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Abnormal Tubulovesicular Particles in Brains of Hamsters with Scrapie (42507)

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Abstract. Abnormal tubulovesicular particles of an average diameter of 23 nm have been observed in brains of mice with scrapie as well as in other animals with spongiform encephalopathies, but they were thought to be absent from the brains of hamsters with scrapie in which the highest known concentrations of the infectious agent occur. We observed in neuronal processes of hamsters as well as mice clusters of those tubulovesicular structures, most often in postsynaptic terminals. Such particles have now been seen regularly in both experimental and natural scrapie in all species examined as well as in other spongiform encephalopathies.

The subacute spongiform encephalopathies are caused by replicating agents that are subprotist in size. The structure of the pathogens is not known. It has been proposed that they are smaller than conventional viruses (1), devoid of nucleic acid (2), and may be unique infectious proteins (3–6). That hypothesis, however, has not yet been substantiated (6–8). It remains important to search for abnormal structures that might represent the etiological agent in tissues containing the infectious agents. Since our original observations of "viruslike" particles in brains of animals with scrapie (9–15) and Creutzfeldt–Jakob disease (16), similar structures have been reported by other investigators (17–21). However, those particles seemed unlikely to represent the infectious agent (22), or even a consistent expression of the pathology of spongiform encephalopathies, because they were thought to be absent from brains of hamsters with experimental scrapie (20, 21), in which the highest reported concentrations of agent occur (23). We found aggregations of 23-nm diameter tubulovesicular particles, identical to those previously described, in thin sections of brains of hamsters with scrapie. Thus, it appears that 23-nm tubulovesicular particles are consistent and unique findings in brains of animals with spongiform encephalopathies.

Materials and Methods. Swiss–Webster mice were inoculated with Chandler's original mouse-adapted strain of scrapie agent (24); brains were collected from mice at various times during incubation period and illness and fixed as previously described (12, 13, 15). Outbred golden Syrian hamsters were inoculated intracerebrally into the left frontal region with 0.03 ml of a 10% suspension of 263K strain (derived from Chandler's mouse-adapted strain) of scrapie-infected hamster brain (23), or with freshly prepared normal hamster brain; scrapie-inoculated animals had ataxia about 60 days later and died with severe wasting and immobility by about 80 days, while controls remained well. Hamsters showing signs of illness were anesthetized, and brains were fixed either by perfusion with 4% glutaraldehyde in phosphate-buffered saline or by immersion in glutaraldehyde after rapid removal. Some brains were fixed, rinsed, and postfixed in fluids containing 0.05% ruthenium red to enhance contrast as previously described (12, 13). Tissues were postfixed in 1% osmium tetroxide, dehydrated, and embedded in Araldite (hamsters) or Epon (mice) (12, 13, 15). Thick sections were stained with toluidine blue, and thin sections were prepared from areas showing vacuolation of gray matter and hypertrophy of astroglia. Sections were stained with uranyl acetate and lead

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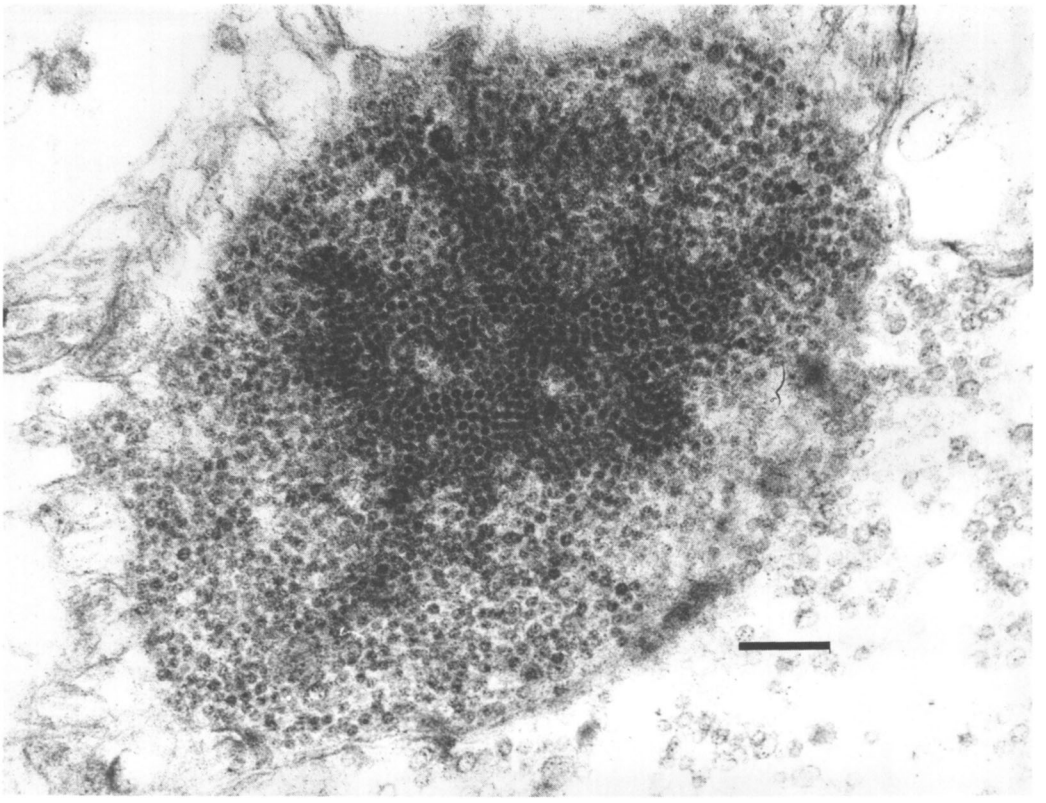

