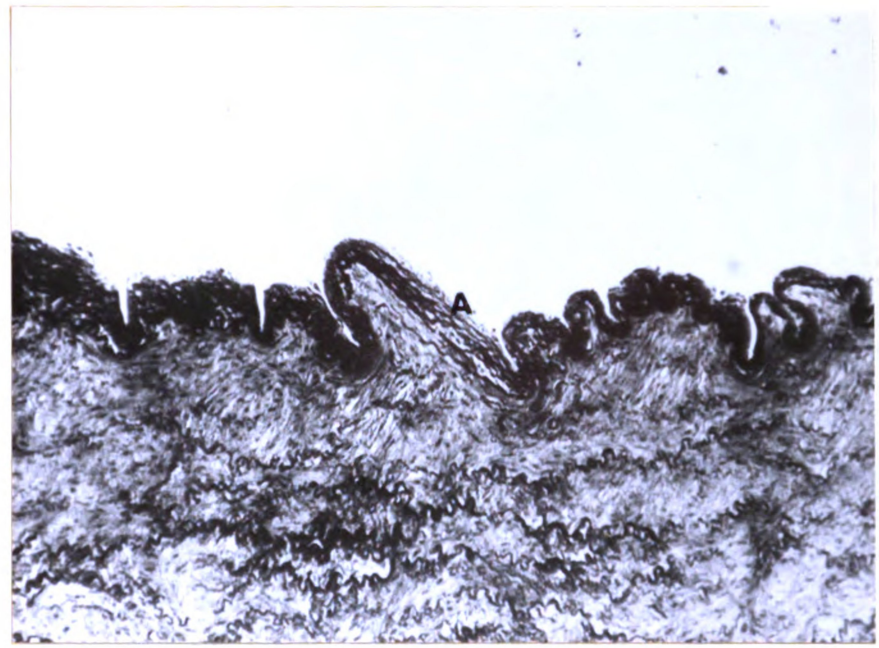

Figure 25 Calcification and fragmentation of elastic fibers (A) in the renal artery. (MSU-6320 Verh. x140)

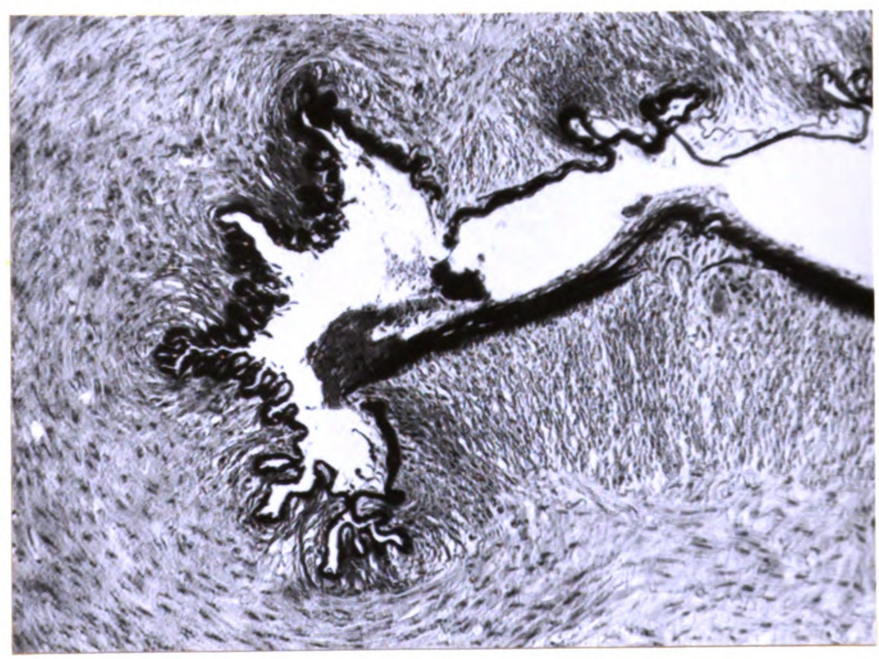

Figure 26 Calcification and fragmentation of elastic fibers. Thrombus formation (A) may indicate intimal injury. (MSU-6785 Verh. x140)

