SUSCEPTIBILITY OF SUCKLING MICE TO VARIOLA VIRUS

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Received for publication December 3, 1960

ABSTRACT

MARSHALL, RONALD G. (Army Chemical Corps, Fredrick, Md.), AND PETER J. GERONE. Susceptibility of suckling mice to variola virus. J. Bacteriol. **82**:15–19. 1961.—The susceptibility of suckling mice inoculated intraperitoneally or intracerebrally with variola virus was investigated. Data are presented that define the death patterns, the relationship of incubation period to dose of virus inoculated, the multiplication of virus in suckling mice, and the influence of the age of suckling mice on their susceptibility to this virus. Additionally the results indicate that a variola virus neutralization test is feasible using the young suckling mouse as an indicator host.

A report by Rabinovitz and Bernkopf (1952) describes the use of suckling mice for studies on variola virus. Because most of the common laboratory animals are relatively resistant to this virus, and because of the need for a susceptible host in our work, a more extensive and quantitative study on the susceptibility of this host to variola virus was conducted. Data are presented on the susceptibility of mice of various ages depicting the occurrence and rate of mortality as a function of dose of virus inoculated. In addition the multiplication of virus in suckling mice was studied. Based on these data, a virus neutralization test has been developed.

MATERIALS AND METHODS

Virus. The Yamada strain of variola virus was primarily used in these studies. This strain had been passed five times in embryonated eggs since its isolation from man. A few titrations were carried out using the Kali-Muthu strain which had been passed three times in embryonated eggs. Virus inocula were in the form of 10% infected chorioallantoic membrane suspensions or decimal dilutions of these suspensions in beef heart infusion broth (Difco).

Mice. Swiss Webster albino mice, ranging in age from 2 hr to 6 days, were used. Litter sizes varied from 7 to 11 mice. One litter was used for each concentration of virus inoculated. The young were fed on uninoculated mothers that were held in the cages with the suckling mice for the duration of the experiment.

Mouse inoculations. Mice were inoculated by the intracerebral or intraperitoneal routes. Volumes of inocula used were 0.01 ml for intracerebral inoculations and, depending on the age of the mouse, 0.025, 0.05, or 0.1 ml for intraperitoneal inoculations.

Virus assay. In addition to titrations performed in suckling mice, seed inocula and mouse tissues were titrated by chorioallantoic membrane inoculation of 11- to 12-day-old embryonated eggs. This procedure has been described by Hahon, Louis and Ratner (1957). Concentrations of virus measured by this technique are expressed as pock-forming units per unit volume of a suspension or weight of a tissue. The LD_{50} values were calculated by the Reed and Muench (1938) method.

RESULTS

Intracerebral titrations. In Table 1 are the results of titrations of a seed of variola virus in suckling mice of different ages inoculated by the intracerebral route. The LD_{50} values in mice less than 24 hr old are about ten times lower than chorioallantoic membrane pock counts (4×10^7 LD_{50}/ml vs. 5×10^8 pock-forming units/ml). The sensitivity of titrations decreased rapidly in mice older than 24 hr.

The death patterns of mice inoculated intracerebrally with variola virus are shown in Table 2. The incubation time, that is, the time from inoculation to the average day of death, was inversely related to the concentration of the inoculum. As the inoculum was reduced, the percentage mortality decreased in a manner consistent with results obtained in other established mouse LD_{50} virus titrations.

Intraperitoneal titrations. The results of some typical intraperitoneal titrations in suckling mice

TABLE 1. *Susceptibility of suckling mice inoculated* by the intracerebral route with variola virus*

Virus strain	Egg titer, PFU†/ml	Age of mice	Mouse titer, LD_{50}/ml
		hr	
Yamada	5×10^8	2–6	$>3 \times 10^7$
		6–22	4×10^7
		8–22	3×10^7
		24–48	Irregular‡
		24–48	$<1 \times 10^6$
		54–56	1×10^6
		52–70	5×10^5
		76–94	$<3 \times 10^4$
Kali-Muthu	4.6×10^8	6–22	1×10^7

* Inoculation of 0.01 ml per mouse.

† PFU = pock-forming units.

‡ Irregular death pattern; LD_{50} value not calculated.

TABLE 3. *Susceptibility of suckling mice inoculated* by the intraperitoneal route with variola virus*

Age of mice	Volume of inoculum	Mouse titer LD_{50}/ml
hr	*ml*	
3–13	0.05	2.5×10^6
12–18	0.05	6×10^6
18–20	0.05	6×10^6
24–48	0.025	8×10^5
24–48	0.05	2×10^5
24–48	0.1	1×10^6
24–48	0.1	Irregular†
48–72	0.1	1.2×10^5
72–96	0.1	2.5×10^5
72–96	0.1	1.2×10^5
96–120	0.1	3×10^4

* Seed virus titered 5×10^8 pock-forming units per ml.

