Pathological Studies on Mineralization in Soft Tissues of Young Calves

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Abstract. Mineralization of soft tissues was investigated in 7 young calves histologically and electron microscopically. It occurred to collagen and elastic fibers of cardiovascular organs. Circumscribed mineral deposition was present in the subendocardial and myocardial stroma and the aortic media. Foreign body reaction was noted around the mineral deposit in the heart. Mineral deposition was also seen in blood capillaries of renal interstice, splenic trabeculae, thymic medulla, and germinal centers of mesenteric lymph nodes.

Calcium and ferric phosphates were detected from mineralized lesions by histochemical analysis and x-ray microanalysis. Acid mucopolysaccharide was also demonstrated histochemically.

Electron microscopy revealed mineral deposit in collagen fibers and crystals aggregated dispersedly or massively in fibrillar extracellular spaces in the heart. Collagen and fibroblasts were present around the aggregated crystals. Mineralization in elastic fibers was recognized in the aortic wall.

Widespread mineralization in soft tissues has been found in hypervitaminosis D [4-7, 9, 10, 12, 13, 15, 16, 19], hypomagnesemia [16, 18], and such enzootic diseases as Manchester wasting disease in Jamaica, Naalehu disease in Hawaii, and enteqe seco in South America [1-3]. Excessive administration of vitamin D [4-7, 9, 10, 12, 13, 15, 16, 19], deficiency of magnesium in feed [16, 18], or a vitamin D-like component of herbage [3] was known as an etiological agent of the respective diseases. The imbalance among calcium, phosphorus and magnesium in the

blood serum level was also recognized in these diseases [3, 4, 6, 16].

The present paper deals with histopathological and electron microscopical studies on the mineralization in soft tissues of young calves.

Materials and Methods

Seven young calves with macroscopically mineralized lesions in the cardiovascular tissue were encountered in the routine meat inspection at the Shiwa Abattoir during a period from May, 1969 to October, 1972. They had manifested no clinical symptoms. They were well nourished Holstein

males 105 days old. They had been fed a commercial mixed diet for white veal, which was mostly composed of skim milk powder and butter milk powder, and enriched with several minerals and vitamins. The vitamin D contained in the diet amounted to 8 international units (I.U.) per gram.

Specimens were collected from various organs of each calf for histological examination. They were fixed in 10% solution of commercial formalin. Paraffin sections were made and stained with hematoxylin and eosin, periodic acid-Schiff, alcian blue, orcein-van Gieson, von Kossa's stain and Prussian blue for histological and histochemical observation.

For electron microscopy, specimens were collected from mineralized lesions of some calves. Ultrathin sections were prepared from them by the routine methods and observed by a Hitachi 125E electron microscope. Some of them were also examined by an EDAX x-ray microanalyser attached to a Hitachi 2B scanning electron microscope.

Results

Macroscopic findings

Heart: The endocardial surface of the left ventricle was diffusely white, with numerous elevations varying from a poppy seed to half a rice grain in size. The elevations were whitish and occasionally circumscribed with a narrow reddish brown area. They were situated beneath the endocardium, being spherical on the cut surface. They were also chalky and hard in consistency, and differentiated readily from the surrounding tissues. Lesional distribution varied from one case to another. A few lesions were scattered on the endocardial surface of the left ventricle in 2 cases. In the remaining cases, numerous lesions were densely distributed around the papillary muscles in the ventricle. The endocardial surface had corrugate streaks formed by their coalescence in these cases (Fig. 1). Several lesions were also present in the aortic and mitral valves and the left atrium in 2 cases.

Blood vessels: Nodular elevations of poppy seed size were scattered on the intimal surface of aortic sinus, aortic arch, and

brachiocephalic trunk in 2 cases. On the cut surface, lesions were present beneath the endothelium. They were chalky and hard in consistency. Furthermore, shallow hollows of rice grain size were dispersedly distributed on the intimal surface of the thoracic aorta in one case. The aortic wall was hard on dissecting.

Microscopic findings

Heart: Lesions of various severity were present in the subendocardial and myocardial stroma and occasionally in the myocardium. Mineral deposition was noted in subendocardial collagen and densely stained with hematoxylin. Subendocardial hemorrhages and fragmentation and mineralization of elastic fibers were also observed occasionally. In the affected areas, proliferating fibroblasts and a small number of lymphocytes appeared (Fig. 2). Atrophy of Purkinje's fibers was noticed occasionally. On the other hand, nodular lesions in the subendocardial and myocardial stroma were circumscribed with mineralized collagen, normal collagen, fibroblasts and a few lymphocytes. Amorphous and partially crystalline minerals stained with hematoxylin were found at their centers (Fig. 3). Granulomatous lesions were also noted in the subendocardial and myocardial stroma. They contained an amorphous mineral deposit at their center. They were surrounded with pale nucleated cells and their syncytium, fibroblasts, collagen and a small number of lymphocytes (Fig. 4). Mineralization was also seen in muscle fibers. Degenerated muscle fibers were densely stained with hematoxylin, with their nuclei obscure. Destruction of muscle fibers and proliferation of fibroblasts were recognized around those lesions.