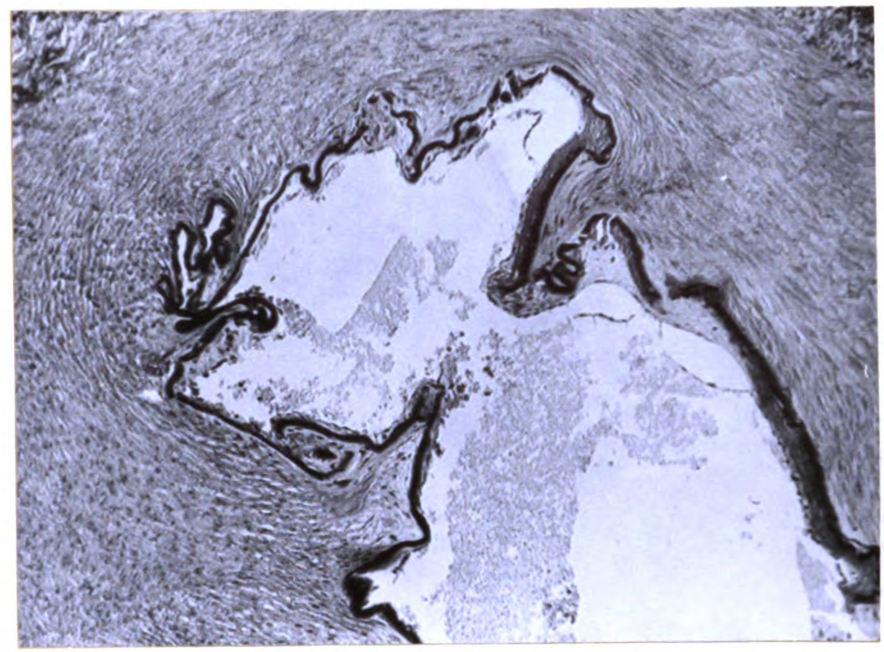

Figure 27 Calcification of internal elastic membrane with distortion of the intimal surface. (MSU-6785 Verh. x140)

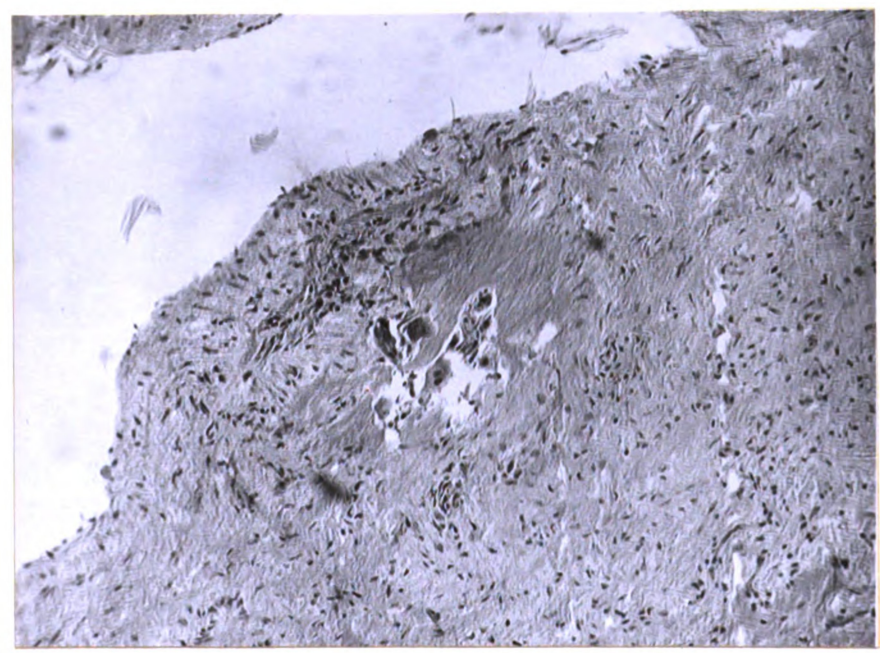

Figure 28 Calcified plaque in the sub-intimal tissues of the jugular vein. (MSU-6785 H&E x140)

