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Failure to Transmit Infectious Hepatitis to Chimpanzees.*

W. P. HAVENS, JR., AND ROBERT WARD. (Introduced by Francis G. Blake.)
From the Section of Preventive Medicine, Yale University School of Medicine.

The etiologic agent of infectious hepatitis is filtrable[1,2] and probably may be classified as a virus. It may be transmitted to man in serial passage[1] by feeding or parenteral inoculation, and to date man is apparently the only susceptible host. Unconfirmed reports have described the propagation of the agent of this disease in developing chick embryos,[3,4] canaries,[5] and pigs.[6] The general experience, however, has been a consistent failure to transmit this virus to incubating eggs and to a variety of small animals, including guinea pigs, white mice, rats, rabbits, hamsters, kittens, gerbils, baboons, rhesus monkeys, and other species such as *cercopithecus, erythrocebus,* and *colobus* monkeys.[7,8]

The present report describes an attempt to produce demonstrable disease in chimpanzees by the administration of materials known to contain an agent of infectious hepatitis. Two of the chimpanzees (No. 1 and 3)† were adults of 12-15 years of age, weighing from 65 to 70 lb. They had been in this country for several years. The other 4 animals (No. 4-7) were estimated to be between 2 and 3 years of age, weighing from 22-30 lb. They arrived from West Africa one month before the beginning of the experiment.

Infectious materials employed here were obtained from naturally occurring and experimentally induced human cases of infectious hepatitis. They are described in Table I.

TABLE I.
Source of Material.

Material	Material obtained from: Exp. case of infectious hepatitis	Material obtained from: Natural case of infectious hepatitis
Stool B		+8
" K	−9	
	−3	
" Bd	+2	
" Kh	−1	
	+2	
" Z	−1	
	+2	
Serums M	−4	
	−1	
	+1	
" K	−5	
" L	−5	
" G	−4	
" Mn	−5	

(−) indicates days before jaundice.
(+) indicates days after jaundice.
Multiple specimens from the same patient were pooled before administration.

Serum and stools were stored at dry-ice box temperature for periods ranging from 4-16 months. The serum was sterile and the stools contained no pathogenic bacteria before use.

The experiment consisted in administering

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1 Havens, W. P., Jr., PROC. SOC. EXP. BIOL. AND MED., 1945, **58**, 203.

2 Findlay, G. M., and Wilcox, R. R., *Lancet*, 1945, **1**, 212.

3 Siede, W., and Meding, G., *Klin. Wchnschr.*, 1941, **20**, 1065.

4 Siede, W., and Luz, K., *Klin. Wchnschr.*, 1943, **22**, 70.

5 Dresel, E. G., Meding, G., and Weineck, E., *Z. Immunitätsforsch u. Exp. Therap.*, 1943, **103**, 129.

6 Andersen, T. T., and Tulinius, S., *Acta M. Scand.*, 1938, **95**, 497.

7 van Rooyen, C. E., and Gordon, I., *J. Roy. Army M. Corps*, 1942, **79**, 213.

8 Unpublished observations of the authors.

† These 2 chimpanzees, No. 1 (Pop-Eye) and No. 3 (No-No), were obtained from the Department of Physiology, Yale University Medical School.

TABLE II.
Inoculum and Route of Administration of Infectious Materials.

Chimpanzee No.	Inoculum	Route
1	Stool B, K, Bd	I.N. and Oral.
3	Stool B, K, Bd, Z, Kh	I.N. " "
	Serum M, K	Par.
4	Stool Z	I.N. " "
	Serum L, G, Mn	Par. " "
5	Stool Z	I.N. " "
	Serum L, G, Mn	Par. " "
6	Stool Z	I.N. " "
	Serum L, G, Mn	Par. " "
7	Stool Z	I.N. " "
	Serum L, G, Mn	Par. " "

I.N.—Intranasally.
Par.—Parenterally.
Oral.—Orally.

were anesthetized with ether or ether and nembutal at the time of each testing; the young chimpanzees were handled without anesthesia.

All of the animals with the exception of No. 7 remained healthy throughout the period of observation. Tests of liver function in these 5 healthy animals were normal throughout the experiment with the exception of the cephalin flocculation which was positive in one adult consistently both before and after inoculation. Occasional falsely positive reactions to this test were encountered in the 3 young animals (No. 4, 5, 6) when an extremely sensitive antigen was used. The results of serial determinations of these various tests of liver function are recorded in Table III. It is of

TABLE III.
Results of Serial Determinations of 4 Tests of Liver Function in Chimpanzees.

Chimpanzee No.	No. of tests	Serum Bilirubin mg %			Bromsulfalein* dye % retained in 30 min.			Thymol turbidity units			Ceph. Floc. 24 hr
		Low	High	Avg	Low	High	Avg	Low	High	Avg	
1	16	.13	.34	.23	3	8	5.4	—	—	—	—
3	12	.20	.41	.28	2	5	3.	—	—	—	++++
4	16	.07	.34	.20	0	2	1.6	0	3	1	0
5	16	.07	.89	.29	0	4	1.9	0	3	1	0
6	16	.14	.62	.29	0	3	1.9	0	3	1	0
7	10	.13	.55	.33	4	16	12	2	8	4	++++

* 5 mg of bromsulfalein/kilo of body weight was injected intravenously.

these materials by different routes to 6 chimpanzees. The inoculum which each animal received is defined in Table II. Inoculation or feeding with these materials had produced infectious hepatitis in human volunteers.

Chimpanzees No. 1, 4, 5, 6 were observed for 4 months, chimpanzee No. 3 for 3 months and chimpanzee No. 7 for 65 days. Certain functions of the liver of each animal were determined before the administration of infectious material and every 7-10 days thereafter throughout the period of observation by the following tests: 1. the quantitative serum bilirubin determination,‡ 2. cephalin-cholesterol flocculation, 3. thymol turbidity,[10] 4. bromsulfalein dye retention. The adult animals

‡ According to the method of Malloy and Evelyn[9] using the photoelectric colorimeter.

[9] Malloy, H. T., and Evelyn, K. A., *J. Biol. Chem.*, 1937, **119**, 481.

[10] Maclagan, N. F., *Nature*, 1944, **154**, 670.

interest that in an equal number of determinations made contemporaneously in normal adult men by the same methods the average total serum bilirubin was 0.8 mg%, and the bromsulfalein dye retention was 5.4%.

Chimpanzee No. 7 was sick on arrival in the laboratory with a severe parasitic infestation with *Balantidium coli,* strongyloides, and hook worm. Both before and after inoculation there was intermittent slight increase of bromsulfalein dye retention, consistently positive cephalin flocculation, and occasionally positive thymol turbidity test. The serum bilirubin was normal throughout. This animal remained thin and weak, dying on the 65th day after inoculation. At autopsy there was a diffuse hemorrhagic pneumonia with sections of unidentifiable worms visible histologically in the lungs. The liver was essentially normal grossly and microscopically. The spleen showed numerous collections of hematin, suggestive of

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.

HERBERT A. WENNER.* (Introduced by H. A. Howe.)

From the Poliomyelitis Research Center, Department of Epidemiology, The Johns Hopkins University, Baltimore, Md.

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 ± 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 (⅝″ 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

* Fellow, National Research Council, Division of Medical Sciences.