INFLUENCE OF DIETARY MAGNESIUM ON CARDIAC AND RENAL LESIONS OF YOUNG RATS FED AN ATHEROGENIC DIET*

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Plates 93 to 95

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Evidence has been presented that the feeding of an atherogenic diet to weanling male rats increased the requirement for magnesium and that the atherosclerosis produced was made less severe or was delayed by the administration of large amounts of dietary magnesium (1).

The present communication describes the morphology of the renal lesions found in rats fed the atherogenic diet (1). These lesions are consistent with magnesium deficiency despite the fact that the diets contained magnesium in excess of the amount for normal diets. The lesions of the heart, the aorta, and the liver are also described.

Magnesium deficiency in animals, especially in rats, has been the subject of investigation since before 1932 (2–10). The histologic emphasis has been upon the renal lesions: these included calcific cast deposition in the corticomedullary zone extending into the pyramids and cortex, and renal epithelial degeneration and regeneration. Tubular dilation and finally hydronephrosis developed. Myocardial degeneration with fibrosis has been described (9) through lesions of atherosclerosis were not mentioned. The lesions of magnesium deficiency in calves included calcification of the yellow elastic fibers of the aorta and calcification in a few hearts (10).

The possibility that metals may be implicated in atherosclerosis has aroused only limited interest and research activity. The concentrations of calcium and magnesium were found to be increased in atheromatous rabbit aortas (11) and with progressing age and atherosclerosis were also increased in the human aorta (12, 13). Dietary vanadium reduced the aortic cholesterol of cholesterol-fed rabbits, and vanadium

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iron, chromium, and manganese affected significantly rat hepatic cholesterol metabolism (14, 15).

The present study establishes more than just an association of atherosclerosis with a change in metal concentration; it implicates a relative deficiency of a metal, magnesium, in the pathogenesis of atherosclerosis experimentally produced in young rats. Further, it shows that when this deficiency, as evaluated by the magnesium content of the serum and by renal lesions, is overcome by means of additional amounts of magnesium in the diet, the development of the lesions is retarded.

Material and Methods

Weanling male rats (50 to 55 gm.) were fed an atherogenic diet containing 1 per cent cholesterol, 0.3 per cent cholic acid, and 20 per cent fat (Spry), and casein at 10 to 20 per cent. The composition of the diet and other pertinent details have been described (1). Briefly, the dietary group divisions were the following:

Group I —10 per cent protein
Group II —10 " " " and 2 mg. per cent thryoxine
Group III—20 " " "
Group IV—20 " " " and 2 mg. per cent thyroxine

Each of the above groups was divided into subgroups by varying the percentage of dietary magnesium as follows:

Subgroup A— 24 mg. per cent magnesium
Subgroup B— 48 " " " "
Subgroup C—192 " " " "
Subgroup D—384 " " " "
Subgroup E—768 " " " "

Autopsies were performed at 24 to 26 days on 6 animals per subgroup for a total of 120 animals. No more than 10 per cent of the rats in any subgroup died prior to the completion of the experiment. These are not included in this report. Fixation of the tissues was in formaldehyde solution U.S.P. (1:10). Paraffin sections were stained with hematoxylin and eosin. The left ventricle and aorta were opened, fixed in formalin, and stained with Sudan IV solution. Then each aortic cusp, the endocardium below the aortic cusps, the mitral leaflets, and the aorta were examined under the dissecting microscope at magnification of 10. The sudanophilia of the internal aspect of each of these was judged on a 0 to 4 plus scale. These various estimates were then totalled. The sudanophilia of the heart and aorta, or "heart score," of rats not on an atherogenic diet was zero.

RESULTS

The Effect of Varying Dietary Magnesium, Protein, and Thyroxine on the Kidneys.— The kidneys showed tubular calcium deposition in the zona intermedia, the outer portion of the medulla. This deposition was lessened by increasing the percentages of magnesium, protein, or thyroxine, either singly or in combination, in the diet (Table I).