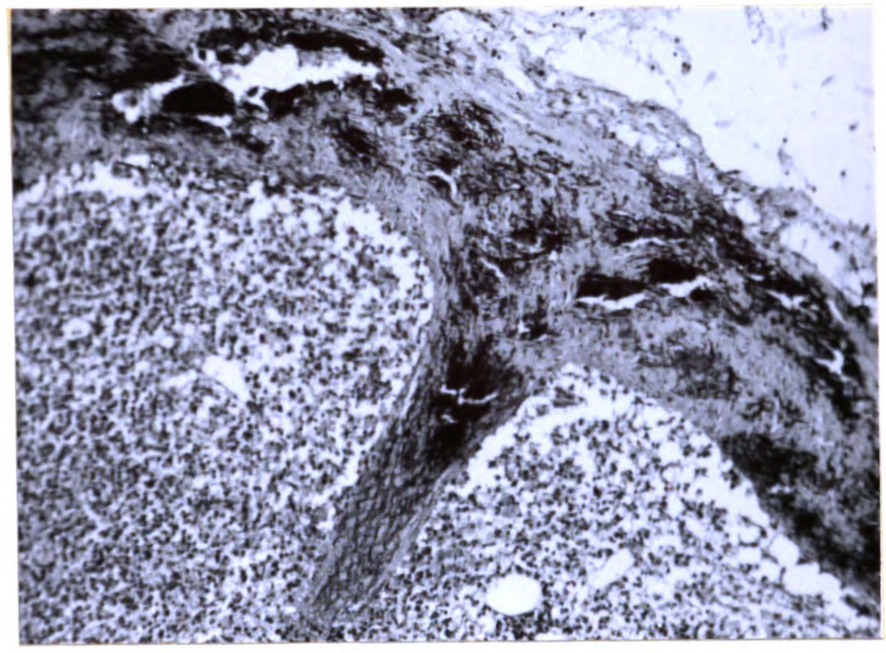

Figure 29 Calcification in the capsule and trabeculus of the spleen.
(MSU-6365 $AgNO_3$ x140)

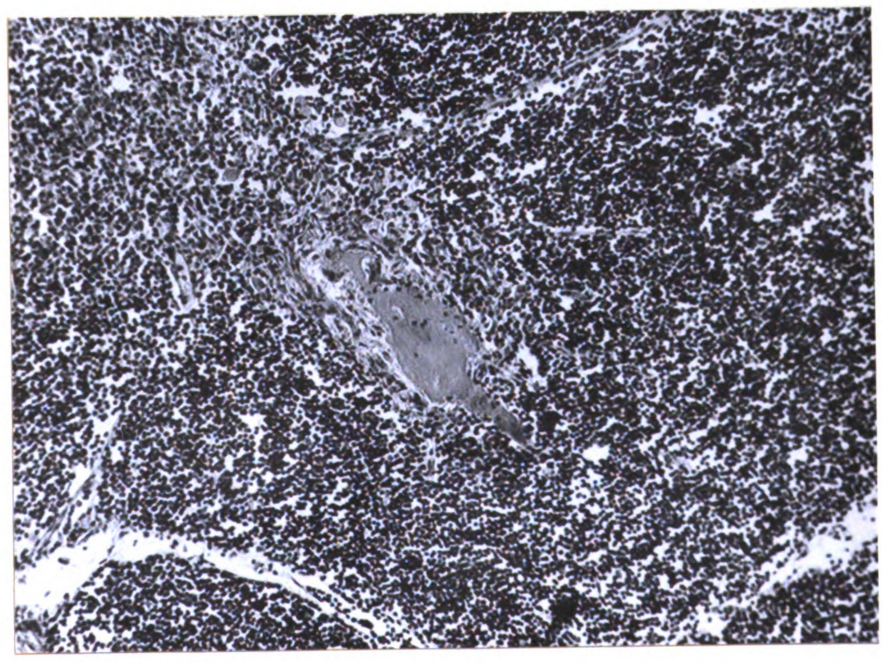

Figure 30 Large calcified body observed in the thymus.
(MSU-6785 H&E x140)

