Ultrastructural Studies of Spleens, Brains, and Brain Cell Cultures of Mice with Scrapie

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Abstract. Mouse brains, cell cultures of mouse brains, and spleens from mice with scrapie were examined by electron microscopy. Brains and spleens of 10 scrapie-inoculated and control mice were studied. Seven brain-cell cultures, four of which were from mice inoculated intracerebrally or subcutaneously with scrapie, were examined. Status spongiosus and vacuolated neurons were found in the brains. Structures 35 nm in diameter were seen in the brains of mice inoculated intracerebrally. They were not evident in cell cultures, although a vacuolated structure was found in one such culture. No significant changes were found in the spleens.

Through electron microscopy, investigators have sought to show scrapie virus and its associated changes. The established histological diagnostic criteria – neuronal vacuolation, astrocytic hypertrophy and status spongiosus – have been studied also by this means. Natural cases of scrapie have neuronal vacuolation and intravacuolar budding of vesicles and cytoplasmic processes, with some vacuolated neurons containing membrane-bound accumulations of 35-nm particles [2]. There are other reports of similar findings without the intravacuolar budding [4], and particles 15–26 × 75 nm were found both in astrocytic processes and nerve cell terminals [20].

Electron microscopic examination of experimentally infected animals has included studies of the mouse and rat. Reports on mice indicated areas of status spongiosus, ruptured plasma membranes and curled fragments of membranes in areas of presynaptic and postsynaptic neuronal terminals, and 35-nm vesicular and tubular structures suggestive of viral particles [10, 17]. One report suggested that the small vesicles found within a nerve cell were formed by dilatation of endoplasmic reticulum [13]. In rats, neurons were found that contained small inclusion bodies composed of a folded membrane and accu-
mulations of a longitudinal particle $20 \times 60$ nm with a 4-nm central core [12]. Others have reported a similar particle [21]. Pleomorphic structures [7] and amyloid bodies [14] have been found in neurons and glial cells. In both mice and rats, large intracytoplasmic masses of amorphous material have been seen in ependymal cells [8].

In a recent report [16] of experimental Creutzfeldt-Jakob disease in four chimpanzees, the characteristic changes included vacuolar neuronal degeneration, astrocytic proliferation, and status spongiosus. The vacuoles were bounded by fragmented membranes and contained accumulations of membranes. Circular and tubular profiles 30–40 nm in diameter were found in the cerebral cortex of one animal.

Electron microscopic observations supported changes previously postulated from light microscopic studies. Of interest, however, is the finding of some particulate matter in each of the animals studied and, in particular, 30–35-nm vesiculotubular structures reported in natural and experimental scrapie and in experimental Creutzfeldt-Jakob disease. These structures have been suggested as the scrapie agent [1].

The present study was undertaken to find similar particulate structures in splenic cells and in cell cultures of brains of mice affected with scrapie. It was first necessary to find the particles in the brains of infected mice. If they were present and were scrapie particles, they might well be demonstrable in spleens of scrapie-affected mice and in cell cultures established from their brains. Scrapie virus has been associated with the spleen [11, 19, 22–24] and has been shown to replicate in cell culture [6, 9].

**Materials and Methods**

The inoculum was obtained from brain tissue of mice of the third and fourth serial mouse passage of the Klenck (0–2374–68) agent isolated from a purebred Suffolk ram in 1968.

Female ICR mice were obtained from Harlan Industries, Cumberland, Ind. Brains and spleens were collected for electron microscopic study from mice inoculated intracerebrally or subcutaneously with 0.03 ml of supernatant fluids from a centrifuged 10% suspension of finely ground mouse brains. Two mice inoculated intracerebrally were killed 183 days after inoculation when they were in an advanced clinical stage of the disease. Brains of three mice inoculated subcutaneously were collected 150 days after inoculation. Fifty percent of such inoculated mice had cord lesions histologically diagnostic for scrapie. Controls were five mice that were not inoculated or that received 10% normal brain suspension intracerebrally. The mice were anesthetized with pentobarbital sodium and perfused intracardially with 6% phosphate-buffered glutaraldehyde for 5 min. The tissues were
removed, minced, and stored for 2 h in the fixative. They were then put in osmium tetroxide, processed for electron microscopy, and embedded in Epon 812.

