

Susceptibility of Mice Aged 0-14 Days to Infection with Junin Virus¹ (34942)

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Newborn mice are often used in attempts to isolate Junin virus from natural sources (1) and are also extensively employed in experimental work with this agent. Thus far, however, no systematic study of their susceptibility to infection with the virus has been reported.

The purpose of the present experiments was to determine the specific age levels, within the range 0-14 days, at which mice are most susceptible to such infection in terms of route of inoculation, height of virus titer in the brain tissue, length of incubation period, and average survival time (AST).

Materials and Methods. Mice derived from the Charles River CD (R)-1 strain were random bred in a barrier colony at the Yale Arbovirus Research Unit. Litters born during the preceding night were taken from the breeding room with their mothers on 15 successive days and held for inoculation of virus until the accumulated groups were respectively 14, 12, 10, 8, 6, 4, 3, 2, 1, and 0 days old (0 day = no more than 12 hr). After inoculation all mice were returned to their mothers, but on the next day each litter (10-14 animals) was reduced to 8; in this way, no losses resulting from inoculation trauma affected the uniformity of group size.

The strain of Junin virus used, XJ, has had 2 passages in guinea pigs and 46 in newborn mice. The viral stock consisted of a 20% suspension of infected newborn mouse

brain tissue in phosphate-buffered saline solution, pH 7.2, with added 0.75% bovine plasma albumin. The stock was kept at -70° in sealed glass ampules, each containing 0.5 ml. Increasing 10-fold dilutions to 10^{-10} were made from a pool of several ampules, using the same diluent.

Inoculations were done by three different routes: intracerebral (ic), intraperitoneal (ip), and subcutaneous (sc). The volume of the inoculum was 0.02 ml in all instances. When the ip or sc route was used, a simultaneous ic titration was carried out in 2- and 4-day-old mice as a control of infectivity. Owing to the large number of mice, about 320 litters, the study was divided into three tests, one for each route.

After inoculation, the mice were observed daily for 30 days for signs of disease and death. At the end of this time, all healthy-appearing survivors were bled out, the bloods of littermates were pooled and centrifuged, and the sera were stored at -20° until examined for presence of Junin virus antibodies in complement-fixation (CF) test (2). The Junin antigen was prepared by sucrose-acetone extraction of brain tissue from mice infected with strain XJ (3); sera were inactivated for 20 min at 60° before testing. Antigen in dilutions 1:16 and 1:32, containing 16 and 8 units, respectively, was tested against serial increasing 2-fold dilutions of serum beginning at 1:4. All sera from mice inoculated by a given route were tested simultaneously.

Mice that survived at 30 days but showed paralysis were held for a maximum of 4 months. Their sera were tested individually in CF test with the Junin antigen and also with sucrose-acetone antigens (4) for the fol-

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TABLE I. Observations on Mice Aged 0-14 Days Inoculated ic with Junin Virus, Strain XJ.

Age of mice (days)	Virus titer ^a	AST, all fatalities (days) ^b	Optimal dilution ^c	
			Dil. ^d	AST
0	5.3	14.8 ± 2.3	-1	13.3 ± 2.5
1	5.2	15.4 ± 2.6	-2	13.4 ± 1.9
2	4.6	13.8 ± 3.2	-2	11.5 ± 2.6
3	4.9	13.7 ± 2.3	-1	11.5 ± 2.0
4	4.9	12.7 ± 2.7	-3	11.1 ± 2.1
6	4.7	11.1 ± 2.6	-1	8.6 ± 0.9
8	4.4	11.5 ± 3.0	-1	9.2 ± 1.2
10	3.9	10.7 ± 1.7	-2	10.0 ± 0.8
12	2.3	10.6 ± 3.0		None
14	≤0.9	10.5 ± 3.5		None

^a Log₁₀ LD₅₀ per 0.02 ml.

^b Average survival time.

^c Optimal dilution defined as a given dilution that killed all mice in a litter with the shortest AST.

^d Exponent to the base 10.

lowing viruses: mouse poliomyelitis (GD 1), mouse encephalomyocarditis (Kissling), mouse hepatomyelitis (Tr 23421), lymphocytic choriomeningitis (Bulgaria), and ecmortelia (Rumania 176).

Results. Mice inoculated ic. Table I shows the LD₅₀ titer for each age group of mice; the AST of all mice that died in each age group, regardless of virus dilution inoculated; and the "optimal dilution" (5), meaning the

dilution that caused death of all mice in the given age group—with the shortest AST. It can be seen that with mice aged 1, 2, 4, and 10 days the optimal dilution was 10⁻² or 10⁻³, rather than 10⁻¹. The table also shows that the AST at the optimal dilution fell from 11.1-13.4 days in mice aged 4 days or younger to 8.6-10 days in older mice.

