

of selected tissues taken from hyperreactive animals consistently revealed hyperemia and hemorrhage of the adrenals. The described hyperreactivity appears to be a manifestation of the generalized Schwartzman reaction.

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Action of Sodium Desoxycholate on Arthropod-Borne Viruses. (23483)

MAX THEILER (Introduced by J. Casals)

From The Rockefeller Foundation Virus Laboratories, New York City

As a by-product of studies on the course of infection of monkeys with virulent yellow fever virus(1), it was discovered that bile from normal rhesus monkeys had a marked virucidal action on the virus. Bile in a dilution of 1:50 was shown to inactivate from 3.0 to 5.0 logs of several strains of yellow fever. In extending the experiments to determine the action of bile on other agents, it was discovered that viruses could be divided into two groups—one resistant and the other susceptible to the action of bile. Viruses which were resistant were the Lansing and MEF₁ strains of poliomyelitis, the GD VII strain of mouse encephalomyelitis and the Mengo strain of encephalo-myocarditis. Agents found to be susceptible were yellow fever, Uganda S. Ntaya, West Nile, dengue 1, dengue 2, St. Louis, Japanese B. Ilheus, EEE, Bwamba, Bunyamwera and Anopheles A. The method of determining the degree of virus inactivation was to make parallel titrations of the virus in the presence or absence of a 1:50 dilution of bile. A summary of such an experiment appears in Table I. The diluent used

in these experiments was either 10% normal monkey serum in saline or a 0.2 solution of bovine albumen (bpa) in saline.

In the course of these experiments it was found that samples of bile from different normal rhesus monkeys varied in their capacity to inactivate a bile susceptible virus. This observation led to the search for a more clearly defined chemical substance. The use of sodium desoxycholate (DCA) was a logical choice as this salt had been used extensively in the study of the bile solubility of the pneumococcus. The results of a preliminary experiment to determine the action of DCA are shown in Table II. In this experiment, the action of DCA in two concentrations (1:1000 and 1:10,000) was tested on yellow fever virus and the higher concentration on GD VII. In addition, the yellow fever virus was titrated in the presence of a 1:50 dilution of bile. It will be observed that the 1:1000 dilution of DCA inactivated the yellow fever virus to approximately the same extent as the 1:50 dilution of bile, whereas this concentration of DCA had no action on the GD VII virus. The

TABLE I. Action of Bile on Yellow Fever, Bwamba, GD VII Strain of Mouse Encephalomyelitis and MEF₁ Strain of Poliomyelitis.

Dilution of virus	Yellow fever		Bwamba		GD VII		MEF ₁	
	Bile 1:50	Control	Bile 1:50	Control	Bile 1:50	Control	Bile	Control
10 ⁻¹					6/6	12/12	11/11	12/12
10 ⁻²	0/8	10/10	0/12	12/12	12/12	"	"	"
10 ⁻³	0/6	"	"	"	"	"	12/12	11/11
10 ⁻⁴	0/7	9/10	"	"	"	"	"	8/12
10 ⁻⁵	0/8	1/10	"	5/12	"	"	8/11	2/12
10 ⁻⁶	"	0/10	"	0/12	"	11/12	4/12	1/12
10 ⁻⁷			"	1/12	12/13	1/11		
Titer	<10 ⁻²	10 ^{-4.5}	<10 ⁻²	10 ^{-4.9}	>10 ^{-7.4}	10 ^{-6.6}	10 ^{-5.8}	10 ^{-4.4}

higher dilution of DCA had no effect on the yellow fever virus. In these experiments, an equal portion of the virus dilution was mixed with the solution of DCA or bile, and, hence, the final dilution of these was 2-fold higher. In further work, to determine the action of DCA on viruses, this salt was used in a dilution of 1:500, so that when mixed with an equal quantity of the virus dilution, the concentration in the mixture was 1:1000. Certain factors were of vital importance to obtain clearcut results. It was found that the DCA at times failed to inactivate an agent which had previously been shown to be susceptible. In these instances, it could be readily shown that the failure of the DCA to inactivate the virus was associated with the method of preparing the virus suspension. In every case of DCA failure, the virus had been prepared by light centrifugation. When the same virus suspension was submitted to an hour's centrifugation at 10,000 rpm in an angle centrifuge, it was readily inactivated by the DCA. This method of preparation has been adopted as a routine. Another factor,

which has been shown to be of importance, is the concentration of protein in the mixtures. It was found that the action of DCA could be completely inhibited by the presence of high concentrations of protein in the mixtures. When a 1:500 dilution of DCA in 0.75% bpa was mixed with virus dilutions made in 7.5% bpa, no inactivation occurred. Similarly, the action of DCA in 10% normal monkey serum was completely inhibited when mixed with virus dilutions made in undiluted normal monkey serum. Consequently, as a standard procedure, centrifugation at 10,000 rpm and a 0.75% bpa diluent has been adopted. The rate of inactivation of a virus by DCA has not been determined, but it has been found that incubating the mixtures for 1 hour at 37°C has given satisfactory results.

