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Membrane-Free Scrapie Activity

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Determinations of scrapie activity in subcellular fractions from infected hamster brains through the asymptomatic and symptomatic course of infection revealed the presence of substantial amounts of scrapie infectivity in the 100,000 × *g* supernatant fractions, indicating that association with physically discernible membrane structures is not necessary for the transmission of the scrapie agent. An increase of scrapie infectivity in the 100,000 × *g* supernatant fractions after vigorous homogenization of infected membrane-rich fractions suggests that the agent is identical in membrane-rich and 100,000 × *g* supernatant fractions.

The association of scrapie activity with membrane fractions of infected cells has been well established (4, 9). Studies of the distribution of the agent have concentrated on membrane-rich subcellular fractions and the determination of a specific membrane location. However, no exclusive associations between scrapie infectivity and a specific subcellular membrane have been found (1, 5, 8, 10, 11). In light of the seemingly ubiquitous distribution of scrapie activity, the following study was undertaken to determine the scrapie infectivity in several subcellular fractions during the asymptomatic and symptomatic course of scrapie infection.

The scrapie agent used in these studies originated from the Chandler strain of mouse-adapted scrapie as previously described (6). Outbred (LVC:LAK) Syrian hamsters (Lakeview Hamster Colony, Newfield, N.J.) 3 weeks of age were inoculated by the intracerebral route with 0.05 ml of 250-1,000 × *g* fractions obtained as described below from scrapie-infected or healthy hamster brain homogenates. Antibiotics (100 U of penicillin and 100 g of streptomycin per ml) were added before inoculation.

At varying time intervals post-inoculation, four hamsters were killed with chloroform; their brains were removed, halved, and immediately cooled on ice. Two half-brains from individual hamsters were placed in formaldehyde for histological examination. The remaining brain tissue was homogenized with a Polytron (Brinkmann Instruments Inc.; 20 s, setting 4) in 54 ml of homogenization buffer (10 mM Tris [pH 7.5]-2 mM dithiothreitol-20 mM 2-mercaptoethanol-0.1% bovine serum albumin-0.4 M sucrose) and then centrifuged at 250 × *g* for 20 min. The pellet, containing predominantly nuclei and myelin fragments, was resuspended; the nuclei were pelleted through 40% sucrose at 82,500 × *g* for 1 h, leaving the myelin at the sample zone-sucrose interface. The 250 × *g* supernatant fraction was sequentially centrifuged at 1,000 × *g* and 13,000 × *g* for 10 min each. The 13,000 × *g* supernatant fraction was sequentially centrifuged at 40,000 × *g* for 20 min and at 100,000 × *g* for 1 h. All pellets were suspended by three strokes of a Vitro Teflon homogenizer in resuspension buffer (5 mM Tris [pH 7.5]-2 mM EDTA-1 mM dithiothreitol-15% sucrose). Scrapie infectivity was determined by intracerebral inoculation of weanling outbred hamsters, as described previously (10).

The 250 × *g*, 250-1,000 × *g*, and 1,000-13,000 × *g* subcellular fractions were enriched with nuclei, rapidly sedimenting membranes, and mitochondria, respectively. The 13,000-40,000 × *g* fractions were enriched with slowly sedimenting membranes, and the 40,000-100,000 × *g* fractions were enriched with ribosomes. The 100,000 × *g* supernatant fractions contained the bulk of the soluble cellular material. Figure 1C illustrates a 1,000-fold increase in scrapie activity in both the nuclear and the rapidly sedimenting membrane fractions over a period of 45 days. The mitochondrial fractions exhibited slightly more than a 100-fold increase in scrapie activity over a 29-day period, whereas the slowly sedimenting membrane and ribosomal fractions contained scrapie activity that increased 1,000-fold during the same period of time (Fig. 1B). The scrapie activity of the 100,000 × *g* supernatant fractions was unique in that it increased 10,000-fold over a 29-day period, but with a 1,000-fold increase over a period of only 15 days during the asymptomatic course of infection (Fig. 1A).

