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EFFECT OF DICHLOROTETRAFLUOROETHANE ON THE INFECTIVITY OF VIRUSES AND RICKETTSIAE SENSITIVE TO TRICHLOROTRIFLUOROETHANE

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ABSTRACT

In contrast to the "true" viruses, agents of the psittacosis group and most of the rickettsiae are markedly inactivated by treatment with the fluorocarbon trichlorotrifluoroethane (Freon-113). The results of further tests indicate that these organisms that are sensitive to Freon-113 are not inactivated by comparable treatment with dichlorotetrafluoroethane (Freon-114). Infectivity titers of suspensions of five representative rickettsiae were reduced one to four logs by emulsification with Freon-113, whereas similar treatment with Freon-114 caused no significant titer change for four of the rickettsiae and a one-log decrease for the most sensitive one. Titers of suspensions of psittacosis virus and murine pneumonitis virus, which were reduced one to two logs by extraction with Freon-113, were not affected by treatment with Freon-114. A possible explanation of these differences is that Freon-113 is a much better lipid solvent than Freon-114. Difference in solvent power and/or density of these fluorocarbons is reflected in their relative effectiveness in clarifying yolk-sac suspensions of the organisms tested: Freon-114 removed 50 per cent of the protein and 15 per cent of the lipid in a single extraction compared with 50 per cent of the protein and 78 per cent of the lipid by Freon-113. To remove larger percentages of protein and lipid, multiple extractions with Freon-114 are feasible. The infectivity of psittacosis virus in a yolk-embryo suspension was not reduced by three successive extractions with Freon-114.

The fluorocarbon method of virus purification, originally described by Gessler, Bender, and Parkinson in 1956,[1] has been reported by subsequent workers to be applicable to a number of animal viruses: those of poliomyelitis, Rous sarcoma, foot-and-mouth disease, vaccinia, mumps, influenza, and the ECHO and Coxsackie groups. However, as we indicated in an earlier report,[2] the rickettsiae and viruses of the psittacosis group are, in general, markedly inactivated by extraction with Freon-113 in contrast to the so-called true viruses, which are unaffected. In the same report it was shown that the stability of a virus was dependent upon the nature of the medium in which it is suspended, i.e., partially purified Venezuelan equine encephalitis (VEE) virus suspended in a buffered salt solution was inactivated by Freon-113, although this virus in tissue culture or chick embryo suspension was quite stable to treatment with Freon-113. It was found that the inactivation of partially purified VEE virus by Freon-113 could be prevented by the addition of small amounts of protein or very small amounts of carbohydrate polymers, such as methylcellulose or dextran sulfate. On the other hand, rickettsiae or psittacosis group viruses were not stabilized by the addition of even large quantities of these polymers.

It is the purpose of this paper to report that it is possible to purify viruses and rickettsiae that are inactivated by fluorocarbon extraction if Freon-114 is used instead of Freon-113.

Table I compares the structural formulae and boiling points of Freon-113 and Freon-114. Note that Freon-114, dichlorotetrafluoroethane, is a symmetrical molecule with a boiling point of 3.6° C in contrast to the unsymmetrical Freon-113, trichlorotrifluoroethane, which boils at 47.6°C.

The following general extraction procedure has been employed to compare the effects of Freon-113 and Freon-114. The infectious suspension, usually 10 per cent yolk sac, was mixed with the fluorocarbon in a ratio of two parts suspension to one part fluorocarbon. This mixture was homogenized in a Servall Omni-Mixer for one minute with the blendor bowl immersed in a salt-ice bath at 0° to 3° C. After homogenization, the emulsion was centrifuged at 1800 rpm for five minutes. Centrifugation caused the formation of two layers. The upper, aqueous layer was removed from the lower, semisolid layer of protein and lipid in fluorocarbon and was assayed for infectivity in embryonated eggs of the appropriate age.

A comparison of the effect of Freon-114 and Freon-113 on the infectivity of five rickettsiae and two viruses of the psittacosis group is shown in Table II.

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1. Gessler, A. E,; Bender, C. E.; and Parkinson, M. C. "A new and rapid method for isolating viruses by selective fluorocarbon deproteinization," Traus NY Acad Sci 18:701-703, 1956.
2. Wachter, Ralph F., and Comer, JoAnn F. "Effect of fluorocarbon treatment on the infectivity of several viruses and rickettsiae," Fed Proc, 20/1 pt 1:437, 1961.

