Large Fraction of Scrapie Virus Resistant to Lipid Solvents (36798)

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(Introduced by S. Baron)

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The virus of scrapie has not yet been sufficiently purified to allow chemical and structural characterization. Because of the resistance of a fraction of infectious virus to a variety of chemical and physical agents, several nonviral theories of the nature of the agent have appeared (1, 2). Nevertheless, much of the available data remain consistent with those for a small virus. Its sensitivity to ether (3, 4), therefore, is of interest because it suggests a lipid component. In those reports, losses of 90–99% of infectivity resulted following treatment with ether. Although clearly significant, these losses are not as great as those of known ether-sensitive viruses. Chloroform is a more efficient lipid solvent, and may inactivate some lipid-containing viruses which are not affected by ether (5). Treatment of scrapie virus preparations with chloroform–methanol or chloroform–butanol mixtures, however, did not result in significantly greater losses of virus than treatment with ether (6–8). Furthermore, fluorocarbon (trichlorotrifluoroethane) had a relatively slight effect on the infectivity of scrapie virus (7), although that solvent extracts a broad range of proteins, lipids, and particulates, including membranes, from tissue suspensions, and also inactivates some ether-sensitive viruses. Because evidence for the association of lipid with scrapie virus is inconclusive, we reexamined the effects of ether, chloroform, and fluorocarbon on the virus.

Materials and Methods. An homogenate of brains from Swiss mice infected with the Chandler strain of scrapie virus (9) was adjusted to 2% (w/v) in Tris-buffered saline (TBS: 0.05 *M* Tris-HCl, 0.1 *M* NaCl, pH 7.5), and maintained at 4–6° during all procedures. The suspension was sonicated using a Biosonik III sonicator at 70% maximum output for 2 cycles of 1 min each. A portion was twice homogenized with 0.5 vol of fluorocarbon (Freon-113, E. I. Dupont de Nemours Co.) for 30 sec in a Virtis 45 homogenizer at maximum speed. The aqueous phase was collected after centrifugation at 800*g* for 10 min. A fraction of this was sedimented at 105,000*g* for 1 hr, and the pellet was resuspended in TBS and sonicated.

Samples of the crude, sonicated suspension, the fluorocarbon-extracted homogenate, and the resuspended pellet of the fluorocarbon preparation, were treated with ether or chloroform. Equal volumes of solvent and viral suspension at 6° were shaken vigorously for 10 min. The aqueous phases were collected after centrifugation at 800*g* for 15 min. Ethyl ether (Fisher, certified grade) from a freshly opened container, anhydrous and peroxide-free, was used.

For viral assay in mice, preparations were diluted 10-fold serially in either saline or saline containing normal, homologous mouse brain suspension [NMBr, final 1% (w/v)]. One fraction of the latter mixture was additionally sedimented at 105,000*g* for 1 hr, and the pellet was resuspended in saline. Titrations were performed and read as described previously (9). Ten general purpose Swiss mice were used per dilution. Inoculations were intracerebral and LD_{50} endpoints determined by the method of Spearman and Karber cited in Ref. (10).

Results and Discussion. Table I gives re-

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TABLE I. Sensitivity of Scrapie Virus Preparations to Fluorocarbon, Ether and Chloroform.[a]

Preparation of virus	Control titrations in saline	Ether			Chloroform	
		Saline	1% Normal mouse brain	Sedimentation[b] with 1% normal mouse brain	Saline	1% Normal mouse brain
Sonicated	7.70 (0.17)	5.53 (0.17)				
Sonicated, fluorocarbon-extracted	7.11 (0.16)	5.50 (0.15)	5.75 (0.20)	5.21 (0.11)	6.37 (0.22)	6.40 (0.23)
Pellet of sonicated, fluorocarbon-extracted[c]	6.68 (0.20)	4.70 (0.21)	4.79 (0.18)	4.71 (0.19)		

[a] Crude, sonicated brain homogenates, fluorocarbon-extracted suspensions, and resuspended sediments (105,000g) were treated with ether or chloroform. Titrations were made in saline or saline containing 1% normal brain suspension. Titers are expressed as $\log_{10}$ mouse LD_{50}/ml ($\pm$ SE in parentheses).

[b] Preparations were sedimented (105,000g) with 1% normal mouse brain homogenate, and the pellets were resuspended and titrated in saline.