In the gross the kidneys appeared relatively enlarged and in some cases pale. There was a suggestion of gray-white striation in the outer zona intermedia in sagittal sections of kidneys in subgroups I A, I B, and III A (low magnesium diets).

Microscopic sections, one from each animal, revealed the most marked changes to be in the zona intermedia, especially in its outer third, with deposition of calcium in tubular lumens (Fig. 1). Epithelial damage was absent in those showing minimal calcium deposition. Beyond this minimal stage, the epithelium was flat to absent. Epithelial hyperplasia was rare, but giant cells were sometimes seen close to or surrounding calcium deposits, either laminated or disrupted (Fig. 2). Only in subgroups I A, III A and III B was there a significant, although still relatively slight, infiltrate of neutrophils and eosinophils near these deposits, sometimes extending well into the nearby interstitial tissue. Only in the last two subgroups mentioned above was there, in addition, extensive tubular calcium deposition in the cortex of some animals. Sudan IV stain of the kidney rarely showed fat globules in the tubular epithelium of the

TABLE I

Effect of Dietary Magnesium, Protein, and Thyroxine on Microscopic Calcific Deposition in the Kidneys (on a scale of 0 to 4+) after 24 to 26 Days

Subgroup*	Dietary magnesium	Average kidney score			
		Group			
		10% protein diet		20% protein diet	
		I	II‡	III	IV‡
	mg. per cent				
A	24	4	0–1	3	0
B	48	3	0	1	0
C	192	1	0	0	0
D	384	0	0	0	0
E	768	0	0	0	0

* 6 rats per subgroup; 120 rats total.

‡ 2 mg. per cent thyroxine added to diet.

In addition, all the diets contained 20 per cent fat, 1 per cent cholesterol, and 0.3 per cent cholic acid.

zona intermedia and the cortex. Within the capillaries of the medulla and cortex, predominantly within the glomerular loops and occasionally in large arteries, there were a few minute sudanophilic droplets, some of which under polarized light contained birefringent crystals in needle form.

In several of the kidneys of subgroups I A, I B, III A, and III B there was a developing internal hydronephrosis; *i.e.*, there was dilatation of tubules in the cortex up to an internal diameter of 160 micra. The epithelium of the dilated tubules was flattened. The lumens were either empty or contained eosinophilic material. The proximal convoluted tubules did not appear affected.

The basalis, the inner portion of the medulla, showed only a few calcium deposits. These were limited to a few severely affected kidneys.

Examination of Table I shows that renal calcification was prevented by increasing the dietary magnesium. For example, in the animals receiving a 10 per cent protein diet, group I, there was 4 plus calcification of the kidneys in the subgroup receiving

24 mg. per cent of dietary magnesium, the scoring being made on the basis of the degree of calcification. The calcification dropped to 3 plus, 1 plus, and zero as the magnesium percentage was increased to 48, 192 and 384 mg., respectively.

Increasing the dietary protein caused a decrease in renal calcification. Animals on the relatively low magnesium diets had less calcification with a 20 per cent than with a 10 per cent protein diet (group III *versus* group I).

The addition of 2 mg. per cent of thyroxine to the diet had a marked protecting effect on the kidneys. Of the animals receiving 10 per cent protein and 24 mg. per cent magnesium, those without thyroxine showed 4 plus calcification, but only a few of those with added thyroxine showed calcification (subgroup I A *versus* II A).

TABLE II

*Effect of Dietary Magnesium, Protein, and Thyroxine on Gross Sudanophilia of Mitral and Aortic Valves and of Aorta after 24 to 26 Days**

Subgroup‡	Dietary magnesium	Average heart score			
		Group			
		10% protein diet		20% protein diet	
		I	II§	III	IV§
	mg. per cent				
A	24	6	1	3	2
B	48	4	2	3	1
C	192	2	2	4	1
D	384	2	1	4	1
E	768	2	2	3	2

* See Material and Methods for scoring of sudanophilia.

‡ 6 rats per subgroup; 120 rats total.

§ 2 mg. per cent thyroxine added to diet.

In addition, all the diets contained 20 per cent fat, 1 per cent cholesterol, and 0.3 per cent cholic acid.