FIG. 1. Cerebral cortical gray matter of mouse with scrapie. Array of tubulovesicular particles, densely packed in center and loose on periphery. (Uranyl acetate and lead citrate. Bar = 200 nm.)

citrate, and examined at 60 kV in a Philips 201 microscope or 80 kV in a JEOL 100 CX microscope.

Results. Tubulovesicular particles identical to those previously described were readily found in postsynaptic terminals and dendrites of all mice with scrapie; the particles were either densely packed as in Fig. 1 (13, 20) or looser, as in Fig. 2. Tubulofilamentous forms, some as long as 200 nm, were seen as well as transverse circular profiles. Diameters of both forms measured 22 to 26 nm. As before, larger vesicular bodies 100 to 110 nm in diameter (Fig. 2) were frequently found in association with the smaller particles. Intensive search of normal mouse brains failed to reveal any similar structures.

In brains of hamsters early in illness and in terminal stages of scrapie, similar arrays of loosely packed particles were found in neural processes (Figs. 3, 4); densely packed particles were not observed. Transverse circular profiles with diameters of 22 to 24 nm were present, as were smaller numbers of tubulofilamentous profiles and of larger vesicular bodies of 100 to 110-nm diameters (not shown). Particles were identified in all blocks examined, although they were more difficult to find in the brains of hamsters than in mice with scrapie. A careful search in sections from brains of sham-inoculated and uninoculated hamsters revealed no similar particles.

Discussion. Osmiophilic tubulovesicular particles of similar morphology have now been observed in brains of all species of scrapie-infected animals studied (9–15, 17–21) as well as in brains of animals with Creutzfeldt–Jakob disease (16), and in those of patients with that disease (H.K. Narang, unpublished data), most frequently located in postsynaptic terminals