Histochemically, the mineral deposit in collagen and circumscribed lesions was positively stained with periodic acid-Schiff and

von Kossa's stain, and identified as carbonic or phosphoric salt of calcium. An amorphous substance stained with Prussian blue was seen in the peripheral area of the mineral deposit in the lesions. Fine ferric granules were present dispersedly in the mineral deposit. Acid mucopolysaccharide was detected by alcian blue staining in mineralized lesions. It was distributed abundantly in the peripheral area of the mineral deposit in these lesions and irregularly in the central area.

Blood vessels: Mineralized lesions were present in parallel to the intima. The elastica interna was mineralized and densely stained with hematoxylin. There were amorphous or crystalline masses of minerals adjacent to the elastica interna. Mineralized elastic fibers were present around the mineral deposit (Fig. 5). They were also scattered over the whole intima, and occasionally in the elastica externa. Histochemically, the mineral deposit in the lesions revealed the same staining properties as that in the lesions of the heart.

Other organs: Mineralization was also noted in the kidney of one case, and the spleen, mesenteric lymph nodes and thymus of 2 cases. In the kidney, a mineral deposit was shown in capillary lumina of the interstitium. Proliferation of fibroblasts was recognized around capillaries. The mineral deposition in the spleen was seen in collagen of capsule and trabeculae. Lesions of lymph nodes were noted in the central area of the germinal center, which contained concentrically lamellar mineral crystals. In the thymus, the mineralization was observed in Hassall's corpuscles (Fig. 6).

Electron microscopic findings

Mineral deposit in the heart was observed in subendocardial collagen. The collagen increased homogeneously in electron density, or its periodical was irregularly high

in contrast (Fig. 7). Moreover, irregular-shaped crystals of high electron density were frequently observed in fibrillar extracellular spaces. On the other hand, lesions containing an aggregation of mineral crystals were present in the subendocardial and myocardial stroma (Fig. 8). The crystals were spherical in shape and ranged from 100 m μ to, frequently, 60 μ in diameter. Larger crystals had concentric lamellae. Each crystal contained needle-like crystalline structures of high electron density. Occasionally, a few mitochondria were scattered among the crystals. An aggregation of crystals was circumscribed with collagen and fibroblasts. The fibroblasts contained rough-surfaced endoplasmic reticulum remarkably developed and dilated to contain amorphous substance. Mitochondria were distributed diffusely in them. Numerous lysosomes were also present. Some of the fibroblasts were degenerated (Fig. 9).

Mineralization in the aortic wall was noted in the elastica interna and elastic fibers of the media. It was represented by a mineral deposit of high electron density with a distinct outline. Mineralization was occasionally recognized in collagen fibers around mineralized elastic fibers (Fig. 10).

In x-ray microanalysis, calcium, phosphorus and iron were detected from mineralized collagen and crystals in lesions of the heart. Furthermore, these minerals were also found in mineralized elastic fibers of the aorta (Fig. 11).

Discussion

It is generally accepted that mineralization in soft tissues occurs in hypervitaminosis D, hypomagnesemia, and such enzootic diseases in the tropic district as Manchester wasting disease, Naalehu disease, and enteque seco. Administration with a large amount of vitamin D increases the absorp-

tion of calcium and phosphorus from the intestinal tract, which is followed by the elevation of calcium and phosphorus level and the depression of magnesium level in blood serum [4, 6]. On the other hand, hypomagnesemia, or depression of serum magnesium level, is induced by magnesium deficiency in feed [16]. In the enzootic diseases mentioned above, a substance with vitamin D-like activity derived from herbage accentuates the imbalance among calcium, magnesium and phosphorus in serum [1-3]. The mineralization in the present cases should be estimated to be followed by administration with an excessive amount of vitamin D.

There were several opinions concerning the requirement and toxic dosage of vitamin D. In calves, the daily requirement of the vitamin was presented as 400 to 800 I.U. [16] or 3,000 I.U. [13]. On the other hand, administration with 5 or 50 million I.U. [4] of the vitamin in calves, or 600,000 [4], 30 million [5-7], or 50 million I.U. [4] of the vitamin in adult cattle was reported to induce intoxication. In the present cases, the amount of vitamin D administered ranged from 3,000 I.U. per day for a 10-day-old calf to 10,000 I.U. for a 100-day-old one. In this dosage, the initial dose was increased by 1,000 I.U. every 10 days. The excessive amount of the vitamin over the ordinary requirement might have been stored in the body and caused the mineralization of soft tissues.

