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Altered Plasma Membranes in Experimental Scrapie*

P. LAMPERT, J. HOOKS, C. J. GIBBS, JR., and D. C. GAJDUSEK

University of California at San Diego, La Jolla, California
and
National Institutes of Health, Bethesda, Maryland

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Summary. The status spongiosus in the cerebral cortex of mice affected with two different strains of scrapie virus corresponded to focally swollen perikaryal cytoplasm of nerve cells and astrocytes, to swollen neuronal and astrocytic processes and to membrane-bounded vacuoles within pre- and postsynaptic neuronal terminals. The swollen cytoplasm contained uniformly dispersed, finely granulo-filamentous material. A few enlarged dendrites were filled with fragments of membranes or 350 Å wide vesicular and tubular structures suggestive of virus particles. Ruptured plasma membranes and curled fragments of membranes were seen around cleared cytoplasmic regions and within membrane-bounded vacuoles. Neurons or astrocytes that lined affected cells or processes frequently showed similar changes. Confluence of swollen cells or processes occurred after dissolution of their adjacent plasma membranes. Astrocytes reacted to the injury by proliferation whereas nerve cells degenerated. The findings are compared to those seen in other subacute spongiform virus encephalopathies, i. e., mink encephalopathy, Kuru and Creutzfeldt-Jakob disease. The characteristic vacuolar degeneration of nerve cells in these diseases which is associated with fragmentation and accumulation of plasma membranes is discussed with reference to the peculiar properties of the scrapie virus.

Key-Words: Scrapie — Neuronal Swelling — Neuronal Vacuolation — Plasma Membrane — Astrocytic Swelling — Astrocytic Proliferation.

Scrapie is a subacute, progressive infectious disease of the central nervous system with a long incubation period. It occurs naturally in sheep and goats and can be transmitted to mice (Chandler, 1961, 1962; Morris and Gajdusek, 1963), rats, hamsters, gerbils (Gibbs and Gajdusek, 1970) and mink (Hanson *et al.*, 1971). The pathology consists of a vacuolar degeneration of nerve cells and gliosis. Other closely related diseases, designated subacute spongiform virus encephalopathies (Gibbs and Gajdusek, 1970), are mink encephalopathy (Burger and Hartsough, 1965), Kuru and Creutzfeldt-Jakob disease (Beck *et al.*, 1966, 1969, 1970). Electron microscopic studies of the cerebral cortex from chimpanzees affected with experimental Kuru and Creutzfeldt-Jakob disease revealed abnormal arrays of membranes in and around vacuoles that developed within neuronal perikarya, dendrites and axons (Lampert *et al.*, 1969, 1971). The present study was undertaken with the aim of determining whether similar changes of neuronal membranes can be detected in the cerebral cortex of mice affected with scrapie.

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Material and Methods

Two strains of scrapie virus were employed in this study. The Compton strain, obtained from Dr. R. L. Chandler, Compton, England, isolated from sheep (Chandler, 1961) and adapted to growth in goats (Pattison and Jones, 1967) was in the 9th goat brain to brain passage upon receipt. It was easily transmitted to mice and was in the 9th mouse brain to brain passage (SPG9:M9) when employed in this study. The second strain designated 434-3-847 was isolated in mice inoculated with a suspension of brain from a naturally infected Suffolk sheep in the United States (Morris and Gajdusek, 1963; Gibbs, *et al.*, 1965). The fifth mouse brain (M5) to brain passage of the virus was used for this study.

General purpose Swiss mice from the NIH colony and ranging in age from 1 day to 1 week were used for all scrapie inoculations. Twelve mice, 8 inoculated ic and ip with 10^3 and 10^4 dilutions, respectively, of the Compton strain and 4 inoculated intracerebrally with strain 434-3-897, were sacrificed 4 to 5 months after inoculation. All animals were in advanced stages of the disease clinically manifested by wasting, lassitude, arched backs, lethargy, paresis of hind quarters and urinary incontinence.

The mice were perfused via the heart with phosphate-buffered 5% glutaraldehyde. Small fragments of cerebral cortex of well fixed brains were post-fixed in 1% phosphate-buffered osmium tetroxide. These blocks were then dehydrated, embedded in Epon and sectioned for examination by electron microscopy after staining with uranyl acetate and lead citrate. The brain of a few additional affected mice were fixed in formalin and prepared for light microscopic studies. The electron micrographs were obtained with a Siemens Elmiscope 101 operating at 80 kv.

Results

All affected mice showed varying degrees of spongiform changes in the cerebral cortex, basal ganglia, hypothalamus, brain stem and cerebellum. The vacuoles were found within the neuronal perikarya and within the neuropil but rarely in perivascular and subpial regions (Figs. 1 and 2). Leukocytic infiltrates were not encountered. Proliferated astrocytes and microglial cells were present in the damaged areas.