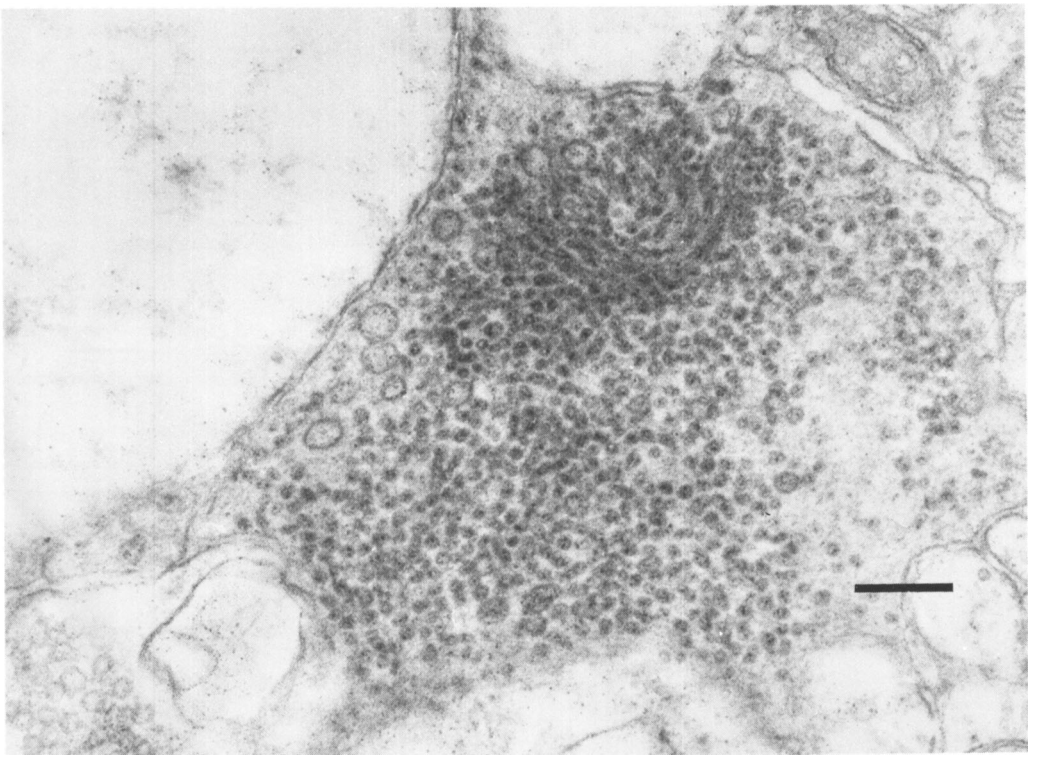

FIG. 2. Cerebral cortical gray matter of mouse with scrapie. Array of loosely packed tubulovesicular particles. (Uranyl acetate and lead citrate. Bar = 200 nm.)

(9, 12, 13, 19–21). Earlier observations reported external diameters of both circular and tubular profiles to be about 30 to 40 nm (9, 16, 17), but more recent studies of morphologically similar particles estimated smaller diameters, between 15 and 27 nm (10–14) with a mean diameter of 23 nm (15, 19, 21). Measured sizes have been reported to vary with conditions of fixation (13). The structures usually had a central lucent core, but sometimes contained a dark central filament (12, 13). It has been suggested that tubular profiles result from overlapping arrays of spherical particles (13, 21). The particles are readily distinguishable from normal cellular structures, such as synaptic vesicles, neurofilaments, glycogen granules, and ribosomes. Although resembling microtubules in size and in having central dense cores (25), the 23-nm particles clearly differed from microtubules seen in the same sections; microtubules were somewhat smaller with less prominent cores and were distributed regularly throughout the cytoplasm as seen in Fig. 4. Failure to demonstrate the 23-nm tubulovesicular structures in previous studies of hamsters (20, 21) may have resulted from sampling problems, or from the selection of animals too early in incubation period. That may also explain failure to find the 23-nm particles in spleen and other organs outside the nervous system, and greater efforts must be made to seek them there. A variety of other viruslike particles have also been observed in brains with spongiform encephalopathies (26–32); those particles, generally larger in size, have been much less consistently reported than the 23-nm particles.

The 23-nm tubulovesicular structures demonstrated here are, at very least, a consistent and unique phenomenon in the ultramicroscopic pathology of spongiform encephalopathies and potentially might even represent individual or aggregated particles or components of the infectious agents of those