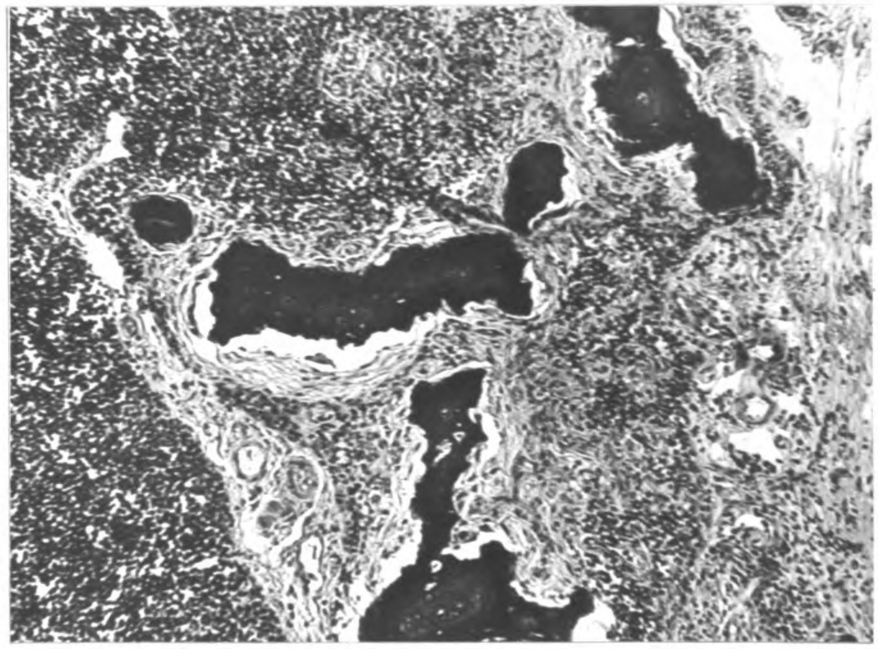

Figure 31 Large bone-like bodies in thymic tissue surrounded by rings of fibrous connective tissue. Nuclear remnants and concentric ring formation present.
(MSU-7042 H&E x140)