By electron microscopy the status spongiosus in the cerebral cortex corresponded to the following neuronal and glial changes. Focal swelling of neuronal cytoplasm was frequently seen (Fig. 3). The swollen cytoplasmic portions, although devoid of organelles, were filled with finely granulo-filamentous material. The surface of the cell next to focally cleared cytoplasmic regions usually revealed a ruptured plasma membrane. Curled fragments of membranes accumulated at the sites where ruptures had occurred (Fig. 3). Rows of vesicles and fragments of membranes demarcated the cleared cytoplasmic regions from the surrounding cytoplasm. Of particular interest was the finding that neuronal and glial processes directly adjacent to cleared portions of neighboring cells were similarly affected (Figs. 4—7). Confluence of adjacent swollen processes occurred after dissolution of their plasma membranes. Such fusion could be demonstrated not only between neurons but in one instance also between a swollen astrocyte and a nerve cell (Fig. 8). Vacuolated neuronal processes further contributed to the light microscopic appearance of status spongiosus. The damaged processes were recognized as pre- and postsynaptic neuronal terminals whenever the respective synaptic junctions were identifiable (Figs. 9, 10). The vacuoles were either completely or partially bounded by membranes. Curled fragments of membranes and vesicular structures were also seen within these vacuoles. Occasional dendrites were entirely filled with fragmented membranes (Fig. 11). In the subcortical white

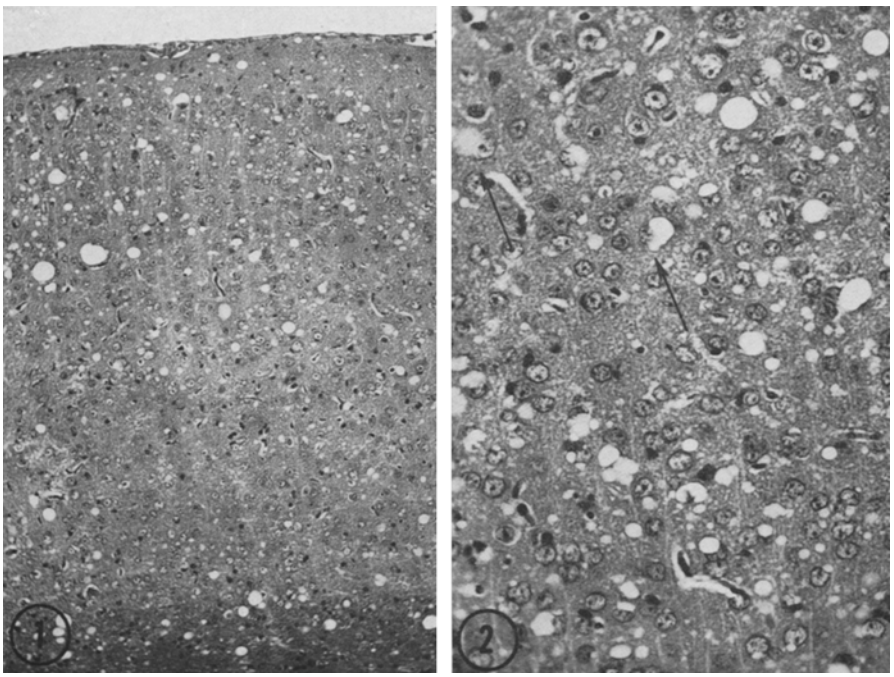


Fig. 1. Status spongiosus in cerebral cortex of mouse affected with scrapie. Note the location of the vacuoles in the neuropil but not around vessels. H.-E. ×100

Fig. 2. Vacuoles in the neuropil and in the perikaryal cytoplasm (arrows) of nerve cells and astrocytes in experimental scrapie. H.-E. ×300

matter most vacuoles as seen by light microscopy corresponded to large spaces located between the axon and the surrounding distended myelin sheath.

Many other pathologic features were observed in the affected cerebral cortex. Apart from the characteristic neuronal swelling and vacuolation, nerve cells had prominent Golgi complexes with abundant cisterns and vesicles. Large conglomerates of lipochrome pigment as well as numerous autophagic vacuoles filled with degenerating membranous structures were seen in the neuronal perikarya. Dendrites filled with vesicles and tubules measuring about 350 Å in width were numerous (Fig. 12). There were axons showing degenerative changes consisting of either clumped, granular disintegration of axoplasmic organelles or accumulations of numerous membranous dense bodies. There were also swollen axons filled with mitochondria, vesicles and filaments. The myelin sheaths about the degenerating axons were generally well preserved unless phagocytosed by microglial cells. These cells contained lipid bodies in different stages of degradation as reflected by their homogeneous, granular or uniformly-layered appearance. Proliferated astrocytes showing cytoplasmic processes filled with glial filaments were present throughout the cortex. Glycogen granules, membrane-bounded dense bodies, mitochondria and filaments were increased in the astrocytes. Except for an increased number of pinocytotic vesicles in endothelial cells, the vessels appeared normal.

