An Occurrence of Cerebrocortical Necrosis in Rearing Calves—Histopathological and Ultrastructural Studies

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(Received for publication January 11, 1977)

Abstract. Pathological studies were carried out on cerebrocortical necrosis in four of five heifers affected in a group of 12 rearing calves. These calves were kept in an isolated barn on a fattening farm of moderate scale, and fed moulded hay harvested two years before. Clinical observation revealed diarrhea followed by the typical nervous symptoms of the disease and leucocytosis. Macroscopically, the edematous turbid meninges yellowish brown in color with hemorrhagic spots, and softening of the yellowish zonal area corresponding to the gray matter were demonstrated in the cerebral hemisphere. Histological observation disclosed neuronal necrosis, edematous degeneration of astrocytes, proliferation of compound granular corpuscles, and vascularization with swollen endothelial cells in the gray matter. Pyramidal cell necrosis, edematous enlargement of the cytoplasm and processes of degenerative glia cells, degenerative disorder of the neuropil in the lesion were observed by electron microscopy. Discussion was made on the pathogenesis of the disease.

Encephalomalacia in sheep was reported by Yamagiwa and Tajima [27] in 1952. Since then, occurrences of the disease have been published in Europe [8, 11, 24–26], North America [6, 12] and New Zealand [20]. The disease is called cerebrocortical necrosis (CCN) or polioencephalomalacia affecting sheep, cattle and other ruminants. Thiamine inadequacy may play a role in causing the disease [4, 5, 7, 14, 17]. It is only Morgan [18] that reported an electron microscopic study on CCN in sheep previously. Hosokawa et al. [10] of the authors’ laboratory performed a pathological study on sporadic ovine cases of CCN in 1971. The present report is concerned with histopathological and ultrastructural studies on CCN which occurred in a group of rearing calves.

Materials and Methods

Four animals obtained from a farm in Shizuoka-shi, Iwate-Ken, were examined. They were 7-month-old heifers of the Holstein-Friesian breed. Autopsy was carried out after brief clinical observation. Blocks of organs and tissues of the whole body were fixed in 10% neutral formalin solution. Paraffin sections were made and stained with hema-toxylin and eosin (H-E) for histological observation. Serial sections of the central nervous system were stained with Luxol Fast blue (L-F). Small tissue blocks from the cerebral cortex of an animal that had been exsanguinated were examined by electron microscopy using the method described elsewhere [1].

Results

1. Clinical history
The farm was located on a hill land and
consisted of four main barns of various sizes in which approximately 200 fattening cattle were kept. There was also a small isolated barn where 12 heifers 7 months old were reared. In this group five animals were affected successively during a period from September 22 to October 10, 1974. As a base fodder, moulded hay harvested in 1972 had been supplied to these heifers exceptionally for more than 5 months. After October 1 when the 4th case showed symptoms of CCN, supply of the hay was suspended. Only one animal developed the clinical symptoms of CCN on the 10th day after the suspension.

The characteristic nervous symptoms were preceded by diarrhea. Lumbar incoordination and staggering gait were rather suddenly recognized. One or two days later, the heifers could not stand, but lay down on the side. Opisthotonos was remarkable with the four legs extended and stiff joints (Fig. 1). Trismus, grinding, blindness (amaurosis), deafness, and loss of sensation of the whole body were observed. The body temperature and pulse rate were within a normal range. Leucocytosis was detected in all the cases examined. White blood cell counts were 17,000 to 25,000/\text{mm}^3 with 72–80% of neutrophils. All the animals were killed in the terminal stage, except a dead animal.

2. Autopsy findings
A noticeable lesion was cerebral edema. The meninges were cloudy and yellowish brown in color with hemorrhagic spots. Cerebrospinal fluid was turbid and increased in quantity. The brain tissue was soft. The zonal area yellowish in color corresponded obviously to the gray matter on the cut surface. There were remarkable lesions in the parietal and occipital lobes of the cerebrum. Similar but slightly yellowish lesions were detected on the cerebellar meninges.