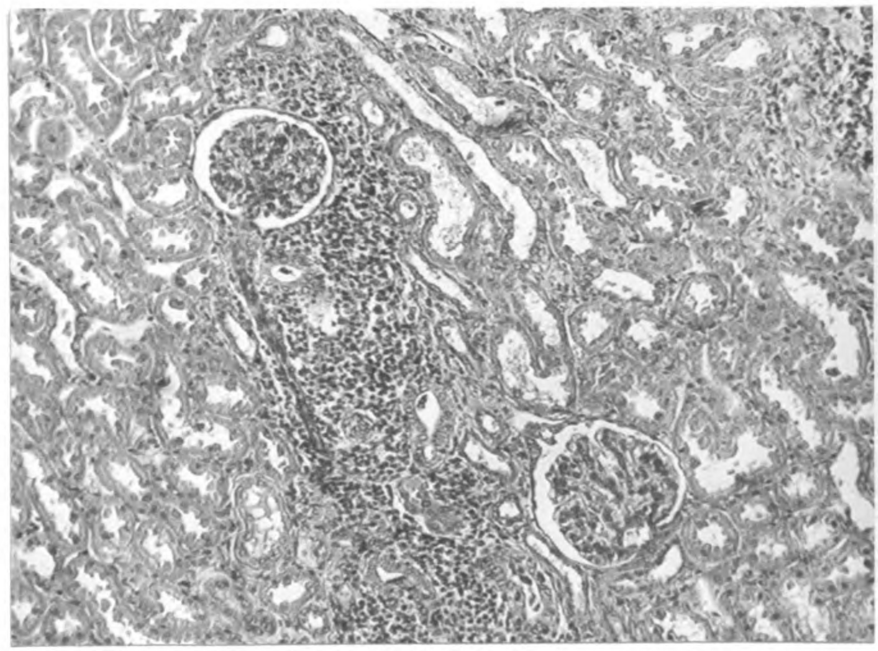

Figure 32 Infiltration of lymphocytes in the interstitial tissue of the renal cortex.
(MSU-6790 H&E x140)

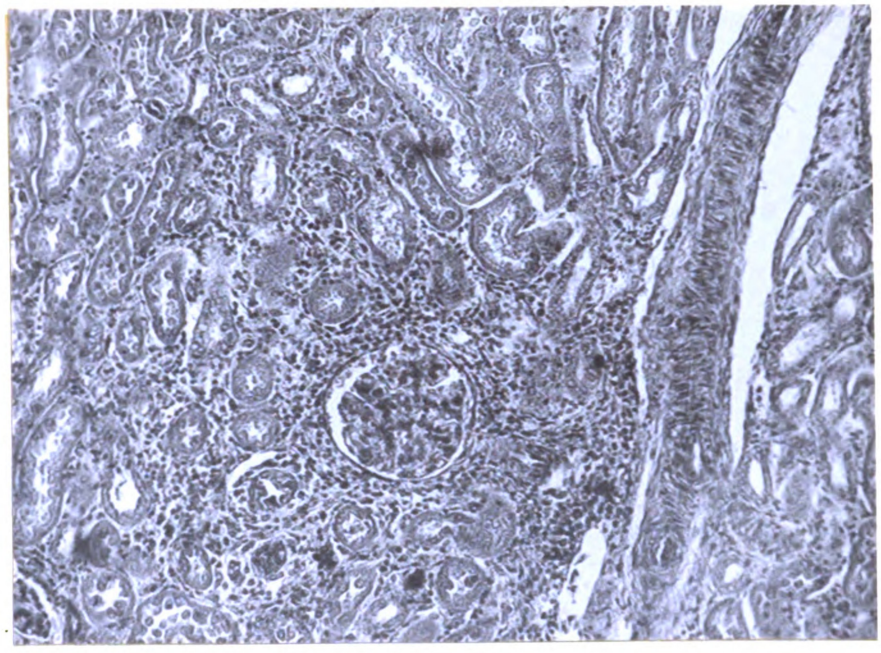

Figure 33 Infiltration of lymphocytes in the interstitial tissues of the renal cortex.
(MSU-6785 H&E x140)