The Effect of Varying Dietary Magnesium, Protein, and Thyroxine on the Heart and Aorta.—Early atherosclerotic lesions of cardiac valves and aorta were present in all subgroups. The extent of the lesions was decreased by adding thyroxine to, or by increasing the magnesium in, the diet. An increase in the percentage of protein caused no consistent change (Table II).

In the gross, the aortic cusps contained most of the heart-aorta sudanophilia (Fig. 3). It was present at the cusp base and/or point of closure. Too few tricuspid valves and pulmonic valves and arteries were examined to allow for detailed statement, though sudanophilia was observed in some. The aortic sudanophilia in this and other experiments involved, especially, the arch near and around the great vessel orifices, additionally, the ascending aorta, and, in only a few cases, the descending aorta.

Sudan IV stain of the few descending aorta lesions showed the initial microscopic lesion to be within the inner half of the media. Here in the interstitial tissue of the media were small globules of neutral fat and irregular birefringent crystals. Adjacent

sections that were Alcian blue-stained showed a probable increase in metachromasia of aortic medial ground substance, although infrequently. The sudanophilia of the ascending aorta was microscopically similar to but more extensive than that just described.

Rarely, Sudan IV stain of the ascending aorta as it merged with the sinus of Valsalva showed a loose fibrillar thickening of the internal aspect in which there were 1 to 8 micra wide sudanophilic globules (Fig. 4). Some, but not all, appeared to be in the cytoplasm of fibroblasts or macrophages. The sudanophilic deposits extended into the inner portion of the media. This appearance is similar to that seen in the early stages of experimental atherosclerosis in older rats. Examination under polarized light showed irregular and needle-like birefringent crystals in some plaques (Fig. 5). Calcium and dense collagen were absent.

The microscopic appearance of the involved aortic cusps was similar to that of the ascending aorta plaques with probably greater involvement of the underlying tissues. When the sudanophilia was at the cusp base and extended over into the nearby endocardium, the internal swelling was more discrete than when elsewhere in the cusp.

A few small coronary arteries deep in the myocardium showed sudanophilic material, some surrounding small irregular birefringent crystals, in the inner half of their walls. A peculiar finding was that of granules of sudanophilic material, 1 to 2 micra wide, in the myocardial interstitial cells of some animals with a high heart-aorta sudanophilia score. The muscle cells were unaffected.

Table II shows that the most marked sudanophilic lesions were in those animals fed the atherogenic diet with low magnesium and low protein; *i.e.*, 10 per cent protein and 24 mg. per cent magnesium. In the 10 per cent protein group, the heart-aorta sudanophilia was reduced by increasing the magnesium content of the diet (group I). In the 20 per cent protein group, an increase in the dietary magnesium had no lowering effect upon the sudanophilia, and the sudanophilia of the subgroups essentially remained constant at a moderate plateau (group III). Hence, at low dietary magnesium levels, the animals fed the 20 per cent protein diet had less sudanophilia than those receiving the 10 per cent protein diet. At high levels of magnesium this did not occur.

The addition of 2 mg. per cent thyroxine to the diet caused a marked lowering of the heart-aorta sudanophilia in both the 10 and 20 per cent protein groups (groups II and IV). Increasing the dietary magnesium had no apparent additive effect on the antiatherogenic effect of thyroxine.

The Effect of Varying Dietary Magnesium, Protein, and Thyroxine on the Liver.— The livers were fatty and no appreciable difference was found between and within the dietary groups.

In the gross the livers were enlarged, yellow-brown, and greasy. They fractured with increased ease and showed no architectural changes.

Microscopically, the cytoplasm of the hepatic cord cells of groups II to IV was fairly eosinophilic and incompletely vacuolated whereas that of group I was clear pale save for a slight eosinophilic reticular pattern. Despite the lack of sharp clear vacuoles of fat in the paraffin sections, Sudan IV stain revealed abundant sudanophilic material within most cord cells. These 2 to 10 micra wide fat vacuoles frequently contained birefringent crystals in a Maltese cross pattern—the crystalline ester cholesterol crystals stressed by Leary (16)—and more frequently in needle form.

