Occlusion of Brain Capillaries by Endothelial Swelling in Mycoplasma Infections
(turkeys/rats/encephalopathy/ischemic necrosis/electron microscopy)

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ABSTRACT  Occlusion of cerebral capillaries due to acute swelling of the endothelial cells has been demonstrated in the brains of turkeys with encephalopathy caused by Mycoplasma gallisepticum (S-6 strain) infection. Similar capillary lesions were seen in rats with rolling disease, caused by M. neurolyticum toxin. Such lesions may be the basis of ischemic necrosis of the brain.

We report here a capillary lesion seen by electron microscopy that is associated with the neurotoxic action of two mycoplasma species: The S-6 strain of Mycoplasma gallisepticum in turkey pouls, and M. neurolyticum in rats. In turkeys, M. gallisepticum infection results in a fatal encephalopathy in which polyarteritis of the cerebral arteries is the most conspicuous lesion seen by light microscopy (1, 2). In rats and mice, M. neurolyticum produces “rolling disease”, an acute neurological syndrome caused by the exotoxin elaborated by this microorganism (3, 4, 5). In both syndromes, there are extensive necrotizing lesions of the brain parenchyma, consisting of vacuolization, demyelination, and neuronal destruction. Previous ultrastructural studies of the M. neurolyticum disease in rats and mice revealed extensive swelling of astrocytes and their processes, associated with necrosis of parenchymal structures throughout the involved areas of the brain (6).

In turkeys, fibrinoid necrosis, which involved virtually all the arteries in the brain, was assumed to be the basis for parenchymal necrosis (2). This destruction of brain tissue, often assuming the appearance of massive infarction, may in fact be due to the capillary abnormality described below.

MATERIALS AND METHODS

Rats, weighing 150–200 g, were injected intravenously with a crude preparation of M. neurolyticum exotoxin, prepared by Millipore filtration of 18-hr broth cultures (4). The animals became ill 1–2 hr after the injection, with convulsive movements of all four extremities, weakness or paralysis of the hind limbs, and occasional episodes of repetitive rolling on the long axis of the body.

Turkey pouls, 6 weeks of age, weighing about 1 kg, were injected by vein with 1 ml of an overnight broth culture of the S-6 strain of M. gallisepticum, containing approximately 5 × 10^9 organisms. With this dose, neurological symptoms occurred 3 or 4 days later, that consisted of ataxia, weakness, torticollis, and, a few hours later, floundering convulsive seizures and coma.

Rats and turkeys with neurological manifestations and healthy controls were killed by perfusion of the brain with Karnovsky’s cacodylate-buffered formaldehyde–glutaraldehyde (7) through the left ventricle of the heart after ligation of the abdominal aorta and opening the right ventricle. The perfusion was initiated with roughly 0.25 strength of the above fixative followed by full-strength fixative, as suggested by Brightman (personal communication). Blocks of tissue from various regions of the brain were post-fixed in osmium tetroxide and embedded in epon; ultrathin sections were stained with uranyl acetate–lead citrate and examined on an RCA 3G electron microscope.

In addition, sick turkeys were intravenously injected with horseradish peroxidase, 200 mg/ml (type II Sigma) in 1 ml of saline (8). Five minutes later the birds were decapitated and the brain was immediately removed, fixed in glutaraldehyde, and processed for tracer studies.

RESULTS

The endothelial cells of the affected capillaries in inoculated rats were swollen to such an extent that the lumen was obliterated partially or completely. Such an occlusive capillary lesion is illustrated in Fig. 1. There was marked swelling and destruction of the pericapillary astrocytic processes, although their mitochondria were relatively spared. Neither the occlusive endothelial lesion nor the astrocytic swelling was seen in any control rats, and their capillaries appeared similar to the normal vessel illustrated in Fig. 2.

The capillaries in a control normal turkey and in two inoculated birds are illustrated in Figs. 2-4. In healthy turkeys, the nuclei of endothelial cells were slender; the cytoplasm showed a poorly developed Golgi apparatus, some thick profiles of rough endoplasmic reticulum containing dense granular material, and mitochondria that were elongate and regular in appearance. Coated vesicles were seen and fibrils could be detected in the cytoplasm. The normal endothelial cells were surrounded by a continuous basilar lamina, which split to contain pericytes or their processes. The basal lamina measured approximately 20–35 nm (200–350 Å). The inner surfaces of the endothelial cells were generally smooth, except for a few cytoplasmic projections. The lumen, as illustrated in Fig. 2, was always patent regardless of the size of the capillary. Tight junctions were readily visualized between most endothelial cells.