Additionally were found intestinal catarrh and small erosive lesions on the mucosa of the abomasum in all the animals, and interstitial nephritis and such circulatory disturbances as subendocardial hemorrhages, hemorrhagic infarct of the pulmonary margin, and hemorrhages of the splenic pulp in one or two animals.

3. Histological findings
Principal lesions were widely distributed in the cerebral cortex, predominating in the parietal and occipital lobes of both hemispheres. Markedly discolored lesions were found in the gray matter. In them most of the neurons presented acidophilic and ischemic changes with an edematous perineurial area. The neurons were stained homogeneously deep blue with L-F stain. Astrocytes with indistinct processes exhibited edematous swelling. Their nuclei were still stainable with cresyl violet, but many of them showed hyperchromatosis of the nuclear wall (Figs. 2 and 5).

The endothelial cells of blood capillaries increased in size and number. The lymph space was dilated. Perivascular and focal hemorrhages were frequently observed. The wall was edematous in some arterioles. The neuropil was loose in the cortex, especially in the internal pyramidal cell layer. Vascular and glia-cell proliferation was observed in some of the lesions (Fig. 4). Migration of neutrophils was occasionally found in the lesions of the gray matter. Crevices and proliferating compound granular corpuscles were observed in some lesions. These corpuscles were sometimes demonstrated in the meninges and molecular layer adjacent to the predominant lesions (Fig. 5). Myelin substances phagocytosed in their cytoplasm were clearly recognized when stained with L-F stain.

Pathologic changes in the white matter
were rather minute. There were spongy lesions and a few necrotic neurons. The cerebellum presented edema of the Purkinje cell layer with ischemic Purkinje cells and partial depletion of myelin sheath in the medulla. Neuronal degeneration and edematous changes were observed in the colliculi nasales of the mesencephalon.

Concurrent lesions other than those recognized at autopsy were myodegeneration or myositis of the skeletal muscle in all the animals examined, acute splenitis in 2 animals, and small focal necrosis of the liver, small ulcerative lesions on the lingual mucosa, coccidial protozoa in the intestinal mucosa in three animals respectively.

4. Ultrastructural findings

Few pyramidal cells in the gray matter maintained the contour of the nucleus and cytoplasm. An edematous watery substance filled up the space around them. The neuropil lost its original structure (Fig. 6). Neurons still maintaining their contour contained numerous intracytoplasmic vesicles and were surrounded by satellite cell processes which were edematous and enlarged. In the astrocytes the chromatin was irregularly aggregated in the nucleus and the cytoplasmic organelles were obscure. The organelles of the axon were frequently dispersed due to axonal swelling. Dilation of the periaxonal space and intramyelinic swelling of the myelin sheath were common (Fig. 7).

Capillaries were rather well preserved in the lesions. Many of the endothelial cells of the capillary were enlarged, and some of them contained edematous cytoplasmic vesicles. Tight junctions of the endothelial cells were well preserved in most of the capillaries (Figs. 6 and 8). Macrophages were also seen attached to the endothelial cells. The perivascular space was dilated. Degenerated glia cells with an indistinct cytoplasmic membrane and macrophages were occasionally recognized in it (Fig. 8).

Discussion

The clinical features, as well as the pathologic changes, of CCN were reported by many investigators. As a result, insufficiency of thiamine has been suspected deeply to be the etiology of CCN [4, 7], though something still remains to be studied on the pathogenesis of the disease [4]. The obstruction of thiamine synthesis in the digestive tract [5, 23] and that of thiamine absorption due to thiamine antagonists produced or ingested [14, 17] are considered to be principal causes of the insufficiency.

Electron microscopy revealed marked degenerative and edematous changes in the gray matter. The lesions observed showed rather progressive features, but the initial ones were not demonstrable. The findings closely resembled those described by Morgan [18, 19] on sheep naturally affected and experimentally with amprolium intoxication.