With the moderate fat and cholesterol deposition seen in all groups, localization to the Kupffer cells could not be determined. In the portal area there was occasionally an increase of macrophages, some fibroblasts, and rare neutrophils and eosinophils. Focal interstitial collections of macrophages were occasionally seen in the lobules of animals from most of the subgroups.

Signs of Disturbances Manifested by the Animals.—The atherogenic diets produced gross signs of magnesium deficiency. These included hyperexcitability, hyperirritability, hyperemia of the ears and feet and ruffled coats. These signs were completely prevented by the addition of 4 to 8 times the normal magnesium requirement.

DISCUSSION

Rats fed an atherogenic diet developed magnesium deficiency. The renal lesions developed despite the intake of dietary magnesium approximately 8 times that normally considered sufficient for similar rats on a non-atherogenic diet (17). The pathologic observations paralleled the biochemical observations of these animals; the serum magnesium was low in animals consuming atherogenic diets containing normal or only moderately elevated dietary magnesium (1). The renal lesions, however, in the present experiment were so well developed at 24 to 26 days that it seems probable that they developed a fair number of days prior to the end of the experiment. Experiments to determine the time of development of the renal lesions are underway. Furthermore, the animals receiving the thyroxine-supplemented diets had low serum magnesium concentrations (1) but had no renal lesions. This is of great interest and is unexplained.

The data in the present experiment do not explain the mechanism whereby renal lesions were produced, nor can it be assumed that cholestrol and cholic acid alone were the responsible factors. Also unexplained is the mechanism whereby increased dietary magnesium decreases atherogenesis.

It might be conjectured that the atherosclerotic lesions observed in the present experiment are the result of hyperlipemia and hypercholesteremia produced by kidney damage. Heymann and Hackel (18) postulated that the nephrotic kidney (created by injection of rabbit anti-rat kidney serum into rat) produces a "hyperlipemia-inducing" agent. Such rats showed glomerular capillary thrombosis and basement membrane thickening and fibrosis as well as tubular fatty infiltrate (18–20). No calcium casts were mentioned. Greenberg, Lucia, and Tufts described proteinuria and hypoproteinemia in magnesium-deficient rats, but made no mention of blood cholesterol and lipide values (5).

However, the kidneys in the present study do not resemble morphologically the kidneys of rats with lipemic nephrosis. Furthermore, heart-aorta sudanophilic lesions were noted in animals which had no apparent morphologic renal lesions. Finally, atherosclerosis in adult rats has been produced without produc-

tion of such renal lesions (21). It is therefore dubious that the rough relationship between kidney damage and atherogenesis is a causal one.

The sudanophilia of the heart and aorta could be lowered by increasing the dietary magnesium, but regardless of the levels of dietary magnesium, protein, and thyroxine, there was a minimum sudanophilia which was never abolished.

The livers, with Sudan IV stain, showed no particular variation between the groups and subgroups. Also, there was no significant variation between the liver cholesterol concentrations of these groups and subgroups (1). Nevertheless, there was a difference in the sudanophilia of the heart and aorta and in the extent of kidney lesions between the groups and subgroups. Similarly, although all the animals had elevated serum total cholesterol, it must be emphasized that with increasing dietary magnesium there was a lowering of the sudanophilia of the heart and aorta despite a still further rise in serum total cholesterol. Thus, in this experiment neither the morphology nor the cholesterol level of the liver, nor the extent of serum total cholesterol elevation would have served to predict accurately the relative extent of the sudanophilia lesions of the heart and aorta. On the same basis, prediction about kidney lesions would have been equally inaccurate. These points need further elucidation.

