

chronic malarial infection, although no parasites had been found in smears of the peripheral blood made during life.

Chimpanzee No. 1 died of acute asphyxia following aspiration of vomitus during anesthesia at the end of the 4-month period of observation. Postmortem examination of the liver was normal grossly and histologically.

Failure to induce infective hepatitis in chimpanzees is in keeping with the failure to infect lower animals.

Summary. 1. Two adult and 4 young chimpanzees were given material known to contain the etiologic agent of infectious hepatitis. Five of the animals remained healthy and showed no evidence of impairment of certain tests of liver function. One animal

which was sick before beginning the experiment, died of a severe parasitic infestation 65 days after inoculation. The liver was normal grossly and histologically. Another animal died accidentally at the end of the period of observation and the liver was also normal. 2. Data are presented on serial determinations of certain tests of liver function in the chimpanzee. Quantitative determinations of serum bilirubin and per cent of bromsulfalein dye retention were of a lower order than similar determinations in normal humans. The cephalin flocculation was consistently positive in one apparently healthy adult animal and in one young chimpanzee with an overwhelming parasitic infestation. Three young healthy animals had consistently negative cephalin flocculation tests.

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Effect of H Ion Concentration on Eluting Poliomyelitis Virus from Cotton Fiber.

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The present report describes an exploratory method in the elution of the Lansing strain of poliomyelitis virus from cotton swabs. These studies were carried out with a view toward finding a method of eluting virus from cotton swabs following their application to the oropharynx of patients with poliomyelitis.

Material and Methods. A pool (20% suspension) of Lansing virus was prepared using the brain stems and cords of mice that became paralyzed following intracerebral inoculation. Aliquots of this pool were stored in ampules at -70°C . The 50% mortality end-point was $1:3750 \pm 1200$.

Iso-osmolar phosphate and acetate buffers were made for the pH range 4.0 to 8.1. Dilutions of virus at the desired pH were prepared using 8 parts of normal salt solution and 2

parts of buffer. The Lansing virus suspension was diluted 1:100 and 1:500 at pH 4.0, 4.6, 5.0, 6.0, 7.0, and 8.0. In tests determining the effect of H ion concentration *per se* on mouse brain-virus suspension the menstrua were kept at room temperature for one hour and then inoculated into mice. In elution studies each virus-pH mixture was divided into two aliquots, *viz.*, (a) to 1.0 cc, 2 cotton pledgets ($\frac{5}{8}$ " surgical sponges) were added, and (b) to the remaining fraction no cotton was added. Following contact at room temperature for 1 hour the cotton sponges were removed and the eluate expressed into clean tubes, using 10 cc syringes. The pH of each solution was re-determined following inoculation of each pair of eluate and control virus suspensions. Slight change in the H ion concentration occurred (*viz.*, virus suspensions at pH 4.0 shifted to 4.2 and from pH 8.0 to 7.8).

Inbred mixed strains of colored mice 4 to 8

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TABLE I.
Infectivity of Eluate Obtained from Cotton Fiber Suspended in Diluent at Various pH's and Containing Lansing Virus. Fate of Mice Inoculated with (a) Virus-pH Diluent and (b) Eluate from Cotton Fiber Suspended in Virus-pH Diluent.

Dilution of virus	Inoculum	pH range												Mean totals				
		4.0			5.0			6.0			7.0					8.0		
		Deaths*			Deaths*			Deaths*			Deaths*			Deaths*				
		No.	%		No.	%		No.	%		No.	%		No.	%		No.	%
1:100	Control (a)	29/31	93.7		22/30	73.5		28/30	93.5		21/24	87.5		28/32	87.6		128/147	87.0
"	Eluate (b)	17/32	53.3		16/31	51.7		27/36	75.2		41/57	72.0		29/32	90.6		130/188	69.3
1:500	Control (a)	26/32	81.2		20/32	62.5		27/32	84.3		23/34	67.6		22/32	68.7		118/162	72.8
"	Eluate (b)	8/31	25.8		10/30	33.3		6/29	20.6		17/56	30.3		17/32	53.1		58/178	32.5

* Surviving 24 hours after inoculation.

weeks of age, obtained from the Roscoe B. Jackson Memorial Laboratory, were used in these experiments. Following intracerebral inoculation mice were observed for 28 days.

