Monkey Pathogenicity of Various Strains of Murine Poliomyelitis Virus. I. Experiments with Lansing, MEF, and Yale-SK Group.* (17489)

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Adaptation of human poliomyelitis virus from monkey or man to rodents results in the production of murine strains which differ significantly in the degree of their pathogenicity for mice and rhesus monkeys. One group, with low potency in mice, *i.e.* Lansing, MEF and Yale-SK virus, retain to a large extent their original ability to paralyze rhesus monkeys, even though continuous passage from mouse to mouse may cause some deterioration of this property.(1) Another group, Columbia-SK and MM virus, with high potency in mice, produce in rhesus monkeys rarely flaccid paralysis but set up a subclinical infection characterized by massive intraneural and extraneural virus multiplication as well as by abundant antibody formation.(2) A third group, transferable only to suckling mice, apparently has no pathogenicity whatsoever for rhesus monkeys.(3) The following communication deals with a study of the pathogenicity for rhesus monkeys of serial mouse passages of viral strains belonging to the first group, *i.e.* Lansing, MEF and Y-SK virus.

Experiments with the Lansing Virus. The strain was obtained in 1945 in its 36th mouse passage through the courtesy of Dr. G. Dalldorf, State Laboratory, Albany, N. Y. The virus has since been carried in this laboratory in mice by making transfers at irregular intervals from glycerinated material stored in the ice-box. A 10% suspension of infected brain and cord tissues was used routinely and 0.03 cc injected intracerebrally into 11-14 g Swiss albino mice. During the past 3 years the virus was examined for its pathogenicity for rhesus monkeys on 3 occasions. In each case a 10% suspension was prepared from freshly harvested brain and cord of a paralyzed mouse and 1 cc of the supernatant, after light centrifugation, was injected intracerebrally into rhesus monkeys weighing about 2500-3000 g. One monkey was injected with the 41st, 2 monkeys with the 57th, and one monkey with the 63rd serial mouse passage. None of the injected monkeys showed any signs of disease, such as fever, weakness or paralysis. Since the animals were subsequently used for the production of hyperimmune anti-Lansing serum there was no opportunity for examining any sections of the spinal cord for possible subclinical lesions or for testing these animals for immunity by reinfection with potent simian virus.

In subsequent experiments the same strain was used in attempts to infect 2 cynomolgus monkeys by intracerebral injection with 1 cc of a 10% mouse brain suspension from the 74th serial mouse passage. Both monkeys remained free from symptoms. It will be of considerable interest to further examine this apparently monkey-nonpathogenic Lansing strain for its ability to induce protection, in the absence of paralysis, against a pathogenic strain.

At the time these observations were made the virus always presented the typical clinical, biological and serological characteristics of the strain. Paralysis occurred only after intracerebral and not intraperitoneal injection, infected mice showed flaccid paralysis of the front or hind legs, the incubation period varied from 48 hours to 21 days, and the potency of the virus fluctuated between 10^{-1} and 10^{-2} with a mortality rate of between 80 and 90%. Moreover, the virus was neutralized by hyperimmune Lansing antiserum (obtained from Dr. Isabel Morgan, Johns Hopkins University, Baltimore) and by a majority of normal and polio-convalescent sera collected in the metropolitan area, but not by Col-SK antiserum or

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1. Theiler, M., *Medicine*, 1941, v20, 43.

2. Jungeblut, C. W., and Sanders, M., *J. Exp. Med.*, 1940, v72, 407; *ibid.*, 1942, v75, 611.

3. Dalldorf, G., Sickles, G. M., Plager, H., and Gifford, R., *J. Exp. Med.*, 1949, v89, 567.

by GDVII antiserum.†

Experiments with the MEF Virus. The strain was obtained in 1945 in its 8th mouse passage through the courtesy of Dr. P. K. Olitsky, Rockefeller Institute, New York City. The virus was carried in mice in this laboratory using the same technic as previously described for the Lansing virus, except that passages were made rapidly with freshly harvested material and no intervening storage. On 4 occasions the virus was examined for pathogenicity in rhesus monkeys by intracerebral injection of 1 cc of 10% viral mouse brain-cord suspension. One monkey received the 10th, one monkey the 15th, one monkey the 19th, and one monkey the 20th serial mouse passage. The first monkey injected with the 10th serial mouse passage became paralyzed on the 14th day but no active virus could be recovered from the cord of the paralyzed animal by transfer to another rhesus monkey or to a group of mice. The other three monkeys remained free from any symptoms of disease. Two of the symptomless monkeys were tested, two months later, for immunity by intracerebral challenge with Aycock virus (0.5 cc 1:100). Both developed typical paralysis. The third monkey was used for the production of hyperimmune anti-MEF serum.

As previously described for the Lansing virus, the MEF virus at all times maintained its typical clinical, biological and serological characteristics.‡

Experiments with the Y-SK Virus. The virus was obtained in 1947 through the courtesy of Dr. G. Dalldorf. It was propagated in this laboratory by rapid serial passage in mice as previously described for the MEF virus. Intracerebral tests for pathogenicity in rhesus monkeys were carried out with various generations, inoculating two monkeys with the 5th and one monkey each with the 10th and 15th serial mouse passages. All monkeys developed typical flaccid paralysis within 9 to 13 days after injection, except one monkey inoculated with the 5th passage and another monkey inoculated with the 10th passage. No attempts were made to carry the strain further from monkey to monkey but all efforts to recover the virus by inoculation of mice with the cord of paralyzed monkeys uniformly failed.