The following arbor viruses were inactivated by DCA. The figures in parentheses indicate logs of virus inactivated. *Group A.* EEE (3.0, 5.5), WEE (6.0), VEE (3.0, 4.7), Semliki Forest (4.0, 5.4), Sindbis (2.6, 4.1, 5.0). *Group B.* Yellow fever (4.2, 5.1, 3.1, 4.5), Japanese B (5.0), St. Louis (3.5, 4.1, 3.8), Ilheus (3.8, 4.8), West Nile (5.8), Uganda S (2.8, 3.3, 3.9), Zika (3.7), dengue 1 (3.1), dengue 2 (4.6, 3.7), Russian spring-summer (5.0), Ntaya (2.8). *Miscellaneous.* Anopheles A (3.6), Anopheles B (2.5), Wyeomyia (3.6), California virus of Hammon and Reeves (2.1), Bunyamwera (3.5), Bwamba (3.0), Rift Valley fever (2.0), the Sicilian (2.8) and the Naples strains of Sandfly fever (3.0). In addition, several recently isolated agents thought to be arthropod-borne, at present under study and apparently new to science, have all been shown to be inactivated by DCA.

TABLE II. Action of Sodium Desoxycholate (DCA) on Yellow Fever and GD VII Strain of Mouse Encephalomyelitis.

Virus dilution	Yellow fever				GD VII DCA 1:1000
	DCA 1:1000	DCA 1:10,000	Bile 1:50	Control	
10 ⁻²	6/6	6/6	3/6	6/6	
10 ⁻³	1/6	"	0/6	"	6/6
10 ⁻⁴	"	"	"	"	"
10 ⁻⁵	0/6	"	"	"	"
10 ⁻⁶	"	"	"	"	"
10 ⁻⁷	"	2/6	"	2/6	4/6
10 ⁻⁸	"	1/5	"	1/6	3/6
10 ⁻⁹					0/6
Titer	10 ^{-2.8}	10 ^{-6.9}	10 ⁻²	10 ^{-6.9}	10 ^{-7.7}

Results. The following strains of virus proved to be resistant to the action of DCA. The negative sign before the figures in parentheses indicates that the virus gave a higher titer in the presence of DCA than in the control. GD VII strain of mouse encephalitis (0.0, -0.8, -0.6, -0.6), FA strain of mouse encephalomyelitis (-0.3), Coxsackie virus (0.3, -0.8, -0.3), MEF₁ strain of human poliomyelitis (-1.0, -0.5), Mengo strain of EMC (0.1, 0, -1.0, 0). It is of interest to note that the agents in the DCA resistant group, as a rule, give a slightly higher titer in the presence of DCA than they do in the controls.

The finding that viruses could be divided into bile salt resistant and susceptible groups has been of great value as a preliminary step in identification. In the attempted isolation of viruses by the inoculation of mice, on numerous occasions, strains of encephalomyelitis virus of mice have been accidentally obtained. Being resistant to the action of DCA, this virus can be readily distinguished from the arbor viruses. In recent years, more and more arbor viruses (*e.g.*, Sindbis) pathogenic for infant mice only have been isolated. These can be readily distinguished from the Coxsackie group because the former are susceptible and the latter resistant to the action of DCA. Of unusual interest is the finding that the Mengo strain of EMC is resistant. This agent has been isolated on several occasions from mosquitoes and was for some time considered to be arthropod-borne. However, ex-

tensive experiments have shown that this agent does not multiply in the mosquito and no successful experimental transmission by bite has been obtained. Infection with this agent leads to a viremia and, consequently, it is not surprising that at times mosquitoes will be caught in nature apparently infected with this agent. In many of its characteristics the Mengo virus is closely related to the polio group of agents. It is of interest here to recall that Kerr(2), by means of the complement fixation test, has shown an immunological relationship between the Mengo virus and the GD VII strain of mouse encephalomyelitis.

Susceptibility to DCA is not an exclusive property of the arbor group of viruses. Agents such as influenza A and lymphocyte choriomeningitis are readily inactivated.

Summary. All arthropod-borne viruses at present under study in the Rockefeller Foundation Virus Laboratories, New York, have been shown to be readily inactivated by a 1:1000 dilution of sodium desoxycholate. The only viruses discovered to date which are resistant to the action of this bile salt are strains of human poliomyelitis, mouse encephalomyelitis, Coxsackie and encephalomyocarditis viruses.

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Maze Performance, Emotionality, Audiogenic Seizure Susceptibility in Rats and Mice Treated with 2-Dimethylaminoethanol (23484)

R. P. SMITH AND W. BEVAN (Introduced by C. C. Pfeiffer)

Departments of Psychology and Pharmacology, Emory University, Ga.

The intimate relationship of acetylcholine to the transmission of impulses in the nervous system has led to the expectation that changes in the metabolism of this substance must have pronounced effects upon various facets of behavior. This expectation has given rise to a

number of investigations with confirming results. For example Ginsburg and Hovda(1) and Ginsburg and Huth(2) have described a negative relation between susceptibility to audiogenic seizures and several parasymphathomimetic substances. Acetylcholine and