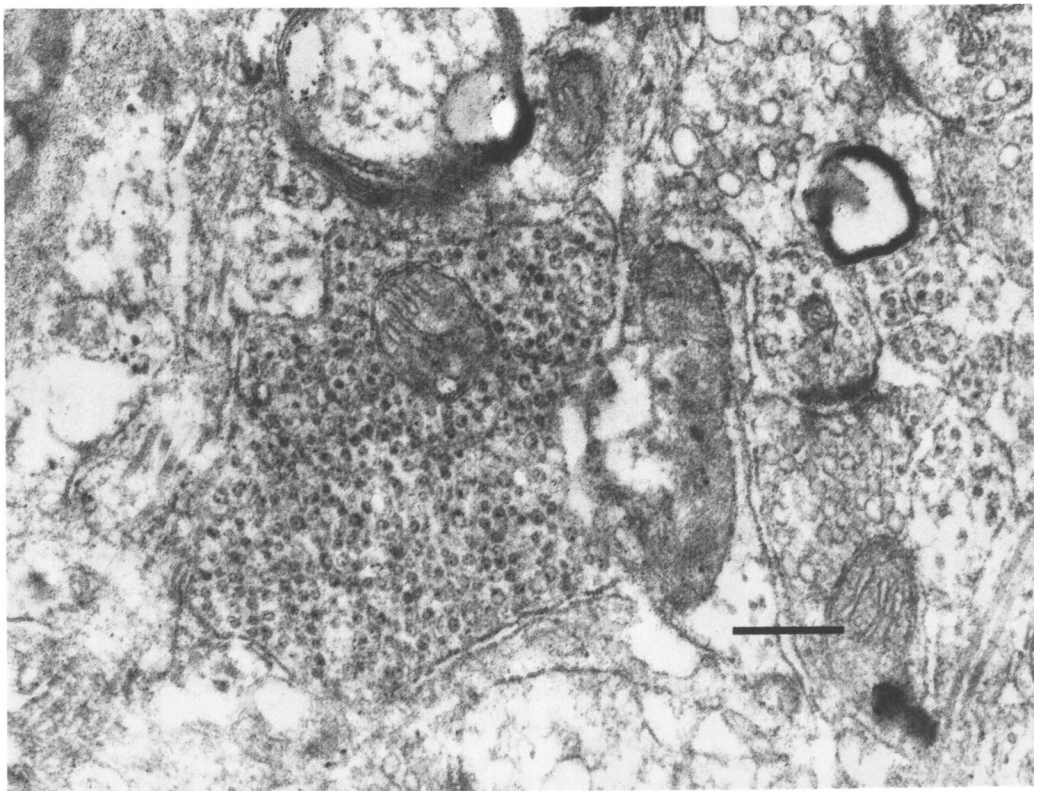

FIG. 3. Cerebral cortical gray matter of hamster with scrapie. Array of loosely packed particles. (Uranyl acetate and lead citrate. Bar = 200 nm.)

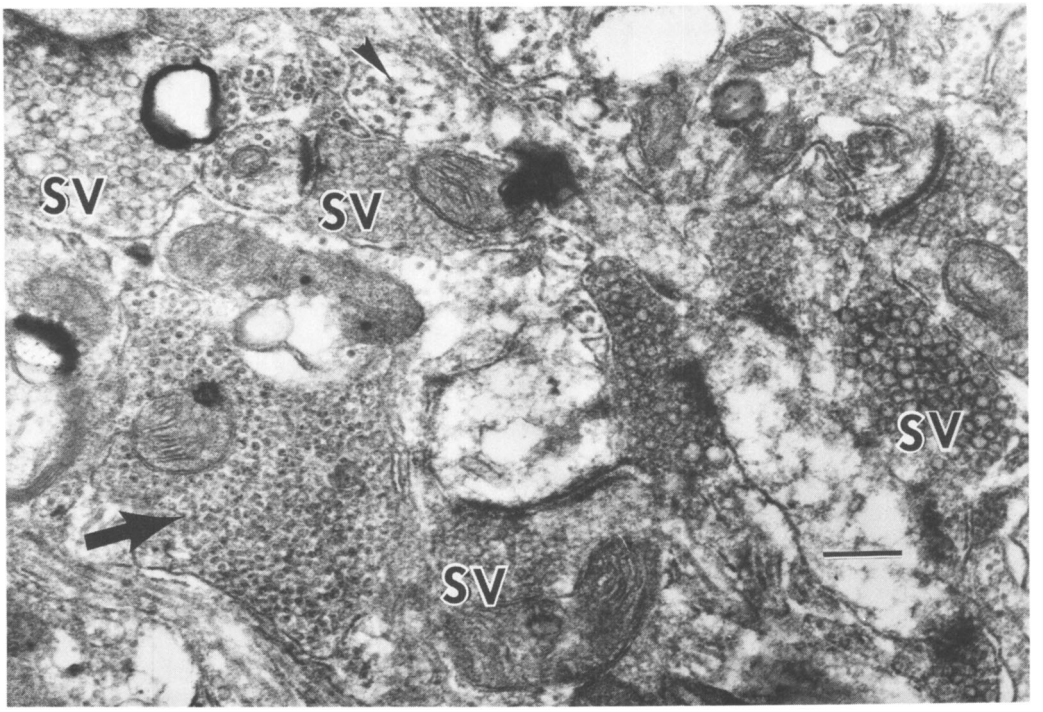

FIG. 4. Cerebral cortical gray matter of hamster with scrapie. Array of particles (arrow) smaller than synaptic vesicles (SV) and slightly larger than microtubules (arrowhead). (Uranyl acetate and lead citrate. Contrast augmented with ruthenium red stain. Bar = 300 nm.)

diseases. In spite of repeated efforts to show that the scrapie agent is subviral, no experimental evidence has convincingly demonstrated the minimal infectious unit to be less than 20 nm in diameter (7, 8, 33–35), a size consistent with that of the 23-nm particles. However, there is still no evidence that the 23-nm particles are specifically related to infectivity.

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