even though this agent is only weakly sensitive to ether at 4°C.

Summary. Of 8 strains of Coxsackie viruses tested including 5 of group A representing four serological types and 3 of group B, only one strain, the group B, type 2, Ohio-R strain proved to be susceptible to diethyl ether. In addition to serving as a means of differentiating the Ohio-R strain from other serological types of C viruses, the use of ether may be a limiting factor in the isolation of this agent.

1. Dalldorf, G., and Sickles, G. M., *Science*, 1948, v108, 61; Dalldorf, G., Sickles, G. M., Plager, M., and Gifford, R., *J. Exp. Med.*, 1948, v89, 567.

2. Melnick, J. L., *Bact. Rev.*, 1950, v14, 233.

3. Sulkin, S. E., and Zarafonetis, C., *J. Exp. Med.*, 1947, v85, 559.

4. Sulkin, S. E., to be published.

5. Sulkin, S. E., Manire, G. P., and Farmer, T. W., PROC. SOC. EXP. BIOL. AND MED., 1950, v73, 305.

6. Wallis, H. C., unpublished observations.

7. Sulkin, S. E., Wallis, H. C., and Donaldson, P., *J. Inf. Dis.*, in press.

8. Gifford, R., and Dalldorf, G., *Am. J. Path.*, 1951, v27, 1047.

9. Godman, G., Bunting, H., and Melnick, J. L., *Am. J. Path.*, 1952, v28, 223.

10. Melnick, J. L., Rhian, M., Warren, J., and Breese, S. S., Jr., *J. Immunol.*, 1951, v67, 151.

11. Robinson, L. K., PROC. SOC. EXP. BIOL. AND MED., 1950, v75, 580.

12. Stanley, N. F., *Aust. J. Exp. Biol. and Med. Sc.*, 1951, v29, 367.

13. Curnen, E. C., Shaw, E. W., and Melnick, J. L., *J.A.M.A.*, 1949, v141, 894.

14. Shaw, E. W., Melnick, J. L., and Curnen, E. C., *Ann. Int. Med.*, 1950, v33, 32.

15. Huebner, R. J., Cole, R. M., Beeman, E. A., Bell, J. A., and Peers, J. H., *J.A.M.A.*, 1951, v145, 628.

16. Andrewes, C. H., and Horstmann, D. M., *J. Gen. Microb.*, 1949, v3, 290.

17. Committee for Nomenclature, National Foundation for Infantile Paralysis, *Science*, 1948, v108, 701.

18. Remlinger, P., and Bailly, J., *C. R. Soc. Biol.*, 1931, v106, 523; *ibid.*, 1935, v118, 1200.

19. Remlinger, P., *C. R. Acad. Sci.*, 1918, v166, 750.

20. ———. *Ann. Inst. Past.*, 1919, v33, 616.

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Interference Between Japanese B Encephalitis Virus and Western Equine Encephalomyelitis Virus in the Rat. (19808)

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It has recently been reported(1,2) that 3- to 4-week-old rats, which are highly susceptible to intranasally instilled Western equine encephalomyelitis (W.E.E.) virus, are much less susceptible if St. Louis encephalitis (S.L.E.) virus is instilled into their noses 72 hours prior to the inoculation of W.E.E. virus. The purpose of the studies reported here was to determine whether Japanese B encephalitis virus which, like S.L.E. virus, induces a fatal infection in 7-day-old but not in 21-day-old rats(3) would "interfere" with W.E.E. virus following intranasal inoculation of both viruses.

Materials and methods. Viruses and animals. The Japanese B encephalitis virus used was the Nakayama strain which had been carried through an unknown number of intracerebral passages in mice. The strain of W.E.E. virus used has already been described (1,2). The virus suspensions were prepared from virus-infected brains of 2- to 3-week-old Swiss mice. The infected brains were placed in a Waring blendor (semi-micro size jar) together with sufficient cold, inactivated, undiluted rabbit serum to make a 10% suspension of the infected brains. The blendor was

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TABLE I. Interference Between Japanese B Encephalitis Virus and Equine Encephalomyelitis Virus Following Intranasal Inoculation of Both Viruses.

No. of rats	Material instilled into nose	Interval (days) between W.E.E. and death 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	Results	% mortality
21	Jap. B × 3* W.E.E.†	0¶	0	0	2	0	0	0	0	1	0	0	0	2	1	1		7/21**	33
20‡	Jap. B × 1§ "	0	0	0	1	1	0	1	0	3	1	0	0	1	0	0	2	10/20	50
20	N.M.B. × 3‖ "	0	0	16	3	1												20/20	100

* Jap. B × 3. Three daily inoculations of Japanese B encephalitis virus prior to inoculation of equine encephalomyelitis virus.

† W.E.E. Western equine encephalomyelitis virus.

‡ One animal in this group became paralyzed but survived.

§ Jap. B × 1. A single inoculation of Japanese B encephalitis virus prior to inoculation of equine encephalomyelitis virus.

