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Rubella Virus Replication in the Brains of Suckling Mice

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Rubella virus is associated with defective development of the fetal brain when infection occurs during the first trimester of human pregnancy and has been isolated from the brains of children infected in utero (J. A. Bellanti et al., Am. J. Diseases Children **110**:464, 1965; L. Z. Cooper et al., Am. J. Diseases Children **110**:416, 1965; D. M. Horstmann et al., Am. J. Diseases Children **110**:408, 1965; G. R. G. Monif and J. L. Sever, Neurology **16**:111, 1966; W. E. Rawls et al., Proc. Soc. Exptl. Biol. Med. **120**:623, 1965). Rubella virus has been recovered from the cerebrospinal fluid of monkeys after central nervous system inoculation (P. D. Parkman et al., J. Immunol. **95**:743, 1965). Intracerebral inoculation of newborn hamsters resulted in a decline in virus titer with no evidence of viral replication (J. S. Oxford and G. C. Schild, Virology **28**:780, 1966).

In the present study, the *growth* of rubella virus in the brains of suckling and adult mice was assayed on MA134 cells (originally obtained from Microbiological Associates, Bethesda, Md.) by means of the hemadsorption-negative plaque technique (P. I. Marcus and D. H. Carver, Science **149**:983, 1965). A stock of rubella virus with a titer of about 2,000 hemadsorption-negative plaque-forming particles (HAD^- PFP)/ml was prepared in green monkey kidney cells by use of the F8 strain (obtained from Dr. Balsamo of New York University) as seed virus.

Suckling mice in the 1st or 2nd day of life were inoculated intracerebrally with 0.025 ml of stock rubella virus. Adult mice with an average brain weight of 0.4 g (four times that of the newborn mouse) received 0.1 ml of virus intracerebrally. Swiss-Webster or ICR mice were used in these experiments.

Mice were killed in a 4 C cold room by decapitation, and the brains were immediately removed and ground in a mortar and pestle with sufficient attachment solution (P. I. Marcus and D. H. Carver, Science **149**:983, 1965) to make 10% suspensions. The suspensions were centrifuged in a refrigerated centrifuge for 10 min at 5,000 rev/min. The supernatant fluid was then centrifuged for 15 min at 10,000 rev/min. The final supernatant liquid was stored in small portions at −60 C, prior to the simultaneous assay of all mouse brain suspensions from a single

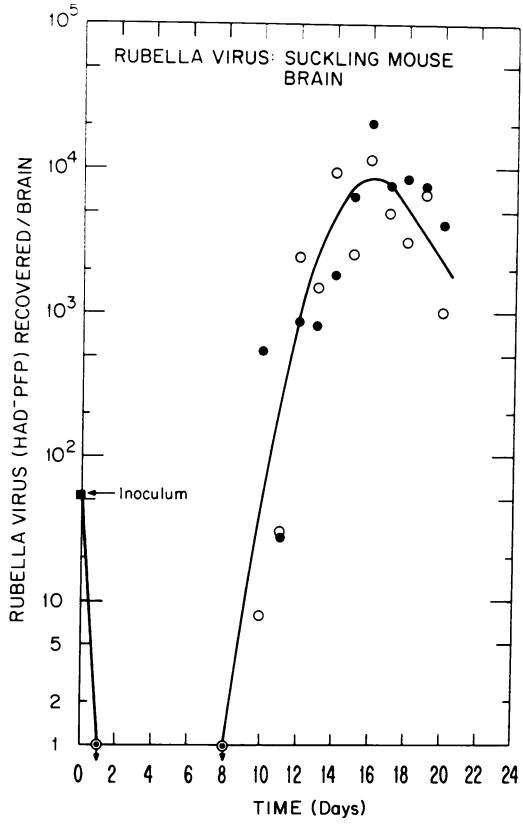

FIG. 1. *Rubella virus recovered from suckling mouse brains.* (○) *Experiment A;* (●) *experiment B.*

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experiment. Except where otherwise indicated, assays were performed on pools of brain suspensions prepared from three mice killed at the same time. The average yield per brain was then calculated.

The results from two representative experiments are illustrated in Fig. 1. After an inoculum of 50 to 60 HAD^- PFP per suckling mouse brain, no virus was detectable for 8 days. Rubella virus was first detected on the 10th day after inoculation and increased essentially logarithmically for the next 5 or 6 days, reaching at the 16th day peak titers some 200- to 300-fold greater than the original inoculum. A significant decline in virus titer was observed from the 16th day to the 20th day postinfection, the last day tested.

In other experiments, virus was first detected on the 8th day after inoculation and was still found on the 45th day. Tests on animals killed on the 47th and subsequent days were negative for detectable virus in the brain suspensions.

Brain suspensions from two litters of rubella-infected neonatal mice were assayed individually to determine the number of mice infected. The mice were killed on the 16th day after infection, and 9 of the 10 brain suspensions from one litter and 3 of the 6 in the second contained virus.

Virus recovered from mouse brains was identified as rubella by means of neutralization tests; "acute" sera, obtained from susceptible persons at the time of their contact with rubella patients, and paired "convalescent" sera, drawn 2 weeks after the subjects developed rashes, were used. The greatest dilution of a serum that neutralized 50% of the HAD^- plaques produced by the virus control was considered its titer against rubella. Neither of two patients' acute sera neutralized a mouse brain isolate at a dilution of 1:8, whereas the neutralizing titers of the convalescent sera were 1:32 and 1:16.

Brain suspensions were prepared daily from pools of three uninoculated suckling mice, and no virus was detected from the 1st through the 20th day of life. The appearance, brain weight, and behavior of control and rubella-infected mice were the same.

Adult mice were inoculated intracerebrally with rubella virus, and daily tests were made to detect the presence of virus in brain suspensions. Virus was not detectable between the 1st and 20th days after inoculation. Sera obtained from uninoculated adult mice did not contain any neutralizing antibody against rubella at a dilution of 1:4.

The present study demonstrates the replication of rubella virus in the brains of suckling mice. Such replication was not demonstrable in adult mice.

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