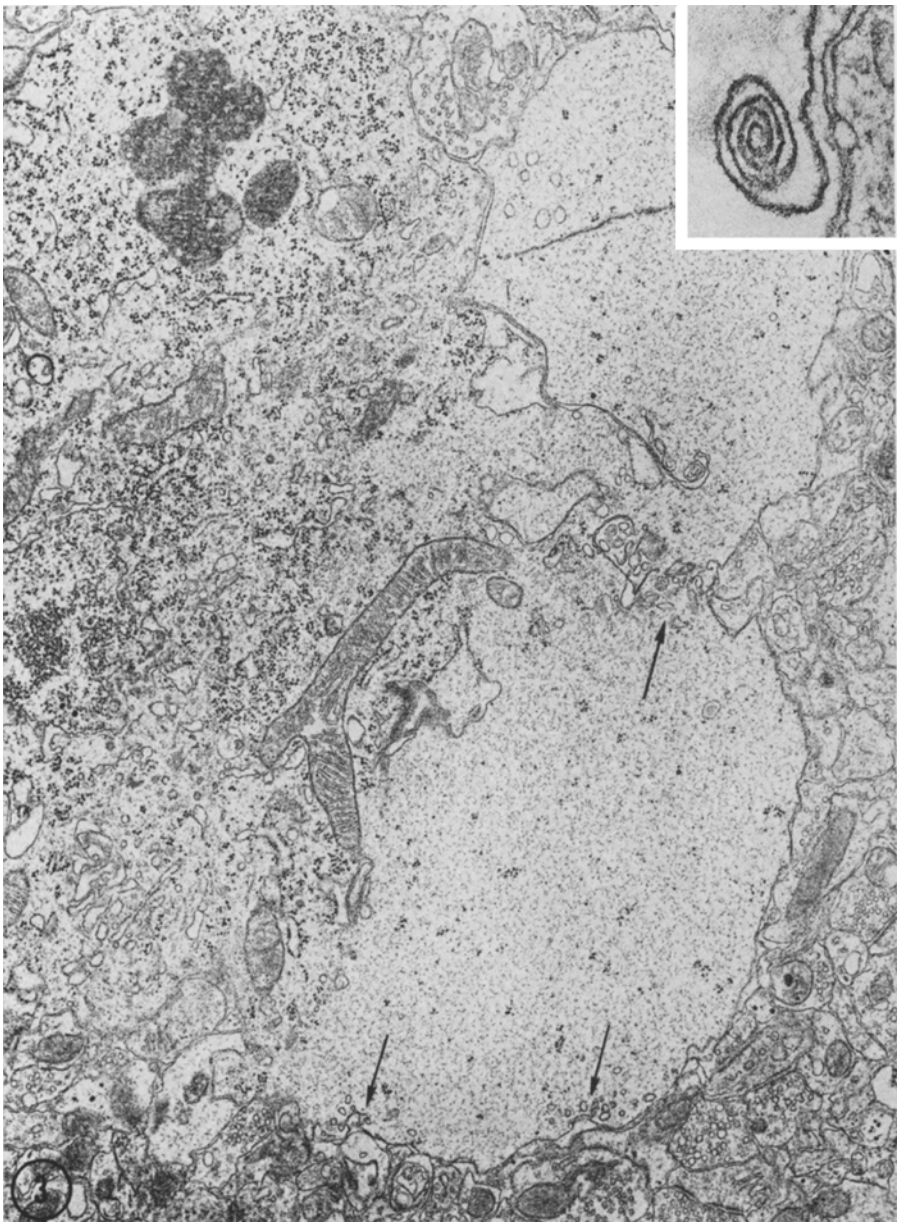


Fig. 3. Focal clearing and swelling of the perikaryal cytoplasm of a nerve cell. Note the curled fragments of membranes at sites where the plasma membrane had ruptured (arrows). ×15,000. Inset shows the curled end of a ruptured plasma membrane. ×60,000

Discussion

Although the virus responsible for the manifestation of scrapie has been isolated and partially characterized, its precise nature has not yet been determined