‖ N.M.B. × 3. Three daily inoculations of normal mouse brain prior to inoculation of equine encephalomyelitis virus.

¶ Zero indicates no deaths on that day. Other number indicates number of deaths on that day.

** Denominator indicates No. of animals tested. Numerator indicates No. of deaths.

run for 90 seconds and the resulting suspension then centrifuged at about 2000 RPM for 5 minutes to sediment gross particles. The supernatant fluid was removed, distributed in 2 ml amounts in screw-capped vials, "quick frozen" in a mixture of dry-ice and alcohol and then stored in a CO_2 cabinet until needed. A single suspension of each of the 2 viruses was used. The mouse intracerebral LD/50 titer(4) of the suspension of Japanese B encephalitis virus, after storage in the CO_2 cabinet for 7 days, was 1 x $10^{-8.4}$. The W.E.E. virus, following 9 days storage in the CO_2 cabinet, titered 1 x $10^{-8.8}$. The 10% normal mouse brain (N.M.B.) suspension was prepared by grinding brains from normal 2- to 3-week-old mice in a mortar with sand and sufficient cold, inactivated, undiluted rabbit serum to make a 10% suspension. It was then treated as described for the preparation of the virus suspensions. Male Sprague-Dawley rats which were shipped when 21 days of age were used. They weighed 38 to 54 g, with an average weight of 45 g at the time they were used. All inoculations were made while the rats were under light ether anesthesia.

Experimental. The 61 rats used in the experiments were divided into 3 groups and inoculated intranasally with a 10% suspension of the material being tested (Table I). One group, consisting of 20 rats, received 0.05 ml of the N.M.B. suspension on each of 3 consecutive days. These animals then received 0.05 ml of the suspension of W.E.E. virus 72 hours after the first inoculation of N.M.B. A second group of 20 animals received a single inoculation of 0.05 ml of the suspension of Japanese B encephalitis virus followed 72 hours later by a single inoculation of 0.05 ml of W.E.E. virus. The third group consisting of 21 rats, received 0.05 ml of Japanese B encephalitis virus on each of 3 consecutive days. These animals were then inoculated with 0.05 ml of W.E.E. virus 72 hours after they had received the first inoculation of Japanese B encephalitis virus. The schedule of inoculations was arranged so that all 3 groups were inoculated with the W.E.E. virus on the same day. The rats were observed for 21 days. The results are presented in Table I and show that the group of 20 rats which received 3 inoculations of N.M.B. before the W.E.E. virus was inoculated were uniformly susceptible to the virus since all of the animals succumbed. The results were different, however, in the 2 groups of animals which had had Japanese B encephalitis virus instilled into their noses before the W.E.E. virus was inoculated. Of the 20 rats which received a single inoculation of Japanese B encephalitis virus followed by W.E.E. virus only 10 of the 20 animals developed a fatal infection. However, one of the 10 animals which survived in this group did not completely escape infection with W.E.E. virus. On the 13th day following inoculation with W.E.E. virus this animal developed flaccid paralysis of both posterior extremities as well as the right front leg. Three days later the animal regained the use of the posterior extremities but irreparable damage had occurred because the animal never recovered the use of

TABLE II. Susceptibility of Rats to a Second Intranasal Inoculation of Equine Encephalomyelitis Virus.

No. of rats	Material instilled into nose	Interval (days) between 2nd inoc. of W.E.E. and death 1	2	3	4	5	Results	% mortality
14	Jap. B × 3 W.E.E. × 2*	0	0	9	3	1	13/14	93
9	Jap. B × 1 W.E.E. × 2	0	0	5	2	1	8/9	89

* Rats reinoculated intranasally with W.E.E. virus 23 days after having previously received W.E.E. by the same route.

the right front leg since muscular atrophy, giving rise to contractures, occurred. It should be noted that this is the first instance in which we have observed a non-fatal infection in the rat caused by W.E.E. virus. In the group of rats which received 3 inoculations of Japanese B encephalitis virus before the W.E.E. virus was inoculated a high degree of resistance was noted since only 7 of the 21 animals tested were found to be susceptible to the W.E.E. virus.

Twenty-three days after W.E.E. virus was instilled into the noses of the above rats, certain of those which survived were reinoculated intranasally with W.E.E. virus to determine whether the original inoculation of W.E.E. virus had rendered them resistant to a second inoculation by the same route. The results appear in Table II and show that the animals which had received 3 inoculations of Japanese B encephalitis virus as well as those which received only one before the original inoculation of W.E.E. virus were highly susceptible to a second intranasal inoculation of W.E.E. virus since only one animal in each group survived.