The Y-SK virus was obtained once more in 1948 through the courtesy of Dr. J. Melnick, Yale University, New Haven, Conn., the material being received in form of frozen pooled mouse brains and cords. The original material proved highly infectious for rhesus monkeys, since 7 of 8 monkeys injected intracerebrally on various occasions between 1948 and 1949 developed typical paralysis. The strain was carried in mice in this laboratory by rapid transfer from mouse to mouse using freshly harvested brains and cords. Tests for rhesus pathogenicity were made with the 6th, 11th and 15th serial mouse passage. One of 2 monkeys each injected with the 6th or 11th mouse passage developed paralysis, as did 2 of 3 monkeys injected with the 15th mouse passage. Again, virus in mouse-pathogenic form could not be recovered from the cords of these animals when completely paralyzed.

During the time of these observations the Y-SK virus maintained its typical clinical, biological and serological characteristics, which made it indistinguishable from the Lansing and MEF virus, except for its somewhat lower virulence in mice.

Conclusions. The data presented above demonstrate that strains of mouse-adapted human poliomyelitis virus may exhibit wide variations in the degree of their pathogenicity for rhesus monkeys. The Lansing virus, for instance, harvested from remote mouse pas-

† This statement refers to the strain under investigation in this paper and not to any rhesus-nonpathogenic, high-titred, serologically atypical possible variants which may or may not have been derived from the original Lansing virus.(4,5,6)

4. Jungeblut, C. W., *Proceedings Fourth Internat. Congress for Microbiology,* Copenhagen, 1947, p. 259.

5. Enright, J. B., and Schultz, E. W., PROC. SOC. EXP. BIOL. AND MED., 1947, v66, 541.

6. Sanders, F. K., *Proceedings Federation of American Societies for Experimental Biology,* Atlantic City, 1948, p. 309.

‡ Reference is made to the original MEF strain and not to a rhesus-nonpathogenic, high-titred possible variant which has previously been described.(4)

sages, gave no evidence of being pathogenic for rhesus monkeys in repeated tests. The MEF virus, originally rhesus-pathogenic, apparently suffered a progressive loss of its pathogenicity for rhesus monkeys during serially maintained rapid mouse passages. The Y-SK virus, finally, seemed to have undergone but little change in its pathogenic power for rhesus monkeys as the strain was passed rapidly through mice. It is realized that the described phenomena may not always be duplicated in different laboratories since the Lansing virus has frequently been reported by others as being fully rhesus-pathogenic. Yet the authenticity of our strains is attested by the fact that all three murine strains had fully preserved their biological and immunological characteristics. The observed differences in the degree of pathogenicity for rhesus monkeys and in the extent to which murine virus returns to mice from paralyzed monkeys may be due to individual differences among strains or reflect an attainment of different levels of perfection in the adaptive process.

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Monkey Pathogenicity of Various Strains of Murine Poliomyelitis Virus. II. Experiments with Col. SK-MM Group.* (17490)

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In the preceding paper(1) data have been presented to show that the Lansing, MEF and Y-SK virus, upon serial passage in mice, may undergo various degrees of deterioration in their ability to produce flaccid paralysis in intracerebrally infected rhesus monkeys. This reduction in monkey pathogenicity, however, was not associated with any demonstrable changes in other biological or immunological properties. It became next of interest to investigate whether the Col-SK-MM group of viruses—agents which since their initial transfer to mice have shown little or no paralyzing power in rhesus monkeys—would be capable of inducing paralytic infection, with central nervous system lesions, in cynomolgus (Java) monkeys.

The cynomologus monkey was chosen for this purpose since the response of this animal to poliomyelitis virus differs in many respects from that observed in rhesus monkeys. Whereas the rhesus monkey, as a rule, is susceptible only to intracerebral infection and the virus remains strictly localized in the central nervous system, the cynomolgus monkey may be infected by peripheral portals of entry, including the gastrointestinal tract, with the production of a systemic disease characterized by wide extraneural distribution of the virus and by fecal excretion. Moreover, isolation of poliomyelitis virus from fresh human or nonhuman sources often succeeds better with the cynomolgus than with the rhesus monkey.(2)

The following communication deals with a study of the pathogenicity for cynomolgus monkeys of current mouse passages of Col-SK and MM virus. Included are also experiments with 2 viruses of as yet uncertain identity but with close serological relationship to Col-SK and MM virus. These are the virus of so-called encephalomyocarditis (EMC virus)(3) which was isolated from chimpanzees suffering from unknown myocardial disease, and the Schultz High Lansing strain, presumably a "high" variant of the original Lansing vi-

* Aided by a grant from the Sister Elizabeth Kenny Foundation.

1. Jungeblut, C. W., PROC. SOC. EXP. BIOL. AND MED., 1949, in press.

2. Melnick, J., *Am. J. Hygiene*, 1949, v49, 8.

3. Schmidt, E. C. H., *Am. J. Path.*, 1948, v24, 97.

4. Enright, J. B., and Schultz, E. W., PROC. SOC. EXP. BIOL. AND MED., 1947, v66, 541.