The meaning of this study for the human being is not clear. Lowered serum magnesium, which from the data on these rats is associated with increased or accelerated atherosclerosis, is seen in humans in cirrhosis and chronic alcoholism, states (22–24) in which it is believed that there is a decreased degree of atherosclerosis (25–27). It is unlikely, though, that the mechanism whereby patients with chronic alcoholism attain a low serum magnesium is a function of ingesting a diet rich in cholesterol or cholic acid. On the other hand, the experimental effect of increasing dietary magnesium and of adding thyroxine to the diet parallel each other in causing a decrease or delay in both atherosclerosis and renal lesions. Similarly, it is stated that euthyroid and hyperthyroid human patients have less atherosclerosis than do those with hypothyroidism and that young patients with coronary disease frequently have laboratory signs of hypothyroidism. (28, 29).

SUMMARY

Young male rats fed an atherogenic diet for 24 to 26 days developed magnesium deficiency.

The renal lesions, calcium deposition in tubular lumens in the outer zona intermedia, are morphologically consistent with those seen in animals fed magnesium-free diets. It was necessary to feed 8 to 16 times the normal requirement of magnesium to prevent completely these lesions. The limitations in the pathogenesis of these renal lesions are discussed.

The addition of 2 mg. per cent of thyroxine to the diet markedly lowered or

abolished deposition of calcium in the kidney; it also lowered atherogenesis to a minimum but did not abolish it.

Early lesions of atherosclerosis of the cardiac valves and of the aorta were observed. These were more prominent in animals on a 10 per cent than in those on a 20 per cent protein diet. The atherosclerosis was diminished but not abolished by exceedingly high dietary levels of magnesium in the 10 per cent protein group. Such a decrease was not noted in the 20 per cent protein group.

There did not appear to be significant variation in the morphology of the fatty livers in and between the various groups and subgroups as judged by staining with Sudan IV.

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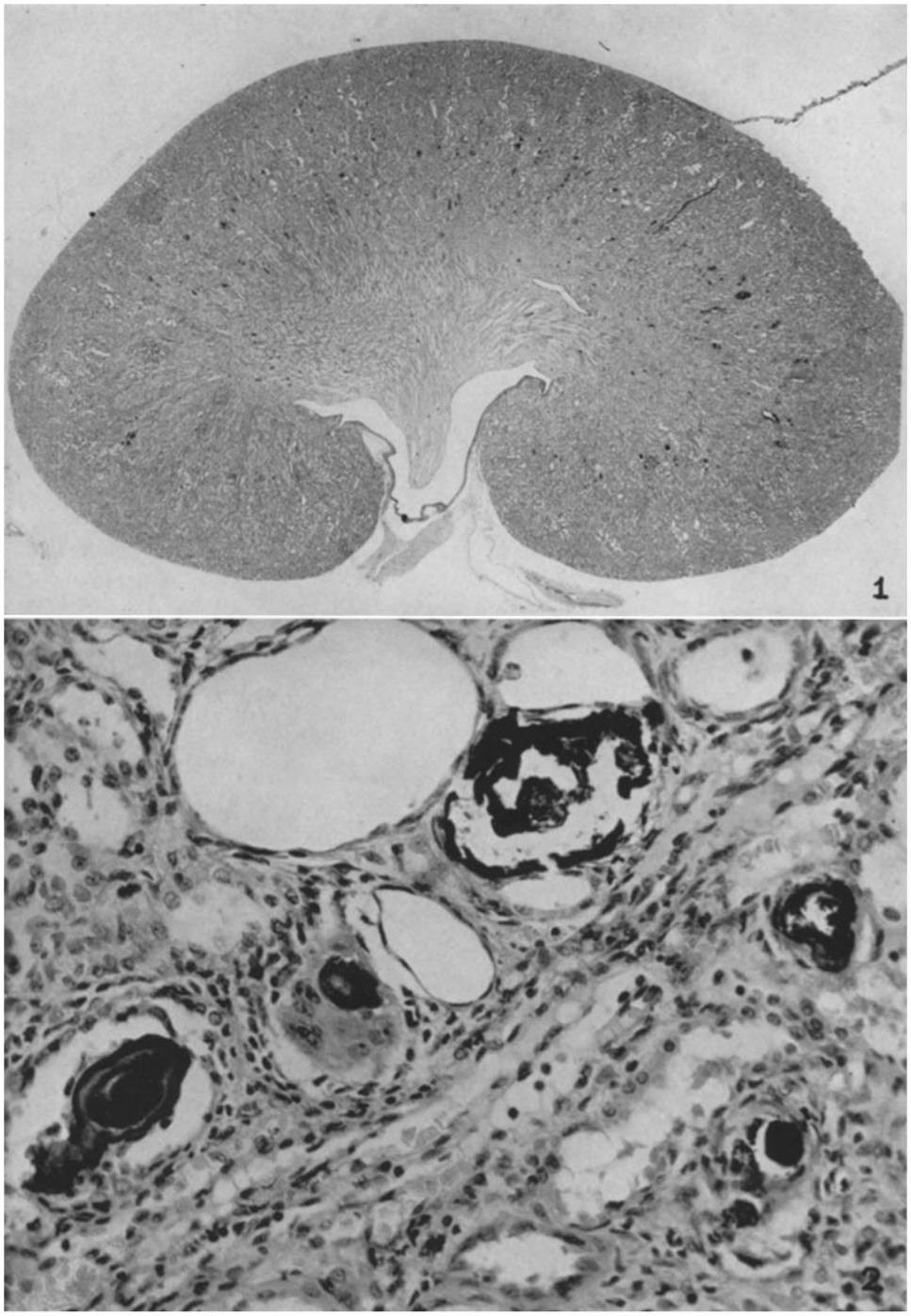
EXPLANATION OF PLATES

