Sensitivity of Various Viruses to Chloroform.* (26459)

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Several authors(1,2) have suggested that identification of certain viruses could be simplified by determining their susceptibility to ether or sodium desoxycholate. Although such sensitivity appears to be a rather constant characteristic of some viruses, it appears to be a group attribute and thus should be of considerable utility in the initial classification of a newly isolated, unidentified agent. Of the 2 systems, that utilizing ether has perhaps been employed more often but with considerable variation in the procedures. These have been in terms of the ether and concentration employed and temperature and time of exposure. Finally, the residual ether must be removed by evaporation. Sodium desoxycholate has its own disadvantages in that solutions have to be prepared and sterilized for this purpose.

It occurred to us that if the method could be more readily standardized and simplified it could be of considerable aid, especially for those working in the respiratory virus field. Chloroform suggested itself as possibly a more efficient substitute for ether because of its greater polarity as a lipid solvent. It offered the additional advantage of being heavy and, therefore, readily separable by sedimentation. It was found that those agents that are susceptible to ether are also susceptible to chloroform while those that are resistant to ether are not affected by chloroform.

Materials and methods. Chloroform. This was of the analytical reagent grade (Mallinkrodt). *Viruses.* The various strains employed were obtained from a variety of sources but their identity was reestablished by appropriate serological tests in this laboratory. *Hemadsorption tests.* These were performed as described by Chanock, *et al.* (3). *Hemagglutination tests.* Those conducted with the influenza viruses were carried out with formalinized human erythrocytes as previously reported from this laboratory(4). Hemagglutination with the hemadsorption viruses was measured as described by Chanock(3).

Chloroform treatment. It was found that a mixture of 0.05 ml of chloroform and 1 ml of either allantoic or tissue culture fluid containing virus represented a suitable proportion. Although a number of mixing times were tested, it was soon learned that 10 minutes of shaking the chloroform-virus containing fluid was sufficient to kill the susceptible but not the resistant agents. It seemed to be immaterial whether shaking was performed by hand at room temperature or in a mechanical mixer at 4°C. Immediately after shaking, the fluid usually was centrifuged at 400 rpm for 5 minutes. The chloroform then appeared on the bottom of the tube, above this was an opaque, interphase layer, covered by the clear, suspending medium. The latter was removed and used for either egg or tissue inoculation or for hemagglutination. Separation, also, can be accomplished by permitting the chloroform to sediment in tubes standing in a rack. This may be particularly advantageous when there are many preparations to handle.

Results. All of the influenza strains tested (PR-8, FM-1, Asian and B(GL)) proved to be non-infectious after undiluted, infected allantoic fluid was shaken with chloroform and the supernate inoculated into the allantoic cavities of 10-day embryonated eggs. This was also true of a PR-8 strain which had been adapted to grow well in embryo bovine kidney cells(5) (Table I). However, ability of these fluids to agglutinate red cells remained relatively undisturbed, even if exposure to chloroform exceeded 10 minutes (Table II). Such treated virus remained suitable for use in the hemagglutination-inhibition test.

The hemadsorption viruses also appeared to be inactivated by exposure to chloroform.

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TABLE I. Effect of Treatment with Chloroform upon Viability of Several Viruses.

Virus	Cell substrate	Virus titer* $CHCl_3$ Treated	Virus titer* $CHCl_3$ Untreated	Chloroform susceptible
Influenza A (PR-8)	Embryo bovine kidney	0	5.0	Sensitive
Parainfluenza† 1	Monkey kidney	0	1.8	"
2	*Idem*	0	1.2	"
3	"	0	2.1	"
Polio I (Mahoney)	"	7.7	7.7	Resistant
Coxsackie A-9	"	7.9	8.2	"
" B-1	"	6.2	6.7	"
ECHO 4	"	4.7	5.2	"
" 6	"	7.2	7.2	"
" 9	"	6.2	6.7	"
Adenovirus 3	HeLa	5.2	5.2	"
" 4	"	4.9	4.9	"
" 7	"	5.2	5.5	"
Coe	"	6.9	6.9	"
Chavis	"	0	5.7	Sensitive
151 J	Monkey kidney	6.6	6.6	Resistant
M. W.	Hep.-2	5.7	5.7	"

* Expressed (except as noted) as log $TCID_{50}$/ml.
† Parainfluenza virus titers expressed as log HAU/ml.

