PATHOLOGICAL STUDIES ON GRASS TETANY (HYPOMAGNESEMIA) IN CATTLE OCCURRING IN JAPAN

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Since SJOLLEMA²³⁾ reported a bovine disease presenting hypomagnesemia with excitation on pasture land in the Netherlands more than 40 years ago, hundreds of papers on the disease have been published by many authors. According to them, magnesium ion in tissues is intimately related to impulse mechanism, and a decline of it caused by a decreased intake of magnesium produces the disease which presents such symptoms as convulsion and tremor and leads finally to death. Magnesium supply is effective and necessary for prevention, as well as treatment, of the disease.

No occurrence of the disease has been recorded among grazing cattle in Japan. Nowadays, improvement of pasture land is promoted in the country and the grass production emphasized in the animal industry. From a viewpoint of animal hygiene, it is anticipated that trace components of grass which are not contained in the chemical fertilizer may be reduced in amount year after year, and that cattle pastured in such grass fields may suffer from the disease¹⁾.

The present authors had a chance to carry out histopathological examination on some cases of the disease which were found in improved pasture lands in the vicinity of Morioka. The epidemiology and clinical signs observed in these cases were already reported by MURAKAMI et al.^{16,17)}

Very few papers were published to deal with histopathological studies on naturally occurring cases of the disease³⁾. Studies on some experimental cases of the disease were reported^{2,11,15,20)}, since the diagnosis can be made without difficulty and an effective treatment by magnesium supply can save diseased animals. The present report deals with a pathological investigation on cases of the first occurrence among grazing cattle in Japan.

MATERIALS

Cases in Pasture S

Grazing was performed over a period beginning with May 14, 1971. Hyperesthesia and spasm began to be observed in 10 cows some time between 4 and 19 days after the start of grazing. Two of the cows died. Serum analysis disclosed a magnesium level of 0.56~0.90 mg/dl in the diseased cows. The remaining 8 cows recovered after administration with $MgSO_4$ ⁷⁾.

Case No. 1: A Short Horn cow 5 years of age showed marked tonic spasm and

convulsion and died on May 27. Blood samples were collected and examined just before her death. The examination revealed 0.3 mg/dl of serum Mg, 4.0 mg/dl of serum Ca, plus 2 in the GROSS reaction, 8.0 mg/dl of serum protein, 1.17 of albumin and globulin ratio, 54% of albumin, 27% of α -globulin, 8% of β -globulin, and 11% of γ -globulin.

Cases in Pasture K

Cows were placed on pasture at the end of May, 1971. During a period from the end of August to that of September, 5 cows died and dozens of hypomagnesemic cattle were detected. They showed incoordination, difficulty in standing, hyperesthesia, clonic and tonic spasm, and convulsion. Treatment with MgSO_4 was very effective for the diseased cases¹⁶⁾.

Case No. 2: In a Short Horn cow 7 years old, incoordination was detected on the morning of September 4. She could not stand up on the next morning and died at 9.00 p.m. on the day.

Case No. 3: In a Short Horn cow 7 years old, incoordination and staggering gait were observed in the hind legs on September 16. She became unable to stand up in the evening. Anorexia and convulsion around the nose and mouth were shown. Body temperature was 37.8°C, pulse rate 92, and respiratory rate 64. Hematological examination revealed a serum magnesium level of 0.4 mg/dl. She was treated daily by injection with 25% solution of MgSO_4 for 3 days after the onset of symptoms. There was no improvement in symptoms. She was killed by bleeding on September 21 for necropsy.

METHODS

Materials were collected from organs and tissues all over the body and fixed in 10% neutral formalin solution. Paraffin sections were made by the routine procedure. They were stained with PAS stain, as well as hematoxylin and eosin, for microscopy.