It is well known that edematous watery fluid stays in the cytoplasm of astrocytes in the cerebral gray matter at the beginning of brain edema [13, 16]. In the pathologic state of the brain, a tracer infused into the blood stream readily flows out into the intercellular space [9]. The lesions observed in the present study may be an expression of edematous changes induced by ischemia or some cytotoxic effects. It is still obscure how the edematous fluid is transfused through the capillary wall into the astrocyte or intercellular space in the brain. The well-preserved capillary endothelium with tight junctions between the endothelial cells and cytoplasmic vesicles occasionally produced in these cells were observed in the lesions of the present study. These
findings suggest that pinocytosis may play a role in the pathogenesis of brain edema in CCN.

Collins and Converse [2] reported a marked glycogen accumulation within glial cell processes in the cerebellum of thiamine-deficient rats produced experimentally. Morgan [18] demonstrated glycogen granules in the glial cell processes of affected sheep. He expressed his opinion that the granules might have been attributed to thiamine deficiency, the consequent impairment of carbohydrate metabolism, and the lowered rate of glucose utilization by neurons. He also presumed that the impairment of neuronal thiamine metabolism or neuronal damage due to local ischemia following capillary compression might account for the increase of glycogen present. Osmiophilic granules similar to those described by Morgan [18] were occasionally observed in glial cell processes and watery spaces in the present study. It was, however, rather difficult to identify them as glycogen granules [21, 22], since the lesions were so destructive.

Davies et al. [3] suggested that some species of fungi requiring abundant thiamine might cause the disease. On the other hand, Loew et al. [15] were in the opinion that a modern high-grain diet might play a role in altering the bacterial flora and possibly cause thiamine insufficiency. Their opinion was based on the results of their experiment in which no fungi isolated from mouldy barley-straws exhausted thiamine.

From an epizootiological point of view, feeding of moulded hay was pointed out as a possible cause of CCN in the present study. This view was not denied, and the direct or indirect effect of a long-term ingestion of the moulded hay might be responsible for the occurrence of CCN in calves.

References


CEREBROCORTICAL NECROSIS IN CALVES


Explanation of Figures

Fig. 1. Affected heifer No. 1019 lying down on the right side and presenting opisthotonus with the extended four legs.

Fig. 2. Ischemic acidophilic necrosis of pyramidal cells, edematous swelling of astrocytes, and rough neuropil are evident in the external pyramidal cell layer of the occipital lobe in heifer No. 1018. H-E staining. ×580.

Fig. 3. Identical lesion to Fig. 2 in a serial section. The neurons stained bluish in color but not with cresyl violet. L-F staining. ×580.

Fig. 4. Vascularization with enlarged endothelial cells, glia cell proliferation, and scattering of necrotic neurons are presented in the external pyramidal cell layer of the occipital lobe in heifer No. 1017. H-E staining. ×145.

Fig. 5. Proliferated compound granular corpuscles in the molecular layer of the temporal lobe in heifer No. 1018. H-E staining. ×290.

Fig. 6. A necrotic pyramidal cell (N) surrounded by edematous space containing granules and enlarged endothelial cells with well-preserved tight junction (arrow) is present in the external pyramidal cell layer of the parietal lobe in heifer No. 1019. ×5,250.

Fig. 7. An edematous satellite astrocyte (left) and a degenerative neuron with cytoplasmic vesicles (center). Irregular edematous processes, axonal swelling, dilation of periaxonal space, and intramyelic swelling of myelin sheaths are recognized in neuropil in the internal pyramidal cell layer of the parietal lobe in heifer No. 1019. ×2,750.

Fig. 8. A macrophage (upper right) in the edematous perivascular space and a degenerated glia cell are shown in a malacic lesion of the parietal lobe in heifer No. 1019. Well-preserved tight junction (arrow) is demonstrated between endothelial cells. ×5,250.