Histologically, subendocardial mineralization, hemorrhage and inflammatory reaction were observed around the mineral deposit of the heart in the case of hypervitaminosis D [5]. Myocarditis was also recognized to have been accompanied by mineralization [9]. In aortic lesions, the internal elastic lamina was interrupted by calcification in the adjacent media. In these lesions, the

mineral deposit was surrounded by collagens, histiocytes, lymphocytes and occasionally eosinophils [5]. A foreign body reaction accompanied by giant cells was frequently noted in aortic and pulmonary lesions [5, 13]. In the present cases, mineralization was noted in subendocardial collagen and occasionally in myocardial fibers in the heart. Subendocardial hemorrhage was also recognized in some cases. Circumscribed mineral deposition and occasionally granulomatous reaction accompanied by pale nucleated cells and their syncytium were also noted. In aortic lesions, mineralization was recognized in the internal elastic lamina, elastic fibers of the media and occasionally the external elastic lamina.

When histochemical analysis was performed on the deposit of the lesion of hypervitaminosis D, calcium phosphate and calcium oxalate [7] or hydroxyapatite [10] were demonstrated. An accumulation of lipid [5] and acid mucopolysaccharide [5, 9] was seen in the lesion. x-ray diffraction indicated the presence of carbonate hydroxyapatite in the lesion, as is clear from comparative studies on bone [5]. In the present investigation, the presence of calcium phosphate and ferric phosphate in the mineral deposit was ascertained by histochemical analysis and x-ray microanalysis. Phosphorus, calcium and ferric ions in serum may have been combined into those salts.

On the other hand, an increase in serum seromucoid occurred to the case of hypervitaminosis D, and calcium was precipitated in tissues in a matrix composed of an unidentified polysaccharide [9]. Both degenerated elastin and mucopolysaccharides were reported to be important for the initiation of mineralization of vascular walls in the case of arteriosclerosis [20] or hypervitaminosis D [5]. Although the mechanism initiating the mineral deposition remained ob-

sure in the present investigation, an increase in calcium and phosphorus ion products might have caused minerals to precipitate in collagen and elastic fibers. Acid mucopolysaccharide deposited abundantly in mineralized lesions might have played a role in the occurrence of mineral deposition.

Ultrastructurally, elastic fibers and tissue were regarded as the first sites of calcification in the arterial wall [10, 14]. Crystals or fine electron-dense granules were present within elastic fibers of the aortic media. They were closely-packed needle-like structures radiating inward from the edge of the fiber. Densely packed crystals were found also in fibrillar extracellular spaces. They exhibited a rosette-like arrangement radiating outward [10]. Crystalline materials arranged to form a rosette and an aggregation of fine electron-dense granules were also seen in the process of medial calcification in bovine arteriosclerosis [17].

In the present investigation, mineralization in the heart took place predominantly in collagens and occasionally in elastic fibers. A few crystals or massive aggregations of such crystals varying in size were distributed in fibrillar extracellular spaces. In the aortas, mineralization was observed predominantly in elastic fibers. Crystals in these lesions were somewhat different in shape from those reported in previous papers [10, 17].

Histopathologically, some investigators recognized a foreign body reaction accompanied by histiocytes and their syncytium in mineralized lesions [5, 13]. In the present study, the foreign body reaction was noted light-microscopically in cardiac lesions circumscribed by pale nucleated cells. No cells, however, formed any obvious syncytium. These cells were different from histiocytes, since they showed a fine structure characteristic of the fibroblast [8, 11].

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Explanation of Figures

- Fig. 1. Mineralized lesion on endocardial surface of the heart.
- Fig. 2. Mineralization in subendocardial collagen. Hemorrhage is also noted. Hematoxylin and eosin staining. H-E, $\times 170$.
- Fig. 3. Mineralization subjacent to endocardium. Mineral deposit is circumscribed with pale nucleated cells. H-E, $\times 170$.
- Fig. 4. Granulomatous lesion in myocardial stroma. Foreign body reaction accompanied by pale nucleated cells and their syncytium is noted. H-E, $\times 70$.
- Fig. 5. Mineral deposit in elastica interna and media of aorta. H-E, $\times 170$.
- Fig. 6. Mineral deposit in Hassall's corpuscle. H-E, $\times 170$.
- Fig. 7. Mineralization in collagen in the heart. $\times 5,600$.
- Fig. 8. An aggregation of crystals in mineralized lesion. $\times 9,000$.
- Fig. 9. Fibroblasts around an aggregation of fine crystals. $\times 8,200$.
- Fig. 10. Mineral deposit in elastic fiber of aortic media. $\times 10,600$.
- Fig. 11. X-ray microanalytic pattern of crystals in cardiac lesions.