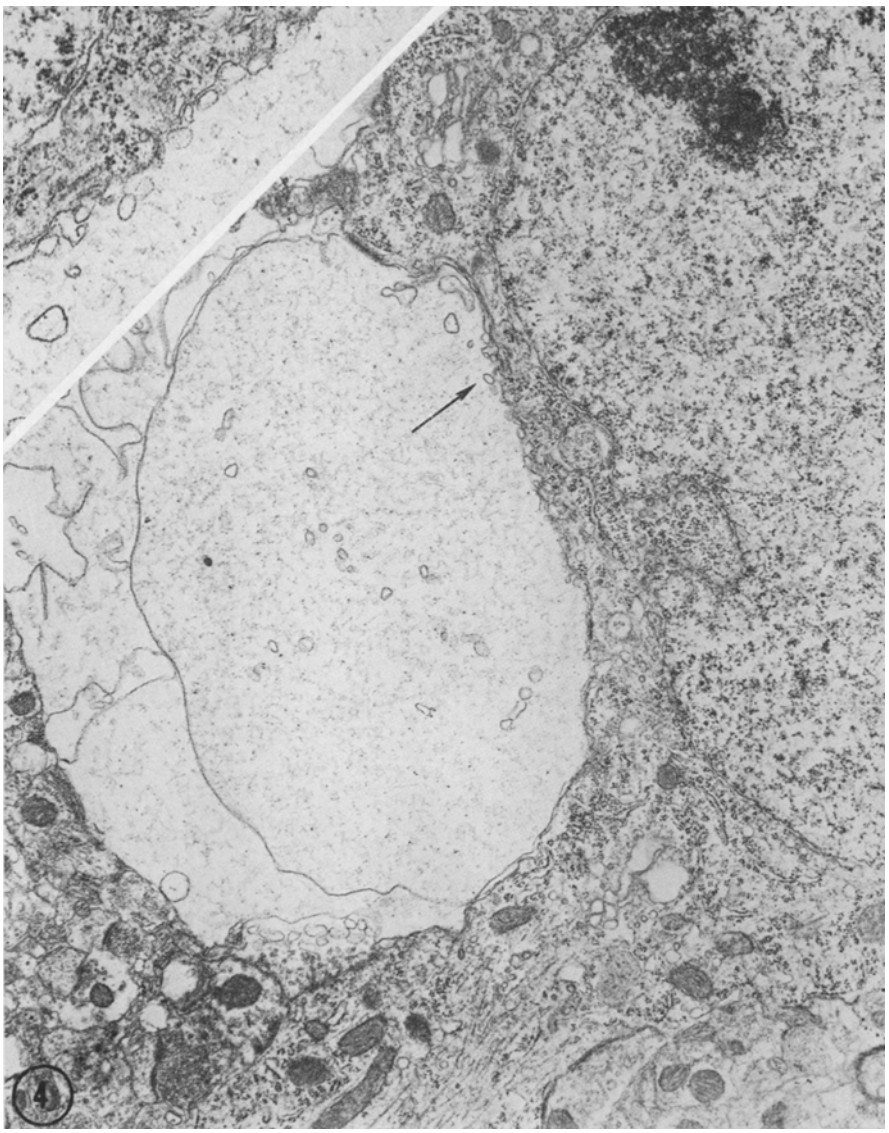


Fig. 4. A swollen and vacuolated cytoplasmic process next to an apparently normal neuron. The adjacent plasma membranes are transformed into rows of vesicles and fragments of membranes. ×12,000. The area marked by the arrow is enlarged in the inset ×25,000

nor has the virus been visualized (Gibbs and Gajdusek, 1970). As reviewed by Hunter (1970), it is a virus with unusual characteristics that strongly implicate plasma membranes as essential building blocks. Our study suggests that altered plasma membranes are the main defect leading to neuronal degeneration. Particularly noteworthy in this regard are the observations that neuronal or glial processes undergo changes in regions where their plasma membranes are in direct contact

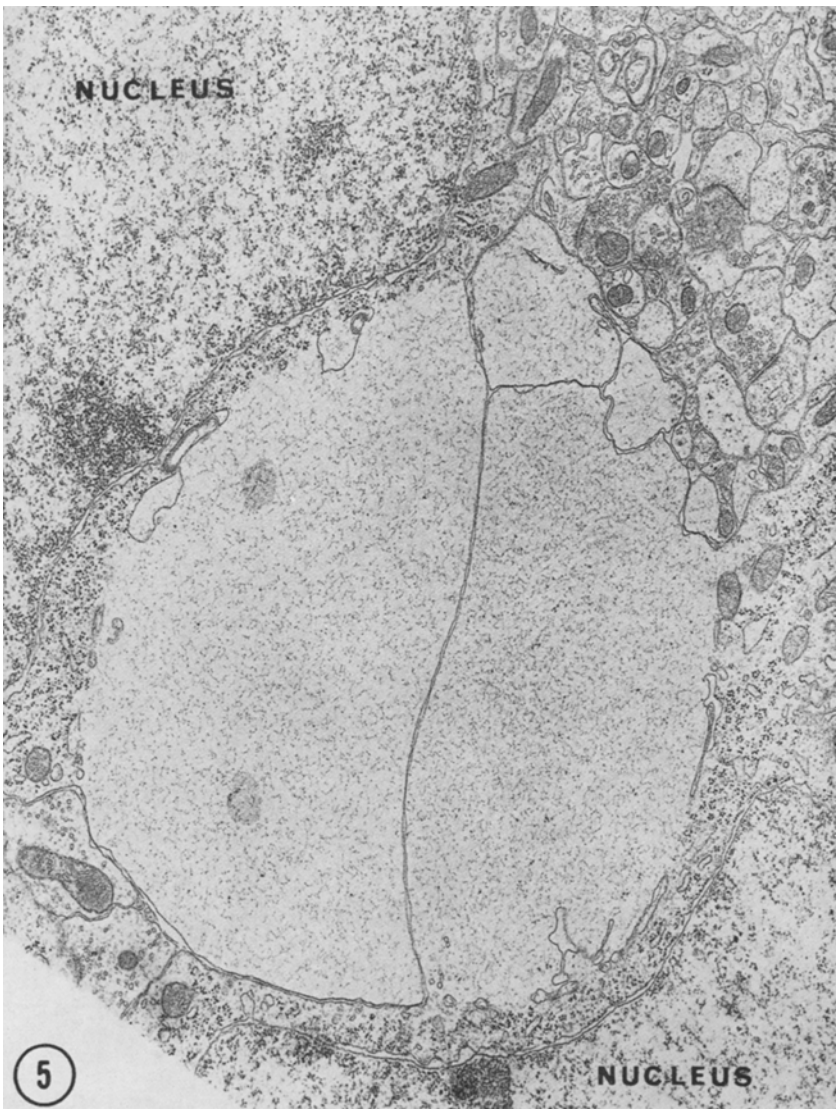
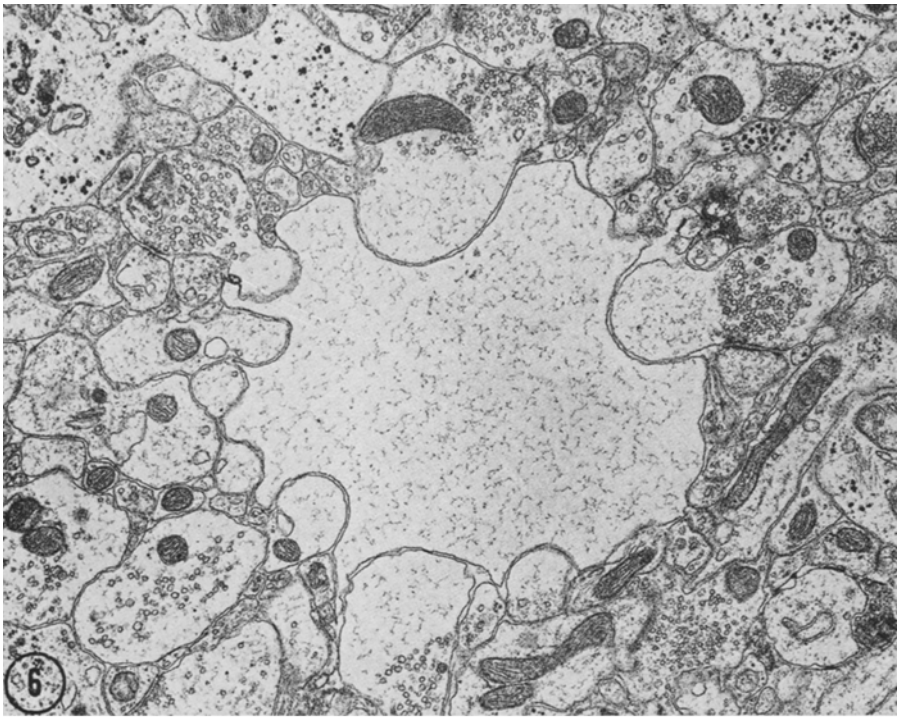