Materials from Case No. 3 were fresh enough to examine by electron microscopy. Specimens in tiny blocks were collected within half an hour after death and fixed, for 2 hours, in a chilled mixture of 1 volume of 25% glutaraldehyde solution, 5 volumes of 0.2 M phosphate buffer solution, and 1 volume of distilled water. After the fixation, they were preserved in 4.5% sucrose solution of 0.2 M phosphate buffer until the next procedure was performed. Post-fixation was performed in 1% OsO_4 solution for approximately 2 hours, as mentioned by PALADE¹⁸⁾. All these procedures were conducted at pH 7.3.

The fixed blocks were dehydrated and embedded in Epon 812 by LUFT's method¹³⁾. A Porter-Blum MT2-B ultra-microtome was used for preparation of ultrathin sections, which were stained with lead nitrate by the method of REYNOLDS¹⁹⁾. A Hitachi HS-8 electron microscope was used for observation.

RESULTS

Autopsy findings

All the cases presented such common lesions as hemorrhage and cloudy swelling of skeletal muscle. Case No. 1 showed (1) hemorrhage on the serous membranes of internal organs and in the abdominal cavity, (2) cloudy swelling of skeletal muscle, and (3) emphysema of the lungs. Case No. 2 showed (1) hemorrhage in the subcutis and in and between the layers of skeletal muscle, as well as on the mucous and serous membranes, (2) focal necrosis in the liver, (3) an increase in abdominal fluid, and (4) hyperemia and edema of the lungs. Autopsy of Case No. 3 killed by bleeding disclosed (1) cloudy

swelling of the skeletal muscles in the hind part of the body, (2) liver flukes harbored in the bile ducts and verminous cholangitis, and (3) the pregnant uterus with foetus about 3.5 months old.

Histological findings

Focal hemorrhages in organs and tissues of the whole body, degenerative lesions on the walls of blood vessels of medium and small size, deposition of calcium salts in soft tissues, and degenerative lesions in parenchymatous organs were recognized.

1. Vascular lesions: Edematous and fibrinoid swelling, hyaline changes, and hemorrhage and desquamation of the intima were noticed on the walls of blood vessels. Most of these lesions were found in arteries approximately 30 to 200 μ in diameter that were accompanied by neutrophil infiltration and fairly active proliferation of histiocytes. Mural or whole fibrinous thrombi were formed in the vessels (Figs. 1~3). Those lesions described above were observed frequently in cardiac and skeletal muscle tissues and less frequently in the muscularis of the diaphragm, tongue, esophagus, stomach, intestine, and bladder.

2. Deposition of calcium salts in soft tissue: Focal deposition of calcium salts was distributed widely in the trabeculae, as well as the capsule, of the spleen in all the cases. No lesions were accompanied by a histiocytic reaction (Fig. 4). Distinct deposition of calcium salts was recognized in the media and adventitia of comparatively large arteries in the vascular layer of the uterus in 2 cases (Fig. 5). In Case No. 2, similar lesions were found in the media of the aorta. In Case No. 3, large amounts of calcium salts were scattered in the interstitial tissue of the medulla of the kidneys, and marked calcium deposition was observed in the muscle fibers of cardiac, skeletal, and glossal muscles (Fig. 6).

3. Retrogressive and other lesions in parenchymatous organs: Retrogressive lesions, which were of fresh focal necrosis and small in size, were detected in the liver, kidneys, and adrenal glands. The principal lesions of these organs observed were as follows.

Liver: Lesions of multiple focal necrosis with neutrophil infiltration were present in the liver in two cases, Nos. 1 and 2. They were of such size as to involve dozens of hepatic cells, exhibiting atrophy of cells, dissociation of cell cords, karyorrhexis, and karyopyknosis (Fig. 7). Thrombi were recognized in portal branches. Marked pericholangitis with fibrosis caused by liver flukes was detected in Case No. 3.

Spleen: Beside the deposition of calcium salts, hemorrhages were detected on trabeculae and capsule. Most of the malpighian corpuscles were inactive, and a few of them presented karyorrhexis in Case No. 2. Hyperemia and abundant hemosiderin pigments were observed in the red pulp.