† Irregular death pattern; LD_{50} value not calculated.

are shown in Table 3. Titrations by this route of inoculation in the younger mice proved to be ten times less sensitive than the intracerebral method, 100-fold less than the chorioallantoic membrane pock count technique, and even less sensitive with increasing mouse age.

Deaths of mice inoculated by the intraperitoneal route (Table 4) occurred a day or two later than those of mice inoculated intracerebrally with comparable doses (Table 2). The incubation period, as in the case of intracerebral inoculation, was inversely related to the concentration of the virus inoculum and directly related to the age of the mice.

Virus neutralization test. The consistent end points and death patterns obtained by the inoculation of 6- to 22-hr mice suggested that not only could virus be titrated in this host but an additional method for detecting and measuring neutralizing antibody could be developed. Therefore, serum samples from a rabbit, collected before and after immunization with vaccinia virus (IHD strain), were used to measure the capacity of antibody to neutralize the lethal effect of the virus in the suckling mice. The control serum was diluted 1:2 in beef heart infusion broth and mixed with equal volumes of variola virus dilutions, 1:10 through 1:100,000. The immune serum, which had a titer of 1:1,280 in a hemagglutination-inhibition test (Kempe, 1956), was similarly mixed with virus. After 1 hr incubation at 37 C, the virus-serum mixtures were inoculated in 1- to 2-day-old suckling mice by the intraperitoneal route (0.05 ml volume). Deaths over an 18-day period were recorded. The virus titer (LD_{50}) in the mixture containing serum before immunization was found to be $10^{4.9}$ per ml; the titer in the immune serum mixture was $10^{2.2}$ per ml. The calculated neutralization index was 500.

Multiplication of virus in suckling mice. Be-

TABLE 2. *Death patterns of suckling mice* inoculated intracerebrally with variola virus*

Calculated dose, PFU†/ 0.01 ml	No. of mice	Deaths occurring on days after virus inoculation																	Avg day of death	Per cent mortality
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	
7,000	10			1				2		2	3	1		1						8.9	100
700	36						3	4	10	3	2	1	4	3	1			2	1	10.0	95
70	40								7	9	4	2	5		3		1			10.2	78
7	21								1	2			1			2	1			12.0	33
0.7	11																1			16.0	9

* Mice less than 24 hr old.

† PFU = pock-forming units.

TABLE 4. *Death patterns of suckling mice inoculated intraperitoneally with variola virus*

Age of mice	Inoculum		No. of mice	Deaths occurring on days after virus inoculation															Avg day of death	Per cent mortality
	Volume	Dose, PFU*		1	2	3	4	5	6	7	8	9	10	11	12	13	14	16	18	
7–23 hr	0.05	1×10^5	9				3	4	2											4.9	100
		1×10^4	9			1	3			1	2	2								6.2	100
		1×10^3	9								1	2			1					9.5	45
		1×10^2	9											2	1					11.3	33
		1×10^1	8											1						11.0	12
24–48 hr	0.05	1×10^6	11				6	5												4.5	100
		1×10^5	8						3	3	2									6.8	100
		1×10^4	8								1	2								8.6	37
		1×10^3	8								2		2		1					9.8	62
		1×10^2	7										1							10.0	14
3–5 days	0.1	2×10^6	9				1	3	4	1										5.6	100
		2×10^5	9								2	3		2						9.6	78
		2×10^4	8								1		1		1				1	12.0	50
		2×10^3	7												1					12.0	14
6–7 days	0.1	5×10^6	9									1		3	3			1		11.8	89
		5×10^5	9												2		3		1	14.0	67
		5×10^4	8																		0
		5×10^3	8																		0

* PFU = pock-forming units.