PLATE 93

FIG. 1. Kidney. Note calcium deposition (black spots) within the tubules of the outer zona intermedia of the medulla. Within the cortex there is tubular dilatation. This kidney was scored as showing 3+ calcium deposition. Hematoxylin and eosin. × 9.

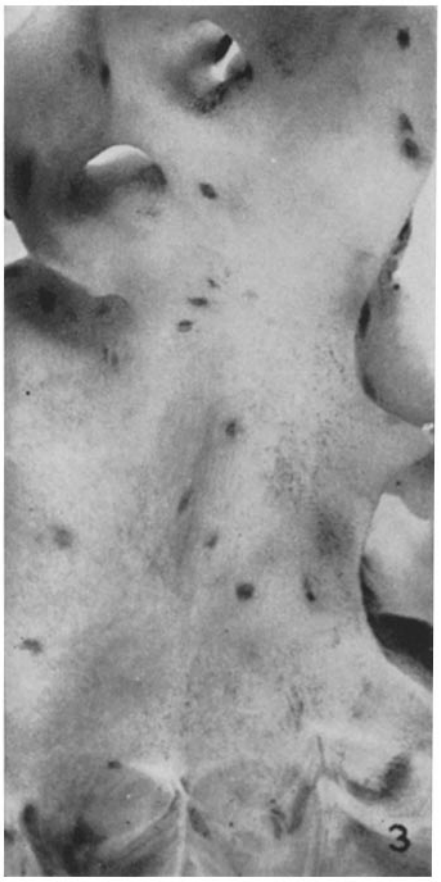
FIG. 2. Kidney. There is calcium deposition with and without giant cell response. Tubular dilatation is also present. Hematoxylin and eosin. × 440.



(Hellerstein *et al.*: Dietary magnesium and atherogenic diet)

PLATE 94

FIG. 3. Aortic cusps and aorta. The left ventricle and aorta have been opened, fixed in formalin, and stained with Sudan IV. Sudanophilic material appears black in the photograph. There is sudanophilia of the aortic cusps and aortic sudanophilia is more prominent than usual. × 15.