Kidney: There was hyperemia. Nephrosis was detected with karyopyknosis in the epithelial cells of renal tubules. In Case No. 3, focal necrosis was seen in the cortex, in which swelling and hyaline changes of the cytoplasm and the disappearance and pyknosis of the nucleus were noticed. Degenerative changes of small arteries and hemorrhages were found in 2 cases and the deposition of calcium salts in the medulla was in a case.

Lung: Edema and emphysema were recognized. Marked hemorrhages in peribronchial tissues and thrombus in a branch of the pulmonary artery which was comparatively large in size were detected in Case No. 2. Focal atelectatic lesions were scattered in Case No. 3.

Heart: There were focal hemorrhages in the subendocardium, as well as the muscularis. Intramuscular histiocytic proliferation was frequently observed. Calcium salts were deposited on muscle fibers in Case No. 3. Vascular lesions with thrombus formation were severe in this case. Degenerated muscle fibers tended to be stained deeply with

eosin and lose striation. Hyaline changes with karyopyknosis were conspicuous around degenerated blood vessels.

Adrenal glands: Focal hemorrhages were observed in the cortex and capsule. Small necrotic foci were detected in the fascicular layer of the cortex in 2 cases. Cell cords were dissociated in these lesions. The cells of them were eosinophilic and atrophic. The nucleus was pyknotic in some of them and enlarged in others (Fig. 8).

Skeletal muscle: Marked degenerative lesions with swelling, fragmentation, and lack of striae of muscle fibers were recognized. Vascular lesions were also remarkable. Moderate histiocytic proliferation was seen in perivascular and intramuscular tissues. Neutrophil infiltration was also demonstrated in the perivascular areas. Calcium salts were deposited on swollen muscle fibers. Many of the sites of deposition were surrounded by proliferated histiocytes. Such lesions as mentioned above were remarkable in muscles of the hind part of the body.

Others: Occasionally, focal hemorrhage and edema were demonstrated in such organs as the central nervous system, pancreas, thyroids, and parotid glands.

Electron microscopic findings

The fine structure of skeletal muscle taken from the hind part of the body was studied. The myofibrils of a muscle fiber showed changes of almost the same degree as one another. In a muscle fiber, each myofibril preserved an almost normal structure with striation, as well as the perimysium did. Myoplasm was arranged regularly and contained mitochondria and endoplasmic reticulum. One or 2 lesions characterized by the disappearance of myofibrils from one Z-band to another were demonstrated in a muscle fiber. The endoplasmic reticulum increased in quantity and a few filaments of the myofibril were present in the space (Fig. 9).

Staining ability was diminished in degenerated muscle fibers. The striae disappeared and irregularly arranged filaments of the myofibril were detectable in these fibers. Areas of myoplasm were demonstrable due to detection of groups of endoplasmic reticulum scattered in the field (Fig. 10). Occasionally, fine electron dense granules were seen there. Mitochondria lost their cristae and it became difficult to find them out.

DISCUSSION

Although many publications on hypomagnesemia have been presented in the fields of epidemiology and clinical science, there are not so numerous papers dealing with this disorder from a histopathological point of view. In their studies, the present authors recognized four principal types of lesions; that is, (1) hemorrhagic lesions, (2) vascular lesions, (3) deposition of calcium salts, and (4) degeneration of parenchymatous organs.

Hemorrhages were remarkable in two cases, Nos. 1 and 3. In the former case were shown marked spasm and convulsion. In the latter case they were manifested after the period of lingering crisis. The occurrence of hemorrhage in hypomagnesemia has already been pointed out by BLAXTER et al.²⁾, ROOK²⁰⁾, and LARVOR et al.¹¹⁾ in their experimentally affected cattle, and by HERD⁷⁾ in his naturally affected sheep with petechiae and ecchymosis observed in the subendo- and subepicardium. In all the cases reported by previous authors, hemorrhagic lesions seemed to be fresh in character and have probably been induced by the vascular contraction resulting from spasm and convulsion in the terminal stage. A similar opinion was expressed by BLAXTER et al.²⁾ It would be impossible, however, to neglect the vascular lesions, as well as the decline in magnesium content in tissue fluid which would make blood vessels contract excessively.