Remarkable changes were observed in the capillary endothelial cells of the turkeys showing neurological symptoms. The cytoplasm and especially the nuclei were greatly enlarged (Fig. 3). The pericytes were also swollen, and the basal lamina was roughly 3 times as wide as in the normal controls.
Endothelial Swelling in Mycoplasma Infections

**Fig. 1.** Rat injected with mycoplasma toxin. An enlarged endothelial cell with a prominent nucleus (N) narrows the capillary lumen (L). There is swelling of astrocytic processes around the basal lamina, in which relatively well-preserved mitochondria (m) float (X 12,000).

**Fig. 2.** Control normal turkey. A capillary is seen with widely patent lumen. Cytoplasm of a typical endothelial cell (E) is seen, as well as tight junctions (*). Splitting of the basal lamina to include cytoplasm of a pericyte is noted (arrows). X12,000.
FIG. 3. Turkey injected with *M. gallisepticum* killed on third day after injection. Distention of endothelial cells (E) with narrowing of the lumen (L) is noted. Both the pericyte (P) and the surrounding basal lamina (arrows) are enlarged. Mitochondria are more round, and the surface of the endothelial cells is irregular. Swelling of the astrocytic cytoplasm with the relative preservation of mitochondria is noted. ×12,000.

The chromatin of the endothelial cells was concentrated in the peripheral part of the nucleus and the center was pale, suggesting distention of the chromatin matrix. Most of the mitochondria were round in shape, but with intact cristae. The profiles of rough endoplasmic reticulum showed some degree of dilation. The tight junctions of the endothelial cells appeared normal. The internal surfaces of the endothelial cells were irregular, and coated vesicles were more frequently seen than in normal vessels. Swollen endothelial cells protruded into the lumen of the capillary and caused marked narrowing of the lumen. This event is illustrated in Fig. 4, taken from a turkey that received horseradish peroxidase in order to visualize the vascular compartment. The tight junctions appeared to be functionally intact, since no peroxidase was extruded into the basal lamina. The lumen was occluded almost completely by the swollen endothelial cells, and only a small, irregular, slit-like lumen containing peroxidase was seen. In Figs. 3 and 4, swelling of the astrocytic processes around the basal lamina was also observed; the mitochondria of these cells showed good preservation.

FIG. 4. Turkey infected with *M. gallisepticum*, injected with peroxidase before death. A markedly enlarged endothelial cell almost entirely occludes the capillary lumen. Its nucleus (N) is markedly distended. Peroxidase is seen in the lumen, which is reduced to a slit (arrows). Peroxidase does not appear in the basal lamina or perivascular space. ×13,000.
DISCUSSION
Although endothelial proliferation has been described in the vessels of cerebral neoplasms, and in the encephalitides associated with rickettsial infections (9), the extraordinary degree of endothelial swelling, not accompanied by any evidence of proliferation of endothelium, seems to be a unique finding in the rats and turkeys affected by mycoplasmas or their toxins. The remarkable speed with which this change can occur is also noteworthy; in rats, fully developed lesions were seen within 2 hr after the injection of *M. neurolyticum* toxin.

The question of reversibility of the vascular lesions observed in turkeys was investigated in tetracycline-treated birds. After the development of the neurological disease, at a time when the birds appear moribund, treatment with tetracycline reverses the disease process, and the cerebral arteries appear to revert to normal (L. Thomas and W. Clyde, to published). The rapidity with which the neurological signs disappear in the treated birds suggests that a toxic property of the mycoplasma may be involved. Two turkeys that were treated with tetracycline on the third day after infection, when neurological symptoms had appeared, were examined 24 hr after the start of treatment, and the capillaries in these birds appeared entirely normal by electron microscopy. Therefore, it is likely that the capillary endothelial abnormality is rapidly reversible.

Since the capillary endothelial swelling causes partial or total occlusion of the lumen, these endothelial changes might in themselves be sufficient basis for ischemic changes in the brain parenchyma. The rapid onset and reversibility of capillary occlusion provide a new and advantageous experimental model for the study of anoxic injury in the nervous system. The existence of such a mechanism offers an approach to certain spontaneous diseases of the brain in which ischemic necrosis occurs without discernible thrombosis.