TABLE I. COMPARISON OF STRUCTURES AND BOILING POINTS OF DICHLOROTETRAFLUOROETHANE AND TRICHLOROTRIFLUOROETHANE

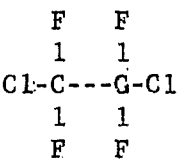
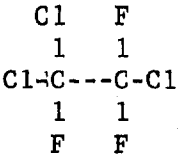
COMMERCIAL NAME	STRUCTURAL FORMULA	BOILING POINT, °C
Freon-114		3.6
Freon-113		47.6

TABLE II. EFFECT OF FREON-114 ON THE INFECTIVITY OF ORGANISMS SENSITIVE TO FREON-113

ORGANISM	INFECTIVITY CHANGE PRODUCED BY:[a]	
	FREON-113	FREON-114
Rickettsia rickettsii	-3.0	-0.3
Rickettsia typhi	-3.0	-0.3
Rickettsia conori	-4.0	-0.3
Rickettsia akari	-4.5	-1.1
Coxiella burnetii	-1.3	0
Psittacosis virus	-2.3	-0.3
Murine pneumonitis virus	-0.9	-0.3

a. Δ log yolk-sac LD_{50} per ml.

The values shown here represent the average infectivity changes obtained in three experiments with each organism. The infectivity titers of Rickettsia rickettsii, Rickettsia typhi, and Rickettsia conori were reduced three to four logs by Freon-113 but not significantly by Freon-114. The infectivity of Rickettsia akari was reduced 4.5 logs by Freon-113 compared with 1.1 log by Freon-114. Freon-114 had no effect on the titer of Coxiella burnetii, but extraction with Freon-113 resulted in an average titer reduction of one log for suspensions of this rickettsia. Infectivity titers of suspensions of psittacosis virus (Borg strain) and of murine pneumonitis virus were reduced one to two logs by Freon-113 extraction but not to a significant extent by extraction with Freon-114.

The observation that Freon-114 does not inactivate these rickettsiae and viruses might be explained on the basis of solvent power. The so-called Kauri-butanol value for Freon-113 is 32, that for Freon-114 is 12. In general, the higher the Kauri-butanol number, the better the solvent. Differences in solvent power and/or density of these fluorocarbons is reflected in their relative effectiveness in clarifying embryo-yolk suspensions of psittacosis virus (Table III). Freon-114 removed 30 per cent of the protein and 10 per cent of the lipid in a single extraction compared with 50 per cent of the protein and 60 per cent of the lipid by Freon-113.

TABLE III. EFFECT OF MULTIPLE EXTRACTIONS WITH FREON-113 AND FREON-114 ON EMBRYO-YOLK SUSPENSIONS OF PSITTACOSIS VIRUS

FLUOROCARBON	NUMBER OF EXTRACTIONS	TITER [a] CHANGE	PERCENTAGE REDUCTION PROTEIN	LIPID
Freon-113	1	-1.8	50	61
	2	-2.5	71	97
	3	-3.0	84	97
Freon-114	1	0	30	10
	2	-0.3	56	34
	3	-0.2	76	84

a. Δ log yolk-sac LD_{50} per ml.

Although Freon-113 caused much more rapid and complete removal of lipid than did Freon-114, three successive extractions with Freon-114 removed 84 per cent of the total lipid and 76 per cent of the protein. However, the infectivity of psittacosis virus was not reduced by the three extractions with Freon-114, in sharp contrast to the inactivation caused by Freon-113. These data suggest that multiple extractions with Freon-114 are feasible with these more sensitive organisms.

As was mentioned earlier, partially purified VEE virus was inactivated by treatment with Freon-113. Since the effects of Freon-113 and Freon-114 on the infectivity of the rickettsiae and psittacosis viruses tested were markedly different, it was of interest to us to determine the relative effect of these fluorocarbons on partially purified equine encephalitis viruses. VEE virus and Eastern equine encephalitis (EEE) virus were partially purified from tissue culture fluids by two cycles of centrifugation and were suspended in phosphate buffer. Aliquots of these samples were homogenized with Freon-113 or Freon-114 as described above. As shown in Table IV, Freon-114 also inactivated these viruses when the suspension fluid was phosphate buffer only, although the average titer decrease produced by Freon-114 was less than that caused by Freon-113.

TABLE IV. COMPARATIVE EFFECT OF FREON-113 AND FREON-114 ON THE INFECTIVITY OF VEE AND EEE VIRUSES

VIRUS	DECREASE IN INFECTIVITY [a] WITH	
	FREON-114	FREON-113
VEE	0.5	1.3
VEE	1.4	2.1
EEE	1.7	2.5
EEE	1.9	2.5

a. Δ log amniotic LD_{50}

In summary, it is felt that fluorocarbon extraction using Freon-114 is of value for the partial purification of rickettsiae and of those viruses that are inactivated by treatment with Freon-113. In addition, these fluorocarbons might be of interest as differential solvents in studying the surface characteristics of viruses and rickettsiae.