[c] Pellet obtained by sedimentation at 105,000g, 1 hr.

sults of titrations for the various preparations of scrapie virus. More than 2 $\log_{10}$ of crude, sonicated virus was inactivated by ether. Only 0.6 log of virus was lost by partial purification with fluorocarbon. Of the virus remaining after fluorocarbon extraction, ether inactivated 1.6 log whereas only 0.7 log was inactivated by chloroform.

As a test of the conditions of ether and chloroform treatments used, viruses with known sensitivities to ether were treated in a manner similar to that of scrapie virus. Type II poliomyelitis virus (ether resistant), Western equine encephalomyelitis virus (WEE) and Newcastle disease virus (NDV) (ether sensitive) were grown in tissue culture, and were suspended in 2% normal mouse brain homogenate in TBS prior to treatment with ether and chloroform as described. Titrations were made in normal saline and assayed on monolayers of HeLa and Vero cells. The titers of WEE and NDV were reduced by 5 to 8 log TCD_{50} by ether or chloroform treatments. A similar result was obtained with WEE which had been propagated in mouse brain. There were no decreases in the titers of poliovirus. As intended, these results show only that ether and chloroform, under these conditions, reduced the infectivity of two lipid-containing viruses in the manner expected.

Thus, in general agreement with the results of others (3, 4, 6–8), ether and chloroform appeared to act on scrapie virus in a manner quantitatively different from their action on other ether-sensitive viruses. It remains possible that the resistant fraction of scrapie virus was protected by association with tissue components, or aggregation with itself. These explanations seem less likely, however, because, as shown in Table I, virus which was partially purified with fluorocarbon was less sensitive to ether than crude virus, and sedimented virus, which might be further aggregated to itself and to nonviral components, was reduced 2 log by ether treatment. A unique sensitivity of scrapie virus to lipid solvents was demonstrated further, in that, following partial purification with fluorocarbon the virus was more sensitive to ether than to chloroform. Furthermore, the same amount of ether-resistant virus ($1 \times 10^{5.5}$ LD_{50}) was found before and after fluorocarbon purification, whereas purified virus might be expected to be more sensitive.

A possible explanation for these findings is that scrapie virus is present in brain preparations in both solvent-sensitive and solvent-resistant forms. An association of scrapie virus with cell membranes has been suggested,

and by centrifugation it appears that membrane-associated virus may constitute up to 90% of the infectivity in some brain suspensions (11, 12). Such readily sedimented, infectious virus at membranes (perhaps incompletely assembled) may correspond to that fraction of virus which is sensitive to the action of ether or chloroform. Conversely, chloroform-resistant virus would not contain lipid as a component, and may represent free, membrane-dissociated virus.

There is no direct electron-microscopic evidence for the existence of membrane-associated or free scrapie virus. Virus which is incompletely assembled at membranes would not be easily detected unless a very large proportion of cells were involved, and the titer of "free virus" is sufficiently low to make purification and visualization difficult. Nevertheless, this hypothesis might be tested by comparing the ether- and chloroform-sensitivities of fast and slow sedimenting virus.

The results do not eliminate the possibility that some component necessary for infectivity is soluble in ether and therefore removed. In another report, however, only a minute amount (0.01%) of infectivity itself was recovered from the ether phase (13). The possibility that there are, normally present in brain homogenates, helper components or "linkage substances" (14) which are ether-sensitive was considered. In the reconstruction experiment (Table I), the infectivity of ether and chloroform treated preprations was not restored by addition of, or sedimentation with, normal brain material. The existence of helper substances which might be present in scrapie-brain suspensions but absent from normal brain will be more difficult to disprove or substantiate.

Summary. An association of lipid with the virus of scrapie was examined by quantitating the effects of ether, chloroform and fluorocarbon on infectivity. Treatment of infected brain suspensions with ether reduced infectivity up to 2.2 $\log_{10}$ mouse LD_{50}. Chloroform had less effect than ether on the virus, and only a slight loss resulted from partial purification with fluorocarbon. In addition, substantial infectivity resisted sequential treatments with fluorocarbon and chloroform. The results suggest that scrapie virus was present in both solvent-sensitive and solvent-resistant forms, and that the latter probably did not contain lipid as an essential component. The infectivity of ether- and chloroform-treated preparations was not restored by addition of, or sedimentation with, normal, homologous brain suspensions suggesting that ether-sensitive helper substances were not present in normal mouse brain homogenates.

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