Fig. 5. Focal clearings of the cytoplasm of two nerve cells. The swollen cytoplasmic regions devoid of organelles are in direct contact with each other. ×15,000

with the membrane that surrounds the swollen cytoplasmic portions of a presumably infected cell. This appearance suggests a virus-membrane interaction with viral subunits incorporated into or spread across membranes. Fusion of the adjacent cells, after dissolution of their respective plasma membranes as illustrated occurs in other viral infections as well (Bunge and Harter, 1969; Baringer and Griffith, 1970). The finding of direct cytoplasmic continuity of a swollen astrocyte with a nerve cell is remarkable. The possibility of an artefact is considered but



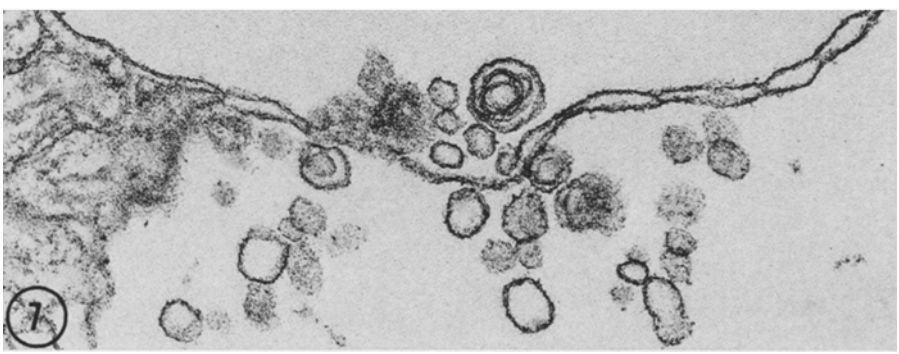


Fig. 6. Swollen cytoplasmic process devoid of organelles in neuropil. Note the focal clearing of neuronal and glial processes that are in direct contact with the abnormal cell. ×15,000

Fig. 7. Vesicular and curly fragments of the plasma membranes of two adjacent swollen cytoplasmic processes. ×60,000

because of the well preserved neuropil around these focally damaged cells, fusion is believed to have occurred *in vivo*.