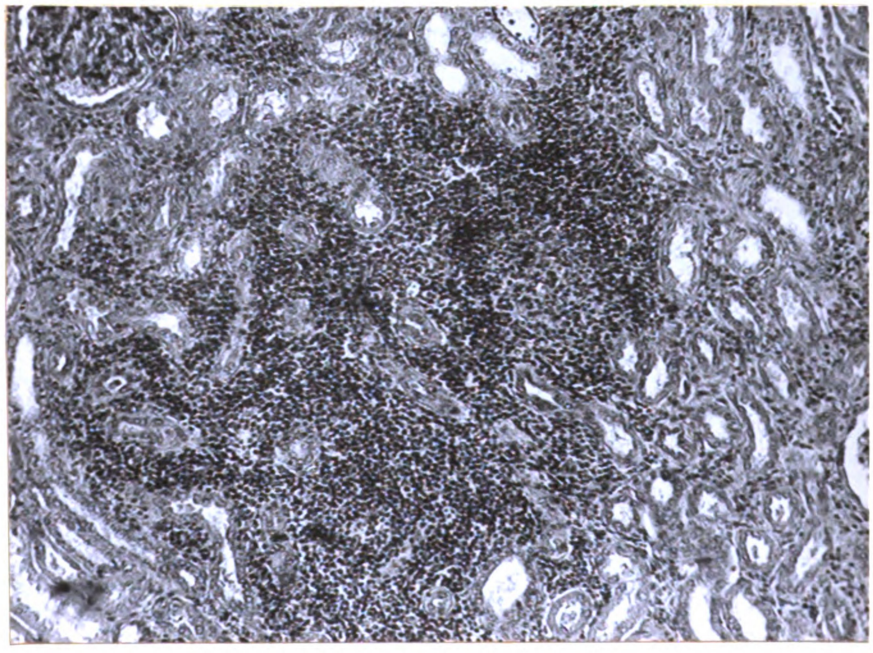

Figure 34 Disruption, atrophy and disappearance of the tubules caused by lymphocytic infiltration.
(MSU-6342 H&E x140)

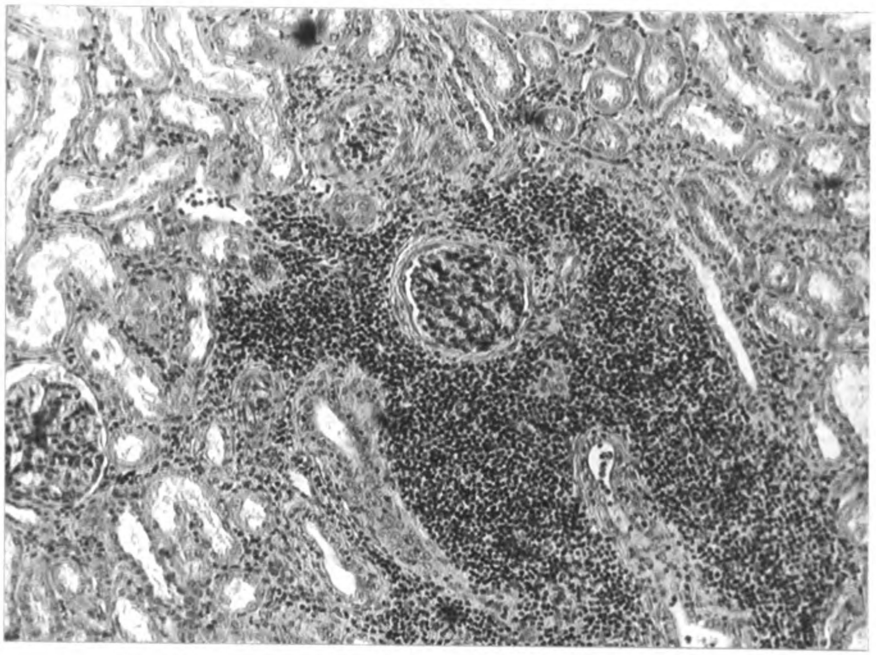

Figure 35 Large foci of lymphocytes causing tubular atrophy and disappearance. Concentric rings of fibrous tissue surround Bowman's capsule.
(MSU-6342 H&E x140)

V. DISCUSSION

The average serum calcium and phosphorous levels in the four groups revealed no apparent fluctuations as the serum magnesium was decreased. All values were within the levels defined as normal (Chart I). Similar relationships have been reported by other investigators (4, 10, 14, 24).

The nature of the lesions made it difficult to accurately define the extent of involvement in each animal. There did appear to be a correlation between the prolonged low serum magnesium level and the number of lesions. The most extensive involvement was noted in those animals with the lowest serum magnesium levels.

The lesions in this study were similar to those described by Moore, Hallman, and Sholl (20, 21) and Langham (18). However, degenerative processes involving skeletal muscle tissue and the Purkinje fibers which they describe, were not found. Correlations between the serum magnesium levels and the lesions observed were not defined by these workers.

The animals reported in this study had been subjected to the addition of many supplements to the basic milk diet. These were in the form of minerals, plant materials, and vitamins. They were added to study the effects of dietary constituents on the serum magnesium level. Due to the wide varation in these constituents and the duration each was fed, it was impossible to draw any valid conclusions concerning the influence of any one particular substance on the lesions.