Discussion. In previous communications (1,2) it was reported that the susceptibility of rats to intranasally instilled W.E.E. virus was altered if S.L.E. virus were inoculated into the noses of the animals before they received the W.E.E. virus. If one inoculation of S.L.E. virus preceded the W.E.E. virus 50 to 65% of the animals survived whereas 95% of the rats were found to be resistant if 3 inoculations of S.L.E. virus preceded the W.E.E. virus. Since S.L.E. virus invades the central nervous system of rats of this age by passing along the olfactory nerve to establish an inapparent infection(5,6) it was postulated that S.L.E. virus, having passed over the olfactory tract and being present in the brain, prevented the subsequent passage of W.E.E. virus over this pathway. Evidence has been obtained which suggests that Japanese B encephalitis virus invades the central nervous system of 3- to 4-week-old rats by the olfactory route following nasal instillation of the virus since the virus was recovered from the anterior rhinencephalon as early as 24 hours after inoculation when it was not recovered from the "remainder of brain" or from the spinal cord(7). It is possible then that the mechanism by which both S.L.E. and Japanese B encephalitis viruses protect a high percentage of rats against intranasally inoculated W.E.E. virus is essentially the same since it would appear that the passage of either of these 2 viruses along the olfactory pathway interferes with the subsequent passage of W.E.E. virus along this route. The observation that the Japanese B virus-treated rats which survived the original inoculation of W.E.E. virus were highly susceptible to W.E.E. virus instilled intranasally 23 days later indicates that the mechanism by which Japanese B encephalitis virus interferes with W.E.E. virus functions in such a manner that the W.E.E. virus is unable to immunize the animals against a second inoculation of W.E.E. virus.

Summary. If Japanese B encephalitis virus is instilled into the noses of 3- to 4-week-old rats 72 hours prior to the inoculation of Western equine encephalomyelitis virus by the same route a high percentage of the animals resist infection with the latter virus. If one inoculation of Japanese B virus preceded the Western equine virus 50% of the rats survived whereas 67% of the animals survived when 3 inoculations of Japanese B virus preceded the equine encephalomyelitis virus. Rats which survived when equine encephalomyelitis virus was instilled into their noses 72 hours following inoculation of Japanese B

encephalitis virus by the same route were found to be susceptible to a second intranasal inoculation of equine encephalomyelitis virus.

1. Jordan, R. T., and Duffy, C. E., *J. Inf. Dis.*, In press.

2. Duffy, C. E., Jordan, R. T., and Meyer, H. M., PROC. SOC. EXP. BIOL. AND MED., 1952, v80, 279.

3. Duffy, C. E., PROC. SOC. EXP. BIOL. AND MED., 1951, v76, 566.

4. Reed, L. J., and Muench, H., *Am. J. Hyg.*, 1938, v27, 493.

5. Duffy, C. E., and Sabin, A. B., *J. Bact.*, 1942, v43, 88, (Abstract).

6. ———, Unpublished data.

7. Duffy, C. E., and Pehrson, G. C., Unpublished data.

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The Size of Serum Hepatitis Virus.* (19809)

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The agent of serum hepatitis (SH) is known to pass through various "bacteria-retaining" filters (Seitz EK(1,2) and Berkefeld N(3)), but the size of the virus, like that of infectious hepatitis (IH), has so far remained undetermined. The present report is concerned with experiments designed to determine the size range for SH virus by means of ultrafiltration of known icterogenic serum through a series of gradocol membranes† of various average pore diameters (A.P.D.)(4).

Materials and methods. Acute phase serum (strain J. W.) was obtained from an American soldier in Germany on the second day of jaundice (30 September 1949). It was kept in a CO_2 dry ice box for 42 days and then at −20°C until the filtrations were carried out in July and November of 1951.‡ A portion of this same lot of serum had previously (1950) produced clinical and laboratory evidence of hepatitis in 3 volunteers, each of whom received 0.5 cc subcutaneously and 0.1 cc intracutaneously(5). The incubation period at that time ranged from 53 to 87 days. In the first series of filtrations whole undiluted serum was used. After clarification through a Seitz EK pad a portion of the serum was passed through a 300 mμ, A.P.D. gradocol membrane under 12 lb positive pressure (nitrogen gas) at 38-39°F. This was then used as a "master" filtrate, portions of which were filtered through membranes of 200 mμ, and 94 mμ A.P.D. under similar conditions. In the second series of filtrations 10% serum in normal saline was used, the procedure being the same as above except for the addition of a 52 mμ membrane. This dilution factor was tried in order to facilitate filtration which is a most time-consuming procedure when whole undiluted serum is used. All filtrates were kept frozen until the time of inoculation. Twenty-nine volunteers, quartered in the same institution,§ participated in the present study. All were healthy males (aged 21 to 30) who gave no history of previous jaundice of any type. All had normal liver function tests (*i.e.*, direct and total serum bilirubin, cephalin fluocculation, thymol turbidity and flocculation, and alkaline phosphatase) at the time of inoculation.

Exp. I. Seventeen volunteers were divided into 4 groups, each receiving a single filtrate

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† Obtained from the Inoculation Department, St. Mary's Hospital, London.

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