The *mortality rate* in mice inoculated with mouse brain-virus mixture at different H ion concentration, and to which cotton may or may not have been added, has been used as an index of the presence of virus in 0.03 cc of inoculum. This index provides an estimation of the activity (and presumably the amount) of virus in the aliquot samples and in the cotton eluates at various H ion concentrations. At least 24 mice, and usually more, have been employed to determine significant differences in the mortality rates in these tests.

Results. A summation of the results found in 4 tests appears in Table I. The Lansing virus was eluted from cotton fiber best at pH 8.0. In 1:100 dilution of virus about 40% of the active infectious agent was removed from the eluate at pH 4.0, 22% at pH 5.0, and about 15% at pH 6.0 and 7.0. At pH 8.0 the amount of virus in the eluate is nearly of the same order as in the aliquot sample to which cotton was not added. Maximal removal of virus by cotton fiber took place in the pH zone (4.0 to 5.5) where flocculation was visible in the mouse-brain-virus suspensions.

The results obtained with 1:500 dilution of virus support and amplify those above, namely, that the amount of virus eluted from cotton fiber increases with rising pH. Maximal elution occurred at pH 8.0. In 1:500 dilution (5-10 MLD) sufficient virus was retained by the cotton fiber at pH 4.0 to 7.0 so as to reduce the virus content beyond the median lethal dose. That the removal of virus from cotton in this dilution is facilitated at pH 8.0 seems clear enough, but it is not possible to say that all available virus was recovered.

The effect of H ion concentration on the mortality rate of mice inoculated with Lansing virus (1:100 and 1:500 dilution) is summarized in Table II. In mice inoculated with virus at pH 4.6 the mortality rate is significantly higher than for other H ion concentrations which were tested. The survival time, particularly among those inoculated with 1:500

TABLE II.
Fate of Mice at Several pH-Virus Mixtures; 1:100 and 1:500 Dilutions of Lansing Virus.

Dilution of virus	pH of inoculum	No. mice inoculated*	No. mice dead	Median day deaths 24 hrs after inoc.	Mortality rate, %
1:100	4.0	85	77	6.6	90.5
"	4.6	59	58	5.5	98.2
"	5.0	88	76	6.1	86.3
"	6.0	81	74	7.8	91.3
"	7.0	70	60	7.5	85.7
"	8.0	79	65	7.5	82.2
1:500	4.0	56	47	9.0	84.0
"	4.6	52	50	3.2	96.1
"	5.0	85	64	8.1	75.2
"	6.0	51	45	10.5	88.2
"	7.0	93	73	9.7	78.4
"	8.0	61	46	9.5	75.4

* Surviving 24 hours after inoculation.

dilution of virus is markedly shortened. For pH values other than 4.6 the differences in mortality rates and survival time can be accounted for by chance.

Discussion. The results point out that the Lansing strain of poliomyelitis virus can be eluted best from cotton swabs at pH 8.0. However, the mortality rate at pH 8.0 (and also 5.0) in a large number of mice inoculated with virus at several pH's has been among the lowest in the series. There were between 16 and 21% fewer mice dying at pH 8.0 than at 4.6. The procedure suggested from the data is to elute virus from cotton at pH 8.0, and then readjust the inoculum to pH 4.6 (or 6.0) before testing in animals.

Hammon and Izumi¹ studied the effect of

pH on the mortality rate in mice inoculated with Lansing virus. They observed a step-like increase in the mortality rate, and progressive narrowing of survival time with increasing H ion concentration. Our results have evolved using a relatively larger number of animals and more concentrated virus preparations than Hammon and Izumi used. The present data do not show a precise increase in mortality rate with increasing pH; however, Hammon's contentions were demonstrated in higher dilutions of virus than were employed in these experiments.

Summary. A method is reported for eluting the Lansing strain of poliomyelitis virus from cotton swabs. This virus is eluted best from cotton when the diluent is alkaline at pH 8.0. To obtain optimal mortality (morbidity) rates the eluate should be made acid (pH 4.6, etc.) before it is inoculated into mice.

¹ Hammon, W. M., and Izumi, E. M., *Proc. Soc. Exp. Biol. and Med.*, 1941, **48**, 579.

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Transmission of Streptomycin from Maternal Blood to the Fetal Circulation and the Amniotic Fluid.*

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Streptomycin, an antibiotic substance obtained from the mold *Actinomyces griseus*,

* The work described in this report was done under a contract recommended by the Committee on Medical Research between the Office of Scien-

supplements penicillin by arresting the growth of many of the gram negative bacilli. It is freely distributed in most body fluids follow-

tive Research and Development and the University of Pennsylvania.