The essential lesion of hypomagnesemia would be the vascular lesion. Among several

authors who reported the pathology of this disease^{2,3,9,11,15,20}), it was only BLAXTER et al.²⁾ that pointed out the occurrence of vascular changes in cattle. Several years prior to them²⁾, LOWENHAUPT et al.¹²⁾ published the results of their experimental studies on rat hypomagnesemia, stating that the essential lesion of this disease was angitis occurring to blood vessels of capillary size. In the present studies, no reactive inflammatory lesions were so conspicuous, but degeneration seemed to be a principal lesion. Anyway, the fact indicated by LOWENHAUPT et al.¹²⁾ that a vascular lesion was the essential lesion of hypomagnesemia is of great interest to the present authors.

Regarding the deposition of calcium salts on soft tissues, MOORE et al.¹⁵⁾ presented results obtained from their experimental cattle with remarkable calcification on the endocardium, as well as on the intima of arteries. BÖMER³⁾ and HORVATH et al.⁹⁾ also reported the calcium deposition of rather low degree on the wall of the aorta and the kidney tissues in cattle. In animals of other species, such as rats^{5,8,14,24)}, dogs²⁶⁾, and sheep¹⁰⁾, similar lesions were demonstrated in the kidney and vascular system. DICK and PRIOR⁵⁾ expressed their opinion that these lesions were induced by metaplastic calcification, and many other workers seemed to agree with them. In the present cases, the authors missed a chance to examine the bony tissue. So they may have to avoid to go too far into discussion. Some previous authors^{16,17,22,25)} reported, however, that mild hypocalcemia was accompanied by hypomagnesemia. There seems to be some points remaining unknown in the pathogenesis of calcification.

Regarding retrogressive lesions in parenchymatous organs, the degeneration of renal tubules was pointed out in rats^{6,8,21)} and sheep¹⁰⁾ previously. The lesions of the liver, kidneys, and adrenal glands in the present cases seemed to have occurred just before death, because they were fresh and coagulation necrosis in nature and demonstrated only in the dead cases, except those of the kidneys. There may be no direct relationship between muscular degeneration and calcification. It may be concluded that the degeneration was induced by circulatory disturbances due to the vascular lesions, as well as to the excessive contraction of muscle.

Electron microscopy disclosed that muscle fibers were in the process of degeneration. Although it was difficult for technical reasons to demonstrate the process of calcium deposition on muscular tissues, the myoplasm might play a role in the process of calcification. The change of myofibrils demonstrated may probably be a non-specific lesion of the disease in question and a common figure in the process of muscular degeneration. At any rate, the fine structure of the lesion of hypomagnesemia mentioned above is the results of the first attempt made by the authors and presents many problems to be solved.

From the discussion described above, the present authors would like to point out the vascular changes and calcification of soft tissues as the essential lesions of the disease in question. DEL CASTILLO and ENGBAER⁴⁾ observed that a vast amount of cholinesterase had been released against an impulse in tissues along with a decrease in Mg ion. Besides, SEEKLES²²⁾ demonstrated that the activity of cholinesterase in synapsis had been elevated in the disease in question. These results^{4,22)} are good for the explanation of pathogenesis of this disease. Repeated contraction of blood vessels would induce qualitative lesions in these vessels. The pathogenesis of calcification, however, does not seem to be known completely. The complicated effects²⁸⁾ of magnesium and other minerals²⁷⁾ and hormonal metabolism³⁾ on the living body should be studied in future.

