Propagation of Measles Virus in Suckling Mice.* (24109)

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Because of the interest in immunological similarity between measles and distemper viruses(1), an attempt to adapt measles virus to mice was undertaken to establish a common animal host. The following experiments indicate that measles virus may be propagated in the central nervous system of suckling mice. Earlier reports on successful transmission of measles virus in mice have been described, but further attempts have failed to produce convincing evidence of its propagation(2). Since the report by Enders and Peebles(3) on successful isolation of measles virus in cultures of human and monkey renal epithelial cells, other cell cultures supported continuous multiplication of the virus(1,4,5,6). Propagation of measles virus (Edmonston strain) in tissue culture has made possible its adaptation to chick embryo(7), chick cells in culture(8), and, as is here reported, its multiplication in the central nervous system of suckling mice.

Materials and methods. Virus. The Edmonston strain(3) of measles virus was propagated in HeLa cell culture(1) and supernatant fluids from infected cultures were used as inoculum for initial passage. *Mice.* Laboratory-bred litters of Swiss albino mice, 1-2 day old, were inoculated intracerebrally in right hemisphere with 0.01 ml of inoculum. *Passages and titrations.* A 10% suspension of infected brain was prepared in brain heart infusion broth. All suspensions were centrifuged at 3,000 rpm for 15 minutes and contained 500 units of penicillin and 200 μg of streptomycin/1 ml of inoculum. For titrations, 10 fold serial dilutions were prepared in brain heart infusion broth. *Tissue culture.* HeLa cell cultures were grown and maintained in medium containing 60% yeast extract medium(9), 20% Scherer's maintenance solution (10), 10% normal calf serum and 10% brain heart infusion broth. The medium was changed every 3-4 days. *Serum neutralization tests.* Mixtures of diluted serum and 20% infected mouse brain, in equal volumes, were held at room temperature for ½ hour at 4°C for additional ½ hour before inoculation of HeLa cell cultures. All serum samples were inactivated at 56°C for ½ hour before mixing with the virus. A standard inoculum of 0.2 ml was added to HeLa cell culture tubes and observed daily for 21 days. The technic for neutralization studies in suckling mice was similar to above procedure. A 20% brain suspension was mixed with dilutions of serum, and incubated similarly before injecting 0.01 ml intracerebrally into suckling mice. Death of animals was recorded for 21 days and neutralization was determined by survival of animals. *Normal and immune sera.* Neutralization tests were carried out with dilutions of acute and convalescent serum from patients with typical measles. Tests were also carried out with sera from ferrets as described earlier(1). Normal ferret serum was obtained from animals prior to immunization and the specific immune serum was obtained 1 month after final injection of tissue culture measles virus.

Results. Passage of measles virus in suckling mice. Supernatant fluid from measles infected HeLa cultures was inoculated intracerebrally into suckling mice of 2 litters. Mice of one litter were sacrificed on 4th and 7th day after inoculation and no virus was detected when brain material was assayed in HeLa cultures. The second litter was observed for signs of illness and 10 days after inoculation, one mouse showed definite signs of irritability. This mouse was sacrificed and a 10% suspension of brain tissue was prepared. The material was inoculated into HeLa cultures in 0.1 ml volumes for detection of virus and also passed serially in suckling mice. The earliest manifestation of illness consisted of running and jumping in the cages.

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TABLE I. Intracerebral Passage of Measles Virus in Suckling Mice.

Passage No.	Ration of infection	Incubation (days)	Mouse titer, log LD_{50} /.01 ml	HeLa titer, log TCD_{50} /.1 ml
1	1/ 7*	10		1.7
2	7/ 7	6		3.2
3	15/20	8		2.5
4	16/20	9–11	1.2	2.5
5	9/16	10		1.5
9	7/17	9–12	1.5	
11	7/13	8– 9	1.4	2.5
12	13/19	8–10	1.3	2.5
17	8/14	7	1.4	2.0
22	11/12	9		3.2
24	11/12	7	2.0	

* Animals either dead of infection or sacrificed when moribund over total No. of animals inoc.

Subsequently, animals became apathetic, lying on their sides and moving very slowly when disturbed. Death generally occurred 2-3 days after initial signs of illness. All passage material when assayed in tissue culture produced a cytopathogenic effect similar to that associated with the original HeLa measles virus(1). Observations of passage study and results of virus titrations in HeLa cultures and suckling mice are summarized in Table I. The approximate LD_{50} titers in suckling mice were quite constant and ranged between $10^{1.2}$ and 10^{2}/0.01 ml for 24 mouse passages. The mouse propagated agent produced cytopathology in HeLa cell cultures and gave TCD_{50} titers between $10^{1.5}$ and $10^{3.2}$/0.1 ml. These results correlate well with the infective titers in suckling mice.