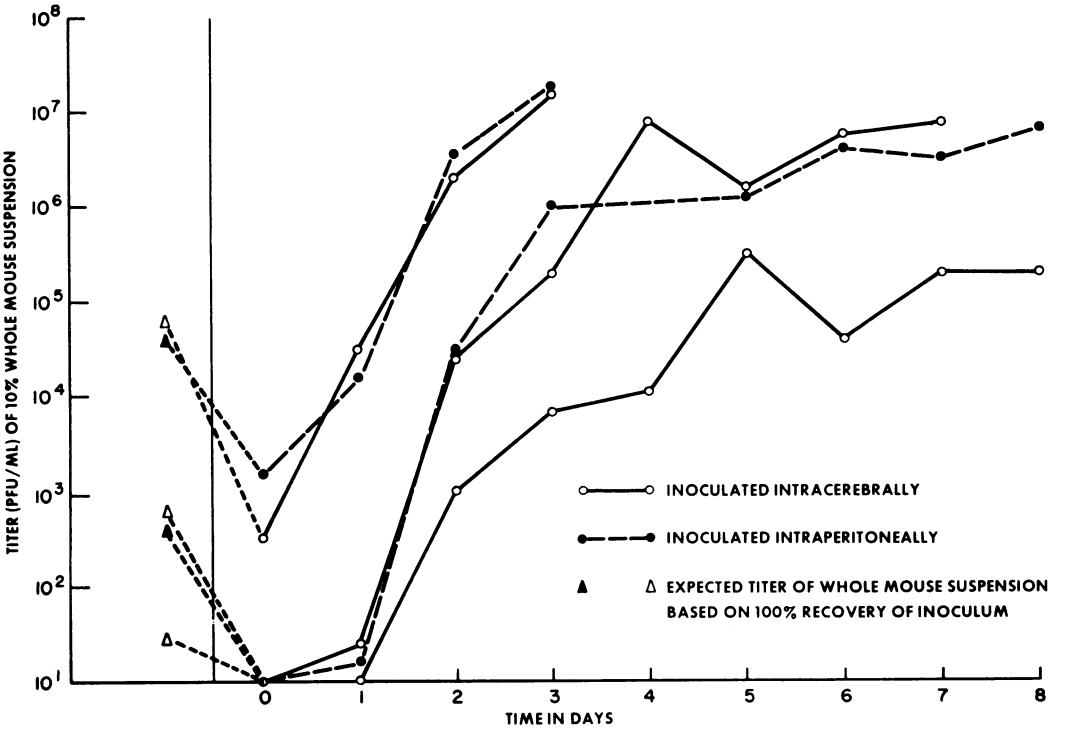

FIG. 1. *Multiplication of variola virus in suckling mice inoculated intracerebrally or intraperitoneally (PFU = pock-forming units).*

cause suckling mice proved to be one of the few hosts susceptible to the lethal effects of variola virus, it was of interest to determine the rate and extent of virus multiplication in this host. Several litters of 1- to 2-day-old mice were inoculated by the intraperitoneal and intracerebral routes. Animals inoculated intraperitoneally were administered 1×10^6 or 1×10^4 pock-forming units, and those inoculated intracerebrally received doses of 2×10^5, 2×10^3, or 2×10^1 pock-forming units. Immediately after inoculation and at daily intervals thereafter, 2 mice from each group were killed and stored in a Dry Ice chest until assayed. The mice from each group were thawed and 10% whole animal suspensions were prepared in beef heart infusion broth containing appropriate amounts of penicillin and streptomycin and assayed by the pock count method. The results of this experiment are presented in Fig. 1. Mice given comparable doses of virus by either route of inoculation showed similar patterns of multiplication. Shortly after inoculation there was a considerable loss of recoverable virus and, in one instance, a 95% loss was observed. In mice receiving the high doses of virus, titers of greater than 1×10^8 pock-forming units per gram of tissue were obtained on the 3rd day after inoculation. With these doses, no survivors were available on the 4th day postinoculation. Virus multiplication in mice receiving the smaller inocula differed in two aspects from those given larger doses. First, there appeared to be a lag of about 1 day prior to the phase of accelerated virus propagation and second, the maximal titers obtained were lower and occurred later.

DISCUSSION

The relative resistance of mice to various strains of variola virus has been known for some time. (Adult mice are apparently highly resistant to the lethal effects of variola virus but highly susceptible to infection (Brown, Elsner, and Officer, 1960).) The results presented in this paper show that very young mice are highly susceptible to this virus when inoculated intraperitoneally or intracerebrally with death as the end point. These results confirm those obtained by Rabinovitz and Bernkopf (1952). An examination of the susceptibility of mice of various ages has shown that the resistance of adult mice is acquired within a few days after birth. Mice inoculated intracerebrally were more sensitive to variola virus than those inoculated intraperitoneally. In mice less than 24 hr old, the intracerebral test and intraperitoneal tests were, respectively, about 10 and 100 times less sensitive than the pock count method of variola virus titration. Regardless of route, the average day of death was earlier when larger doses were employed.

A serum neutralization test utilizing the suckling mouse as the test system appears to be feasible. Additional studies will be undertaken to define this test further and to compare its results with those of other standard serological procedures applicable to studies on variola virus. In future growth and passage studies with variola virus, the susceptibility of suckling mice may provide an additional criterion for comparing possible qualitative genetic alterations of the virus as described by Smith and Sharp (1960) for vaccinia virus and Stoker (1959) in the case of herpes virus.

Multiplication of the virus in suckling mice was found to follow a similar pattern irrespective of the route of inoculation employed. In all cases less than 10% of the inoculum was recovered from mice killed within 1 hr after inoculation. The maximal titers obtained were directly related to the amount of virus injected, i.e., larger doses produced higher maximal titers. Inoculation of $10^{4.5}$ to $10^{5.0}$ pock-forming units per mouse by either route resulted in maximal titers of greater than 10^8 pock-forming units per gram of mouse. Additional studies are presently underway to determine the specific sites of viral replication.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the technical assistance of Helen H. Miller, John Lazuick, Boyd Yates, and Michael Sulima throughout these studies.

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