SUMMARY

Three cows with hypomagnesemic tetany were studied pathologically and one of

them was electron microscopically. This disease occurred in 2 pastures in Iwate Prefecture, Japan, in the spring and fall of 1971.

Degenerative changes of vascular walls were remarkable in muscular tissues all over the body. They were characteristic of the disease in question. At the same time, the deposition of calcium salts was demonstrated on soft tissues, such as splenic trabeculae, arterial walls, and muscle fibers.

Besides, fresh focal hemorrhages were recognized in organs and tissues of the whole body and fresh focal coagulation necroses in parenchymatous organs.

The pathological lesions of the disease reported by previous workers were not always apparent in the present study. Some discussion was made comparatively on the results obtained from the present study.

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牛のグラステタニー（低マグネシウム血症） に関する病理学的研究

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わが国において、はじめて発生が報ぜられた放牧牛の低マグネシウム血症について、病理組織学的研究を行ない、そのうち1例については、電子顕微鏡的観察をあわせ行なった。発生は岩手県における2カ所の改良牧野において、1971年の春および秋に別々にみられたもので、いずれも雌の短角種であった。

本病の本質的病変として、全身とくに、心筋を含めての筋組織における、血管の変性変化が指摘された。また、脾梁材、動脈壁、筋線維などの、軟組織における石灰沈着が認められた。

このほか、全身の臓器組織における新鮮巣状出血、および肝・腎・副腎などにおける新鮮微小巣状の凝固壊死が確認された。

電顕的には、筋原線維のZ板を境とする脱落がみられた。変性変化の高度のものでは、筋原線維のフィラメントの消失と、筋漿における小胞体の集積がみられた。

低マグネシウム血症における病変については、先人の業績のすべてが軌を一にしているわけではないので、今回の所見と対比しながら、本病の病理発生について、若干の論議を重ねた。

EXPLANATION OF PLATES

PLATE I

Sections from Case No. 3 stained with hematoxylin and eosin.

- Fig. 1. Fibrinoid swelling of arterial walls (arrows) and venous fibrinoid thrombus (T) with histiocytic proliferation in the perivascular area of the heart muscle. $\times 400$.
- Fig. 2. Fibrinoid degeneration of the wall of a small artery, and edema and hyaline degeneration of muscle fibers in skeletal muscle. $\times 100$.
- Fig. 3. Magnification of the enclosed square in Fig. 2. Degenerated arterial wall with neutrophil infiltration, histiocytic proliferation, and hemorrhage is shown in perivascular tissue. Granular fragmentation has appeared in muscle fibers (upper right). $\times 400$.
- Fig. 4. Deposition of calcium salts on the trabecula in the spleen. $\times 100$.

PLATE II

Hematoxylin and eosin stain.

- Fig. 5. Deposition of calcium salts in media and adventitia of arterial walls in the vascular layer of the uterus. Case No. 3. $\times 40$.
- Fig. 6. Calcification (dark) and edema (light) of muscle fibers with mild histiocytic proliferation in skeletal muscle. Case No. 3. $\times 100$.
- Fig. 7. Central necrotic focus (center) with dissociation of cell cords, karyorrhexis, giant nuclei, and irregularly arranged wall of a central vein (upper left) in the liver. Case No. 2. $\times 400$.
- Fig. 8. Necrotic focus with dissociation of cell cords, eosinophilic protoplasm, karyopyknosis,

and giant nuclei in the fascicular layer of the adrenal gland. Case No. 2. $\times 400$.

PLATE III

Fine structures of the skeletal muscle of Case No. 3 in sections treated with lead stain.

- Fig. 9. Falling out of myofibrils between two Z-bands (arrows) with scarce filaments and slightly increased organelles in the myoplasm. $\times 33,100$.
- Fig. 10. Degenerated muscle fiber with poorly stained shadow of myofibril filaments and groups of abundant endoplasmic reticulum indicating the area of myoplasm. $\times 35,400$.