Light microscopic examination of formaldehyde-fixed brains, stained with hematoxylin and

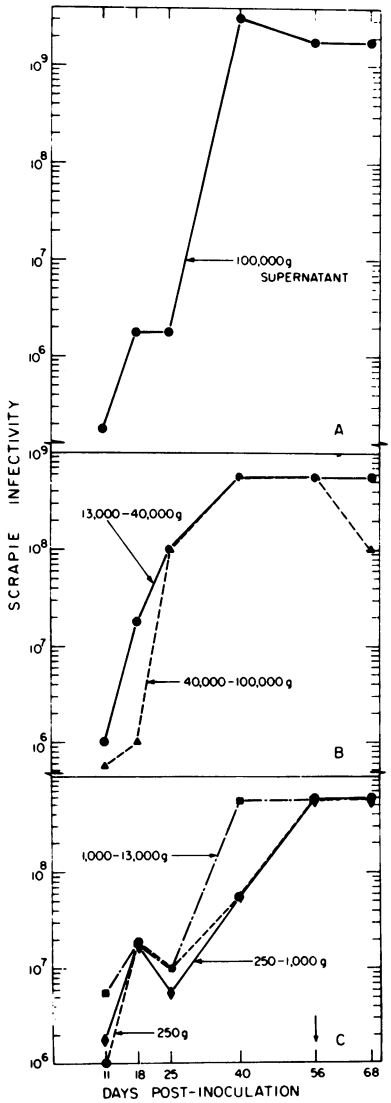

FIG. 1. *Distribution of scrapie activity (log_{10} LD_{50}/fraction) through the course of infection in subcellular fractions. (↓) Onset of clinical disease.*

eosin, revealed astrocytic hypertrophy in the brain stem at 33 days, moderate spongiform degeneration and severe astrocytic hypertrophy at 50 days, and widespread spongiform degeneration, astrocytic hypertrophy, and neuronal degeneration by the onset of clinical signs of disease at 56 days post-inoculation. No difference was observed between lesions produced by inoculation with membrane-rich fractions and those produced with 100,000 × *g* supernatant fractions.

Electron microscopic examination of material pelleted (100,000 × *g* for 16 h) from the 100,000 × *g* (1 h) supernatant fraction revealed that, although the pellets contained a high level of infectivity ($10^{8.3}$ 50% lethol dose [LD_{50}]), neither sectioned nor negatively stained preparations revealed discernible membrane structures, as reported by T. G. Malone (M.A. thesis, University of California, Riverside, 1977).

These data, obtained with the Polytron, a relatively gentle homogenization process, suggest that the rapid increase in scrapie infectivity in the 100,000 × *g* fractions might be due to the in vivo degradation of the membranes and accompanying release of the scrapie agent. Therefore, attempts were made to increase scrapie activity in the 100,000 × *g* (1 h) supernatant fractions by in vitro homogenization of membrane-rich preparations. Brains from hamsters at 3 weeks post-inoculation were used in which visible pathogenic membrane degradation was minimal. Vigorous homogenization of whole brains and infected membrane-rich fractions in the Virtis 45, followed by subcellular fractionization, resulted in a significant increase of 1 to 5 log units in scrapie infectivity associated with the 100,000 × *g* (1 h) supernatant fractions over the values associated with similar fractions after Polytron homogenization of identical tissue (Table 1).