Ruptured plasma membranes, curled membranes and irregular vesicular structures were recognized in the damaged cells or their processes. In this regard our observations in mice affected with scrapie did not differ from those made in studies on the cerebral cortex of chimpanzees affected with experimental Kuru

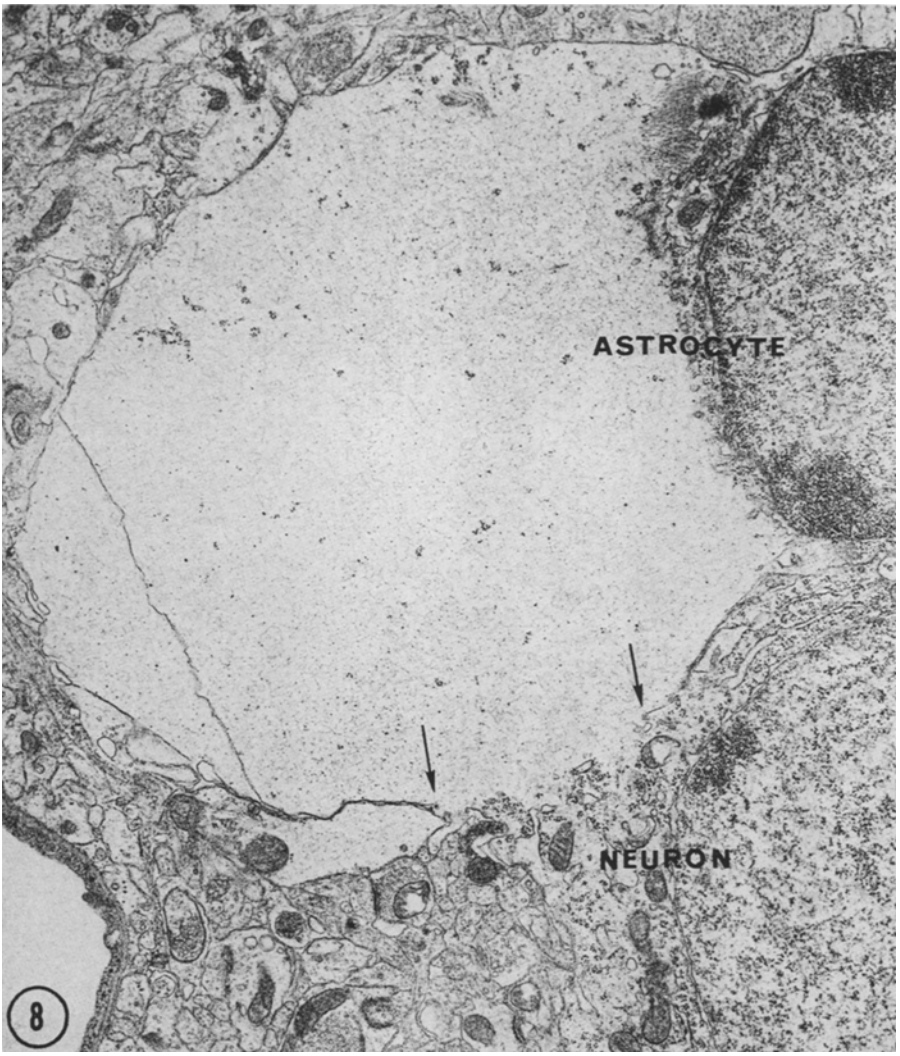


Fig. 8. Confluence of the swollen cytoplasm of an astrocyte with that of a nerve cell. Arrows point to their ruptured plasma membranes. ×10,000

or Creutzfeldt-Jakob disease (Lampert *et al.*, 1969, 1971). These membranes may represent or harbor the infectious agent or a subunit thereof. This has not yet been established, however, and as mentioned below membranous fragments and whorls may also develop secondary to other types of cell injury. The membranous structures revealed the triple-layered appearance of unit membranes and except for being more electron-dense, their morphology did not differ from that of normal plasma membranes. Much more suggestive of viral particles were the arrays of 350 Å vesicles and tubules in dendrites (Fig. 12). These structures have previously been reported in scrapie in mice (David-Ferreira *et al.*, 1968) and in experimental Creutzfeldt-Jakob disease in chimpanzees (Lampert *et al.*, 1971). Their size is in