Cell cultures were obtained from mice that had received 0.03 ml of the scrapie inoculum by either route. Cultures were established, using a reported technique [15], from mice inoculated intracerebrally, 155 and 163 days after inoculation and from mice inoculated subcutaneously, 107 and 149 days after inoculation. The brains of four mice were used to establish each of the cultures. Control cultures consisted of two from normal adult mice that received no inoculum and one, 141 days after inoculation, from mice that were given an intracerebral inoculation of 0.03 ml of a 10% suspension of normal mouse brain. All the cultures were examined between the 4th and 11th serial passages. The 107-day culture was examined at its 11th and 21st passages. The cell cultures were processed for electron microscopy by trypsinization of the cell sheet and pelleting the cells by centrifugation. The pellet of cells was fixed initially in 4% phosphate-buffered glutaraldehyde for 1 h then in osmium tetroxide; after fixation it was processed and embedded in Epon 812.

All the tissues were cut on a Sorvall Porter-Blum MT2-B ultramicrotome, stained with lead citrate and uranyl acetate, and examined in a Philips-200 electron microscope.

Fig. 1. Electron micrograph demonstrating the presence of 30- to 35-nm vesiculotubular structures (S) inside a postsynaptic process.
Fig. 2. Electron micrograph of a scrapie-affected tissue culture illustrating vacuoles (V) containing fine granular material (G).

The scrapie agent titer was determined in the inoculated mice by the Reed-Muench method. During the very advanced stages of the clinical disease, the activity was determined in the brains and spleens of animals inoculated intracerebrally. Activity in the brains and spleens of subcutaneously inoculated mice was determined 150 days after inoculation.

Results

Part of the occipital region of the right cerebral hemisphere was examined in the electron microscope for evidence of the scrapie agent. Status spongiosus and vacuolated neurons were seen in both groups of inoculated mice, but changes were most evident in those inoculated intracerebrally; their brains had particles 30–35 nm in diameter (fig. 1) that had an electron-lucent center.
and were associated with postsynaptic processes. Most of the structures were spherical but some appeared as long rods. No similar structures were seen in the mice inoculated subcutaneously or in the controls.

The spleens from these mice had none of the vacuolar changes or particles seen in the scrapie-affected mouse brains. No differences were seen between the spleens of affected and control mice.

In tissue cultures 163 days after inoculation there was a vacuolated structure in the cytoplasm (fig. 2). The vacuoles consisted of a ruptured double membrane and contained fine granular material. The origin of these cells was not determined. Vacuolation was the only change and was not present in any of the other cultures.

The LD₅₀ in brains and spleens of mice inoculated intracerebrally was \(10^{5.5}\) and \(10^{4.5}\), respectively. The titers 150 days after inoculation in mice inoculated subcutaneously were \(10^{4.5}\) and \(10^{3.3}\), respectively.

**Discussion**

The significance of the 30- to 35-nm particles seen by us and reported by others [2, 10, 16, 17] and their relationship to the etiological agent of scrapie are unknown. The inability to demonstrate the particles in the subcutaneously inoculated mice seems to be related to the stage of the incubation period. In our laboratory, there is extreme variation in incubation time in such mice. The area of the brain examined may also be a factor, since scrapie lesions have been seen first in the thalamic region [5]. Location of the structures in the spleen may be a difficult task because of the varied population of cells. However, Lavelle et al. [18] did find that in populations of splenic cells of lesser density the requirement for a mouse LD₅₀ was 40- to 60-fold less than among the populations of cells with greater density. Our examinations included all cell types, and 35-nm particles were not found.

An interesting finding was the vacuolated structures in the cell cultures of scrapie-affected mice. The number of cells reported to support scrapie replication is low [9] and may account for the low number of cells with fine granules and vacuoles. The significance of these structures is unknown. Further study is warranted, however, since vacuolated membranes have long been of diagnostic importance in the subacute spongiform encephalopathies.

If the particles in brains of inoculated mice are indeed scrapie virus then they are probably masked in the spleen, and are at least rare in cultures of brain cells of mice with scrapie.
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References


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