Morphological Observations by Electron Microscopy of West Nile Virus after Propagation in the Swiss Mouse. (19163)

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The West Nile strain (B 956) of virus used for this study was furnished by the American Type Culture Collection of Washington, D. C. This virus had originally been obtained from Dr. K. C. Smithburn of the Rockefeller Foundation Laboratories in New York(1). He isolated this strain in 1937 from the blood of a patient in Uganda, Africa. On receipt at this laboratory the virus had been passed 25 times in Swiss Albino mice by the intracerebral route of inoculation, and at this laboratory it was passed one additional time before initiating the experiment.

Six Swiss albino mice (3-weeks-old) were inoculated intracerebrally with 0.3 cc of a 10% suspension of West Nile virus of the 25th intracerebral mouse passage. The virus received here was lyophilized mouse brain and was diluted to a 10% suspension with sterile distilled water. The mice showed nervous symptoms of involuntary motor reactions on the 4th day. When symptoms appeared the mice were sacrificed, and the brains and cords were removed aseptically. Six mice were injected intracerebrally with a 10% suspension of normal mouse brains. No symptoms were noted in these normal controls. These 6 mice were sacrificed on the 4th day post inoculation. The brains and cords were removed aseptically as above. The pools of infected mouse brains and cords and of normal mouse brains and cords were ground with alundum, and diluted to 10% suspensions with physiological saline. The 2 suspensions were then subjected to 5 minutes centrifugation in an angle centrifuge at 2000 rpm. The supernatants were removed from the specimens and filtered through type ST size L3 Seitz filters. The filtrates were then subjected to centrifugation for 2 hours at 44,770 rpm under refrigeration. The temperature of the refrigerated outer jacket stayed constant at −8°C during the centrifugation. After the 2-hour centrifugation period, the supernatants from the specimens were discarded and the sediment from each specimen was resuspended in 0.8 cc of physiological saline. Several drops of each suspension were placed on several parlodion film supports which had been prepared 24 hours previously. After all excess fluid had been removed with small capillary pipettes, the films were dried and shadowed with chromium(2) at arc tangent 2/10 and examined under the RCA electron microscope, type EMU. The balance of the material of each suspension was injected intracerebrally into each of six 3-week-old Swiss albino mice.

The mice injected with the concentrated infected material showed nervous symptoms after a period of 6 days. These mice were sacrificed and the brains were removed aseptically, pooled, ground, and diluted to a 10% suspension. This suspension was injected intracerebrally into 6 unvaccinated mice and into 6 mice which previously had been immunized intramuscularly with the original West Nile virus. After 8 days the unvaccinated mice showed symptoms of central nervous system involvement while the immunized group showed no nervous symptoms. This confirmed the concentrated virus to be West Nile virus. The mice injected intracerebrally with the concentrated suspension of normal mouse brain and cord appeared normal and were discarded after a 21-day observation period.

Upon electron microscope examination of the concentrated suspension of the brains and cords of the normal mice, no virus-like particles could be seen. These controls were screened carefully. Upon examination of the concentrated suspension from the infected

1. Smithburn, K. C., Hughes, T. P., Burke, A., Paul, J., *Am. J. Trop. Med.*, 1940, v20, 471.

2. Williams, R. C., and Wyckoff, R. W. G., PROC. SOC. EXP. BIOL. AND MED., 1945, v58, 265.

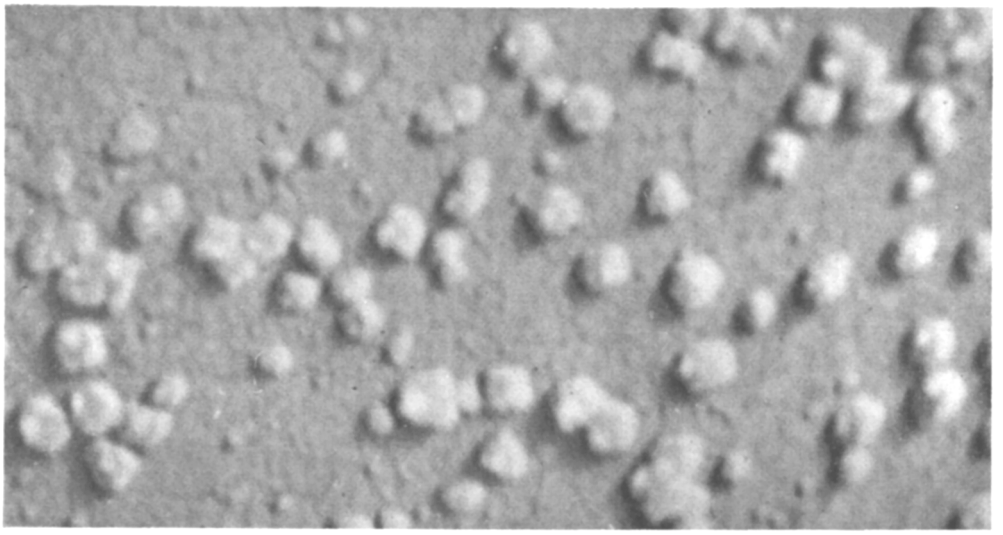

FIG. 1. West Nile Virus shadowed with chromium at arc tangent 2/10. × 150,000.

brains and cords, uniform virus-like particles were demonstrated as shown in Fig. 1. These bodies measure 38-40 mμ by direct measurement and are cube-shaped with irregular contour.

Summary. Studies by electron microscope of brains and spinal cords of mice infected with West Nile virus, B 956 strain, show the virus to be cube-shaped with a diameter of 38-40 mμ. These bodies could not be demonstrated in concentrated normal mice brains and spinal cords subjected to the same procedure of concentration and examination. The bodies from the West Nile preparations resemble the rabbit papilloma virus described by Sharp *et al.*(3). The virus in the concentrated material was infectious for unvaccinated mice but had no effect on mice previously immunized against the West Nile virus.

3. Sharp, D. G., Taylor, A. R., Hook, A. E., and Beard, J. W., PROC. SOC. EXP. BIOL. AND MED., 1946, v61, 259.

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Composition of Mitochondrial Preparations in Relation to Intracellular Localization of Azoprotein in the Mouse.* (19164)

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The tissue localization of labeled proteins has been the subject of extensive investigation, and after parenteral administration, such antigens have been demonstrated repeatedly in the cells of the reticulo-endothelial system and, to a lesser extent, in those of other organs (1-6). It is known that the distribution of

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1. Sabin, F. R., *J. Exp. Med.*, 1939, v70, 67.
2. Smetana, H., and Johnson, F. R., *Am. J. Path.*, 1942, v18, 1029.