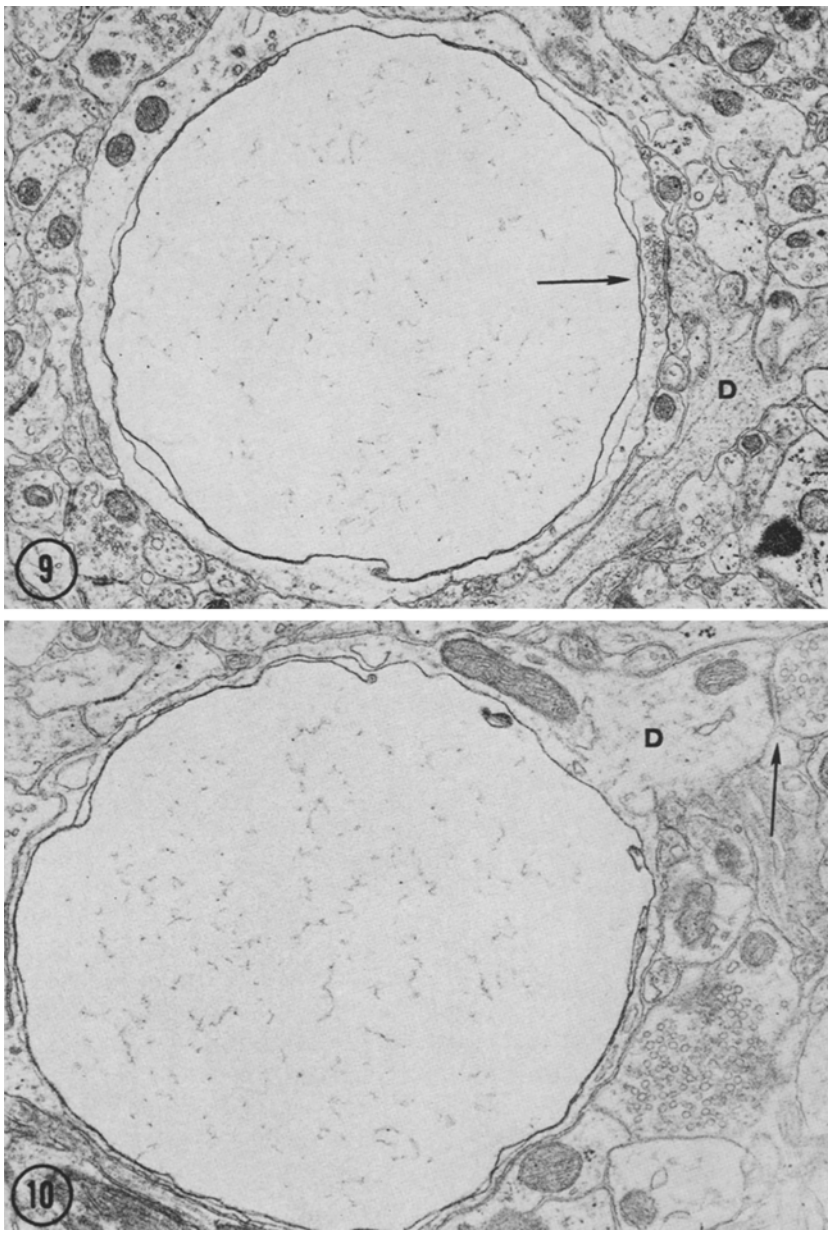


Fig. 9. Membrane-bounded vacuole in an axonal terminal. Arrow points to synaptic junction with a dendrite (*D*). ×12,000

Fig. 10. Membrane-bounded vacuole in a dendrite (*D*). Arrow points to synaptic junction. ×18,000

close agreement with the 20—30 mμ size of scrapie virus as determined by filtration experiments (Gibbs *et al.*, 1965; Hunter, 1969, 1970). Less uniform and larger vesicular structures have been observed within neuronal vacuoles in natural

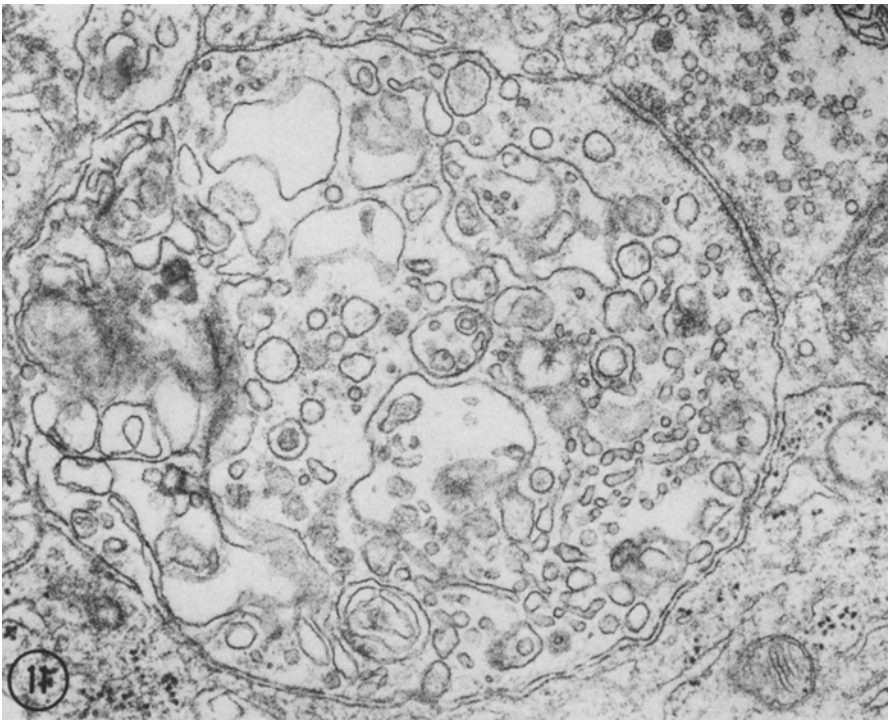


Fig. 11. Dendrite filled with membranous and vesicular structure. Note intact synaptic junction in upper right corner. ×25,000

scrapie (Bignami and Parry, 1970). In our studies the abnormal particles were, however, not found in damaged neurons or astrocytes. In a recent publication Bignami and Parry (1971) also demonstrated 350 Å wide virus-like particles in neuronal processes in natural scrapie in addition to particles of larger size.