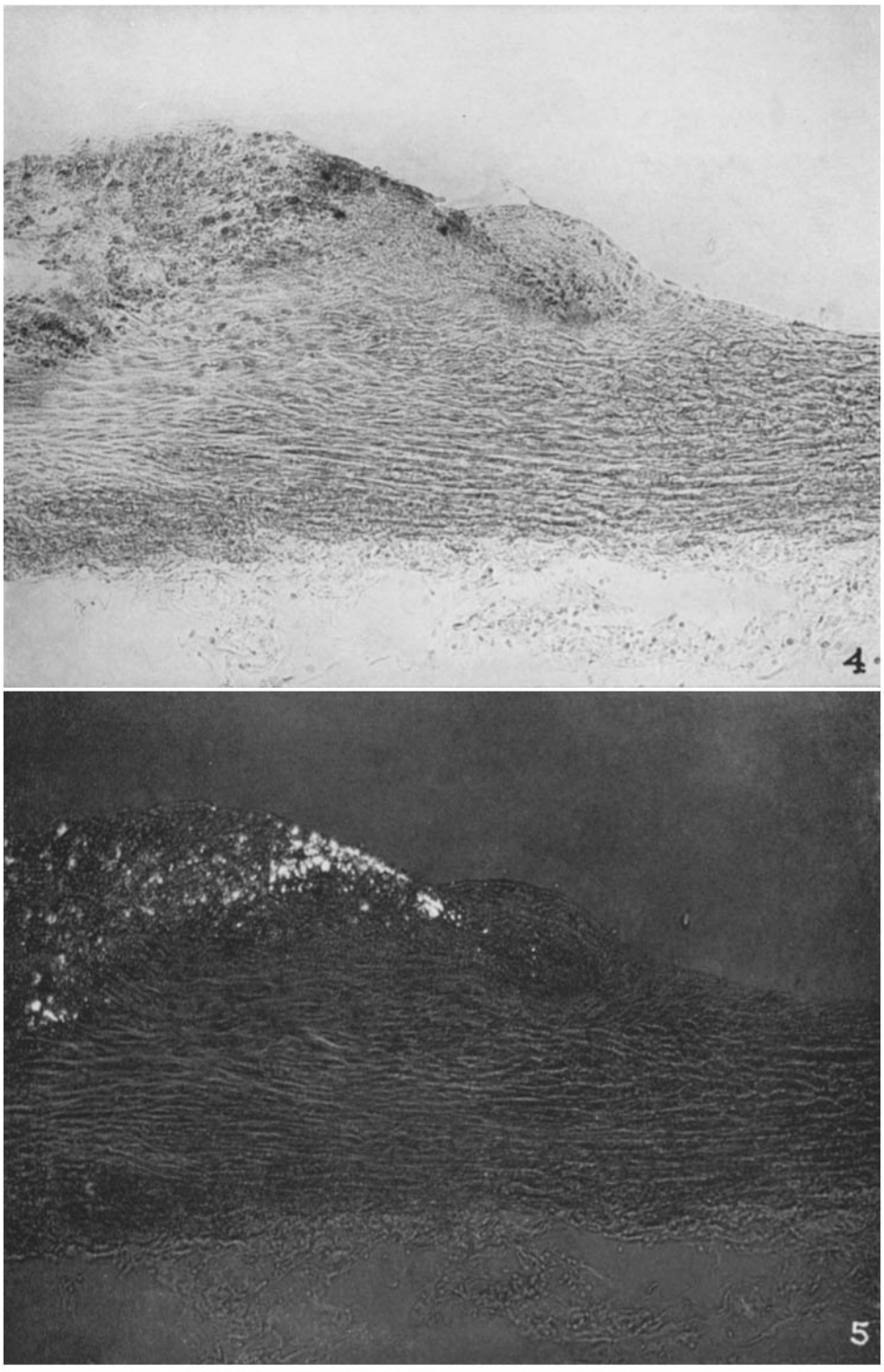


(Hellerstein *et al.*: Dietary magnesium and atherogenic diet)

PLATE 95

FIG. 4. Ascending aorta. The intimal plaque shown is fibrillar and contains sudanophilic globules. The latter are in the inner portion of the underlying media as well. The left edge of the picture is immediately beyond the sinus of Valsalva. Frozen section stained with Sudan IV and hematoxylin. × 220.

FIG. 5. Ascending aorta. The field is identical with that in Fig. 4, but the picture has been taken under polarized light. Birefringent crystals, which appear white in the figure, are extensive in the intimal plaque and some are present in the inner portion of the media on the left. Frozen section stained with Sudan IV and hematoxylin. Polarized light photography. × 220.



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