Preservation of mouse measles virus. Ten percent suspensions of brain material from 3rd and 7th passages were stored at −35°C for 7 and 2 months respectively. When 7th passage material was thawed and injected into suckling mice, the comparative infection rates of original and frozen material showed no significant difference. Virus was still viable after 7 months of storage, however, there was some loss of infectivity as shown by a 60% decrease in death rate.

Identification of suckling mouse propagated virus. Three principal methods were employed to identify mouse propagated virus as measles virus: 1) Serum neutralization tests in tissue culture, 2) Serum neutralization tests in suckling mice, and 3) Protection tests in suckling mice. Neutralization tests in tissue culture were performed with specific measles antisera prepared in ferrets and with human measles convalescent sera. For control purposes normal ferret sera (pre-immunization) and human measles acute sera were employed. Immune ferret sera and human convalescent sera completely inhibited the cytopathogenic effects of the virus, whereas, normal ferret sera and acute human sera showed no significant inhibitory effect. The results are summarized in Table II. Neutralization studies in suckling mice were carried out with virus obtained from 22nd mouse passages. These results correlated well with neutralization studies in tissue culture. Ferret antimeasles sera and human measles convalescent sera protected 50% of mice against lethal effects of the virus at 1/16 and 1/128 dilutions of the sera respectively, whereas, normal ferret sera and acute human sera showed no observable neutralizing effect. In protection tests of suckling mice, adult female mice were immunized by multiple injections of HeLa adapted measles virus. Pups born from these mice were protected from challenge inoculation of mouse propagated measles virus, whereas mice born from females immunized with control material or from untreated females succumbed to the challenge. These results are summarized in Table III.

Serial propagation of mouse measles virus in HeLa culture. After 4th mouse passage, serial transfer of mouse adapted measles virus in HeLa cell culture was difficult when supernatant fluids were employed; however, cytopathogenesis was readily induced when infected cells were used as inoculum. This phenomenon was also observed when suckling

TABLE II. Neutralization of Mouse Propagated Virus with Measles Antiserum in HeLa Culture.

Mouse passage	Serum samples	Neutralizing serum dilution
11	Human acute	1/8
	" convalescent	1/128
17	" acute	<1/4
	" convalescent	1/128
13	Normal ferret	<1/4
	Ferret antimeasles	1/32
17	Normal ferret	<1/4
	Ferret antimeasles	1/16

TABLE III. Protection Test in Suckling Mice.

Mouse measles employed	Suckling mice born from mothers: Immunized with measles virus	Immunized with control material	Untreated
7th passage	0/ 8*		6/6
8th "	0/ 9	5/9	4/9
9th "	0/17	3/9	4/6

* No. dead/Total No. inoculated.

mice were inoculated with tissue culture material, as illustrated in the following experiment. Virus representing 20th mouse passage was back titrated in HeLa cell culture and produced characteristic cytopathogenesis. When cytopathogenicity had reached 4 plus after 9 days of incubation, culture fluids were removed, and centrifuged twice at 3,000 rpm for 15 minutes. The supernatant fluid when back titrated in mice did not exhibit any lethal effect, whereas the sediment consisting of cellular debris produced typical signs of infection and eventual death. Further passage studies showed that these deaths were due to the measles virus.

Discussion. After 24 serial passages of measles virus in suckling mice, an increase of virulence failed to manifest itself. Unlike many other viruses, neither the infective titer of mouse brain material nor mortality rate was appreciably altered. Multiplication of virus in the central nervous system was evidenced by presence of more virus in each passage than the amount present in the original inoculum. In experiments with dengue virus, up to 15 passages were required before uniform clinical signs of infection were evident (11). It is possible that additional mouse passages of the measles virus are necessary before lethal infection will occur uniformly in all mice. In back titration experiments, fluid phase from cultures inoculated with late passage materials did not contain infectious material either for HeLa cells or for suckling mice. Infections, however, were readily induced when cells were employed as inoculum. Failure of infectious agents to be liberated into supernatant fluid is peculiar since culture fluids from back titration of mouse adapted agent were obtained after 4 plus destruction of HeLa cells. In contrast, our unpublished experiments, which agree with data reported by Ruckle(6), show that tissue culture adapted measles virus can be detected in the fluid phase on the same day as the occurrence of first obvious cytopathic changes. The possibility of incomplete viruses being liberated into the supernatant fluid is being investigated.

Summary. HeLa adapted measles virus (Edmonston strain) was propagated in suckling mice through 24 serial intracerebral passages. The virus produced a lethal infection in most animals 7 to 13 days following inoculation. Identity of mouse propagated agent was established by serum neutralization tests in tissue culture and in suckling mice. Further identification was established by protection of suckling mice born of mothers immunized with tissue culture measles virus. Fluid phase from tissue cultures inoculated with late mouse passage materials did not contain infectious material either for HeLa cells or for suckling mice. Infections, however, were readily induced when cells were employed as inoculum.

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