The results of these experiments are consistent with the hypothesis that the scrapie agent is associated with developing membrane. Membrane dissolution is characteristic of scrapie pathology; it has been speculated that the dissolution is due to the action of lysosomal enzymes (7). It is possible, therefore, that the release of membrane components containing

TABLE 1. *Comparison of scrapie infectivity in subcellular fractions after differential homogenization*

Subcellular fraction	Log_{10} LD_{50}/fraction by following means of homogenization:		
	Virtis[a]	Polytron[b]	Polytron and Virtis[c]
13,000–40,000 × *g*	9.3	8.3	8.3
40,000–100,000 × *g* (1 h)	8.5	8.3	8.3
100,000 × *g* (16 h)	8.3	3.5	5.5
100,000 × *g* (16 h) supernatant	3.4	3.4	4.4

[a] Homogenization of whole brains with a Virtis blender (setting 10, 5 min).

[b] Homogenization of whole brains with a Polytron blender (setting 4, 20 s).

[c] Homogenization with a Virtis blender (setting 10, 5 min) of a 13,000 × *g* supernatant (membrane-rich) obtained after Polytron homogenization.

scrapie agent into the cytoplasm results in the 1,000-fold increase in the infectivity of the 100,000 × *g* supernatant fractions from 25 to 40 days after inoculation.

The membrane hypothesis assigns scrapie activity to an assemblage of macromolecules bound in a membrane complex (2, 3). The data presented here are compatible with the hypothesis; however, the importance of membrane structures in the transmission of the scrapie agent must be questioned and must await a careful determination of what components of the scrapie agent complex are critical to the infectious process. The 100,000 × *g* (1 h) supernatant fraction from infected hamster brain, containing high titers of scrapie infectivity, represents a suitable preparation from which to purify and characterize the infectious unit of this unique pathogenic agent.

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LITERATURE CITED

1. **Clarke, M. C., and G. C. Millson.** 1976. The membrane location of scrapie infectivity. J. Gen. Virol. **31**:441–445.
2. **Gibbons, R. A., and G. D. Hunter.** 1967. Nature of the scrapie agent. Nature (London) **215**:1041–1043.
3. **Hunter, G. D., R. H. Kimberlin, G. C. Millson, and R. A. Gibbons.** 1971. An experimental examination of the scrapie agent in cell membrane mixtures. I. Stability and physicochemical properties of the scrapie agent. J. Comp. Pathol. **81**:23–32.
4. **Hunter, G. D., and G. C. Millson.** 1967. Attempts to release the scrapie agent from tissue debris. J. Comp. Pathol. **77**:301–307.
5. **Hunter, G. D., G. C. Millson, and G. Meek.** 1964. The intracellular location of the agent of mouse scrapie. J. Gen. Microbiol. **34**:319–325.
6. **Kimberlin, R. H., and R. F. Marsh.** 1975. Comparison of scrapie and transmissible mink encephalopathy in hamsters. I. Biochemical studies of brain during development of disease. J. Infect. Dis. **131**:97–103.
7. **Marsh, R. F., J. C. Sipe, S. S. Morse, and R. P. Hanson.** 1976. Transmissible mink encephalopathy: reduced spongiform degeneration in aged mink of the Chediak-Higashi genotype. Lab. Invest. **34**:381–386.
8. **Millson, G. C., G. D. Hunter, and R. H. Kimberlin.** 1971. An experimental examination of the scrapie agent in cell membrane mixtures. II. Association of scrapie activity with membrane fractions. J. Comp. Pathol. **81**:255–265.
9. **Mould, D. L., W. Smith, and A. McL. Dawson.** 1965. Centrifugation studies on the infectivities of cellular fractions derived from mouse brain infected with scrapie. J. Gen. Microbiol. **40**:71–79.
10. **Semancik, J. S., R. F. Marsh, J. L. M. C. Geelen, and R. P. Hanson.** 1976. Properties of the scrapie agent-endomembrane complex from hamster brain. J. Virol. **18**:693–700.
11. **Siakotos, A. N., D. G. Gajdusek, C. J. Gibbs, R. D. Traub, and C. Bucara.** 1976. Partial purification of the scrapie agent from mouse brain by pressure disruption and zonal centrifugation in sucrose-sodium chloride gradients. Virology **70**:230–237.