In summarizing their findings, Blaxter and Rook suggested that the lesions described by Moore, Hallman and Sholl were the result of factors other than nagnesium deficiency (2). The syndrome produced by the English workers was one involving extreme depression of the serum magnesium level to approximately .5 to .7 milligrams per cent. It was accompanied by an increasing excitability developing into convulsions, tetany, and a high mortality.

Further studies with the rat and dog demonstrated the development of metabolic upsets thought to be due to the deficiency of magnesium (16). It was noted that the few animals recovering from the initial tetanic phase gradually began losing weight, the composition of their blood lipids changed, increased amounts of calcium was lost, and the urinry nonprotein nitrogen increased. Administration of magnesium eliminated these symptoms and the animals returned to normal. Kruse et al. also noted that older animals did not develop signs of deficiency as rapidly because of the skeletal magnesium reserves which they possessed. Animals on diets containing small amounts of magnesium gradually developed signs of metabolic distrubance but did not develop the tetanic phase.

The data resulting from controlled work with rats and dogs seemed to offer some basis for explaining the conflicting results mentioned by Blaxter and Rook.

Their calves were placed on a diet supposedly lacking

only magnesium. The resultant rapid hypomagnesmia and tetanic syndrome paralleled that seen in rats on similar diets. Moore's calves were on diets deficient, but not totally lacking, in magnesium. These calves were usually one to two months older than Blaxter's before the low level of magnesium was produced. The serum level did not drop to that obtained by Blaxter and Rook.

It appeared that most of Moore's calves were receiving enough magnesium to prevent the tetanic syndrome. Difficulty arises in placing this amount in its proper relationship with the maintainence of metabolic processes. Perhaps more time was required to develop evidence of metabolic failure. Unfortunately the post mortem reports available for the calves in this study made little, if any, mention of the apparent physical condition of these animals. Without such data it is impossible to draw any further parallel between the syndrome of magnesium deficiency as it is observed in rats and calves.

In evaluating the presence of endocardial plaques observed in one animal of the control group, the concurrent uremia must be considered. This animal had an extensive interstitial nephritis. Some upset in calcium metabolism as a result of the nephritis may be responsible for the calcified areas in the heart (26).

The significance of the kidney lesions is difficult to evaluate. Many of the calves were, or had been, afflicted with a diarrhea or pneumonia. Edleman states that lesions

similar to those described as white foci were either fetal remnants or of toxic origin (7). Phenniger described lesions in the calf kidney composed of focal accumulations of fibroblasts and lymphocytes. He stated that B. coli was isolated from them in high percentages. These calves had suffered from a diarrhea known as white scours (23). Langham described similar lesions occurring in calves studied by Moore. He suggested the possibility of a toxic factor or possible anoxic changes resulting from the vascular lesions associated with magnesium deficiency. He described more extensive development of interstitial nephritis with loss of tubular function. No mention of health or concurrent diseases was made (18). The recent recognition of the disease leptospirosis, must also be considered in the evaluation of these lesions.

Perhaps newer techniques will be developed which will allow more critical study of lesions such as those described in the kidney.

VI. SUMMARY AND CONCLUSIONS

A microscopic study was made of tissues collected from thirty-five calves in which a low serum magnesium level had been produced.

Calcification of the endocardial elastic fibers and the internal elastic membrane of blood vessels was observed. In some cases the cardiac lesions were accompanied by adjacent fibroblastic proliferation; this frequently resulted in the formation of elevated plaques. Calcified areas were also noted in the capsule of the spleen, in the thymus, and diaphragm.

An increase in frequency of these lesions was observed as the serum magnesium level was decreased below the normal limits.

Kidney lesions consisting of focal interstitial nephritis were observed in many animals. Previous history of diarrhea or pneumonia was common in these cases.

The effects of stress and concurrent diseases must be considered when evaluating these renal lesions.

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