Focal swelling and clearing of the cytoplasm next to ruptured plasma membranes were observed in astrocytes and nerve cells in experimental scrapie. Such focal cytoplasmic clearings have been recognized as characteristic findings in mink encephalopathy (ZuRhein and Eckroade, 1970), Kuru and Creutzfeldt-Jakob disease in chimpanzees (Lampert *et al.*, 1969, 1971) and significantly also in human Creutzfeldt-Jakob disease (Gonatas *et al.*, 1965). Status spongiosus of nervous tissue reflected by swollen neuronal and glial processes has also been induced by the injection of ouabaine, an agent that inhibits ionic transfer mechanisms across membranes (Bignami and Palladini, 1966). Electron microscopic studies have demonstrated, however, that intracerebral injection of ouabaine produces a generalized swelling of the glial and neuronal processes (Cornog *et al.*, 1968) but not focal clearings filled with granulofilamentous material as demonstrated here. In regard to alteration of plasma membranes, in vitro studies on the effect of ouabaine on renal tubules (Trump and Ginn, 1969) have shown that the plasma membranes of the swollen cells develop focal defects that are associated with membranous whorls.

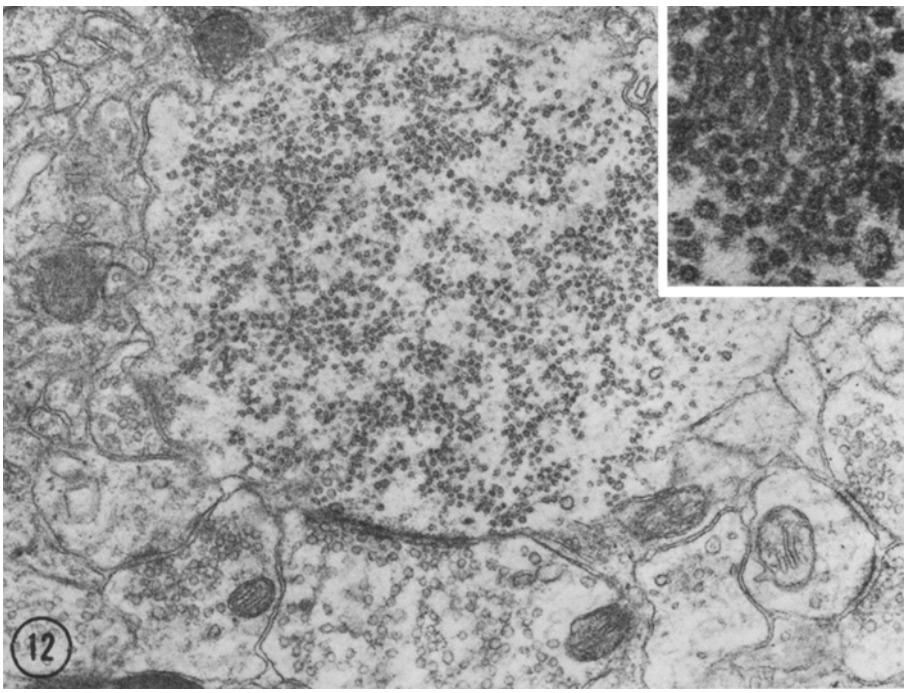


Fig. 12. Dendrite filled with vesicular profiles. A presynaptic process filled with larger synaptic vesicles is attached to the dendrite. ×18,000. Inset shows the tubular appearance of the particles found in dendrite. ×60,000

The formation of membrane-bounded vacuoles in neurons and their processes is believed to develop secondarily to the cytoplasmic clearing. Other studies on cell injury indicate that focally-damaged cytoplasm is sequestered from the surrounding cytoplasm by membranes resulting in the formation of autophagic vacuoles (Arstilla and Trump, 1968). It is possible that the same phenomenon takes place in the injured nerve cells and that only severely affected cells proceed to complete degeneration. In this regard, astrocytes, which may show focal swelling, have thus far not been observed to develop vacuoles in their cytoplasm. Astrocytes also do not degenerate in scrapie. On the contrary they react by swelling and proliferation (Chandler, 1961; Pattison and Jones, 1969) as they do in mink encephalopathy (ZuRhein and Eckroade, 1969), Kuru and Creutzfeldt-Jakob disease (Beck *et al.*, 1970; Lampert *et al.*, 1969, 1971).

Earlier electron microscopic studies on experimental scrapie in mice and rats have been more concerned with nonspecific changes that invariably develop in the central nervous system following injury to neurons (Field and Raine, 1964, 1967; Chandler, 1968; Field, 1969). Axons degenerating after destruction of their cell body develop granular disintegration of neurofilaments and focal accumulations of mitochondria and membranous dense bodies (Lampert, 1967). Various forms of lipid inclusions in microglial cells are consistently observed in macrophages after myelin disintegration. The peculiar layered structures as ob-

served in glial cells in scrapie by Field and Raine (1967) represent but one spectacular stage in the degradation of myelin (Lampert and Cressman, 1967). Although myelin sheaths about degenerated axons in the central nervous system remain intact for long periods of time, they are eventually engulfed and digested by microglial cells. The appearance of the proliferated astrocytes filled with mitochondria, ribosomes, bundles of glial filaments, lipid inclusions and glycogen granules also did not differ from that of astrocytes reacting to other injuries.

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Peter W. Lampert, M. D.
Department of Pathology
University of California at San Diego
La Jolla, California 92037 (U.S.A.)