Another, as yet unidentified, agent which has been obtained from human throats and labelled "Chavis virus" in this laboratory was readily inactivated by chloroform. This agent is also susceptible to ether. On the other hand, poliovirus 1 (Mahoney), Coxsackie A-9 and B-1, echo 4, 6 and 9, adenovirus 3, 4 and 7 and Coe virus all survived exposure to chloroform as they have been reported to do to ether. Viruses 151J and MW recently isolated in this laboratory are resistant to both ether and chloroform. One of these is suspected of being an adenovirus and the other, a member of the echo group.

Discussion. The advantages of being able to categorize a virus by a series of relatively simple physical or chemical measures are apparent and have appealed to other investigators. The apparent efficacy of the method described here is particularly attractive. Whether ether susceptible arbor viruses also would be sensitive to chloroform can not be stated on the basis of this study. They should behave in similar fashion unless treatment of infected mouse brain imposes additional requirements as regards exposure time or concentration of chloroform. Whether the immunogenicity of chloroform-sensitive viruses is altered by such treatment also remains to be determined. Davenport, *et al.*(6) recently reported that influenza virus exposed to ether retained its abilities to agglutinate red cells and also was capable of inducing antibodies to inhibit this reaction. The authors suggest that a vaccine made from virus treated in this way might have some advantages over those currently in use. The relative effectiveness of chloroform treated virus in this regard is to be investigated.

TABLE II. Effect upon Hemagglutination Titer of Shaking Influenza Virus (PR-8) for Various Periods with and without Chloroform.

Reciprocal virus dilution	10 min. $CHCl_3$	10 min. None	30 min. $CHCl_3$	30 min. None	60 min. $CHCl_3$	60 min. None
10	+	+	+	+	+	+
100	+	+	+	+	+	+
200	+	+	+	+	+	+
400	+	+	+	+	+	+
800	+	+	+	+	+	+
1600	0	+	+	+	0	+
3200	0	+	0	0	0	+

Summary. A number of myxoviruses were found to be susceptible to chloroform on short exposure in the same way that they are sensitive to the action of ether. On the other

hand, the adeno, Coxsackie, echo, polio and Coe viruses tested, were resistant to chloroform.

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Effect of Testosterone Propionate on Tissue Protein Synthesis in the Castrated Male Rat.* (26460)

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The fact that nitrogen retention occurs after administration of androgenic steroids to gonadectomized animals is well established (1). This nitrogen retention has been equated with the overall synthesis of total body protein, hence the employment of "anabolic" steroids in attempts to increase overall retention of nitrogen. However, this hormonal induced nitrogen retention has been shown to occur in the castrated rat even during consumption of a protein deficient diet (2) and a positive nitrogen balance could be maintained in spite of a loss of body weight. Furthermore rate of body weight loss was not influenced by the nitrogen retained.

No definite information is available regarding the location of the nitrogen stored by the body during androgenic treatment. The amount of nitrogen retained is greater than can be accounted for by growth of the sex organs. It would be of interest to localize the sites of protein synthesis which are stimulated by testosterone.

For this purpose we decided to utilize the rate of labeling of tissue proteins of castrated male rats receiving a tracer dose of 1-C^{14}-glycine as a measure of the anabolic activity of the tissues under consideration.

Materials and methods. Male Wistar rats were bilaterally castrated under light ether anesthesia when they were 2 months old. Fifteen days after gonadectomy they were implanted subcutaneously with sterile polyvinyl sponges (Ivalon), to study the effects of the hormone on rate of synthesis of a newly formed protein such as collagen.

TABLE I. Effect of Testosterone Propionate on Uptake of C^{14}-Glycine into Protein and Non-Protein Fractions of Tissues in Gonadectomized Male Rats.*

Treatment	Serum	Sem. ves.	Kidney	Heart	Liver	Diaphr.	Perin. musc.	Brain
T.P.	60 ± 8† (1.9)‡	57 ±11 (8.9)	40 ± 9 (6.3)	15 ± 2 (2.1)	29 ± 4 (3.5)	9 ± 2 (2.9)	15 ± 4 (13.2)	4 ±.5 (1.0)
Control	72 ± 5 (2.3)	16 ± 5 (5.8)	39 ±10 (7.3)	23 ± 1 (2.7)	39 ± 4 (4.6)	11 ± 3 (4.0)	5 ± 1 (5.5)	3 ±.5 (.6)

* Sacrificed 5 hr after inj. of C^{14}-glycine.
† Tissue protein specific activity counts/min./mg protein, and stand. error.
‡ Non-protein fraction radioactivity counts/min./mg tissue protein.

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