Taking into account all age groups and all dilutions of virus tested, signs of illness in mice inoculated ic usually appeared first on day 8 postinoculation (pi), but occasionally on day 7. The signs seen in the course of the disease were tremors, loss of postural reflexes, lateralization of walking upon stimulation by pressure on the tail, and paralysis. After becoming ill, most mice showed progressive deterioration and died within an average period of 2-4 days (range of less than 24 hr to 9 days). Some sick mice, however, were observed to recover completely.

In Table II it can be seen that at 30 days pi some of the healthy-appearing surviving mice had developed Junin virus antibody in high titer. In general, the higher concentrations of virus inoculated resulted in the higher antibody titers. With virus dilutions 10⁻⁸-10⁻¹⁰, all mice survived and all were negative.

Mice inoculated ip or sc. The results obtained with these routes of inoculation were

TABLE II. CF Tests with Junin Virus Antigen and Serum (pooled by litter) of Healthy-Appearing Mice Surviving at 30 Days after ic Inoculation with Junin Virus.

Age of mice (days)	Virus dilution inoculated (exponent to base 10)						
	-1	-2	-3	-4	-5	-6	-7
0	(0) ^a	(0)	(0)	(0)	16 ^b (4)	Neg (7)	Neg (6)
1	256 (3)	(0)	(0)	4 (1)	Neg (3)	Neg (5)	Neg (7)
2	(0)	(0)	(0)	8 (1)	Neg (6)	Neg (8)	Neg (8)
3	(0)	128 (1)	256 (1)	4 (2)	Neg (3)	Neg (7)	Neg (8)
4	(0)	(0)	(0)	8 (1)	Neg (4)	Neg (8)	Neg (8)
6	(0)	(0)	(0)	64 (2)	8 (5)	4 (8)	Neg (8)
8	(0)	(0)	8 (2)	64 (3)	8 (5)	Neg (7)	Neg (8)
10	(0)	(0)	8 (4)	8 (4)	Neg (6)	Neg (7)	Neg (8)
12	64 (4)	16 (2)	16 (6)	64 (8)	Neg (7)	Neg (8)	Neg (8)
14	32 (5)	8 (8)	8 (8)	32 (7)	Neg (8)	16 (8)	Neg (8)

^a Figures in parentheses indicate number of survivors, of eight inoculated, represented in serum pool. (0) = no survivors.

^b Reciprocal of serum titer. Neg = no fixation at serum dilution 1:4, lowest used.

TABLE III. Observations on Mice Aged 0-14 Days Inoculated ip or se with Junin Virus, Strain XJ.

Age of mice (days)	ip Inoculation		se Inoculation	
	Virus titer ^a	No. of paralyzed survivors on day 30 pi	Virus titer	No. of paralyzed survivors on day 30 pi
0	2.7	0	3.2	3
1	1.0	1	1.7	2
2	<1.0	1	1.5	0
3	<1.0	0	<1.0	2
4-14 ^b	<1.0	0	<1.0	0

^a Log₁₀ LD₅₀ per 0.02 ml; paralyzed survivors not included.

^b Mice 4, 6, 8, 10, 12, and 14 days old.

similar and are, therefore, presented together. Signs of illness, resembling those already described, were observed only in mice 3 days old or younger; disease was followed in most instances by death, but the AST of 15-17 days was longer than that of mice in the same age groups inoculated ic. As shown in Table III, the LD₅₀ titers for these younger mice were also far lower than those obtained after ic inoculation. No mouse 4 days or more old either showed signs of illness or died of specific cause.

In further contrast to the ic results, nine mice that became paralyzed in one or both hind limbs on day 15 pi or later did not recover completely but nevertheless survived

to day 30 pi (Table III). As noted in Table IV, three of these mice showed progressive deterioration and subsequently died (on days 36 and 74 pi); the remaining six, however, developed normally and, except for the paralysis, appeared healthy at the end of 4 months.

None of the sera of these nine paralyzed mice reacted in CF test with antigens for mouse polioencephalitis, mouse encephalomyocarditis, mouse hepatoenephalitis, lymphocytic choriomeningitis, and ectromelia viruses. All nine sera, however, had titers of 1:16 to 1:64 against Junin antigen.

The results of CF tests with sera of the healthy-appearing survivors at 30 days are shown in Table V. Antibodies to Junin virus were not detected in the sera of mice inoculated with virus dilutions 10⁻⁵-10⁻¹⁰. The majority of the higher reactions observed, 1:16 and 1:64, were given by sera from mice aged 4 days or younger and inoculated with dilutions 10⁻¹-10⁻³; however, reactions of 1:16 and 1:32 were recorded for sera from mice inoculated with dilution 10⁻⁴.

Discussion. The present results indicate that lethal outcome is not the sole criterion for determining infection with Junin virus in infant mice. Consideration must also be given to the development of antibodies (a) in mice that survive ic or peripheral inoculation of the virus without signs of illness; (b) in mice inoculated ic that become ill but recover completely; and (c) in mice that survive

TABLE IV. Paralyzed Mice Surviving on Day 30 after Peripheral Inoculation of Junin Virus: Further Observations.

Mouse no.	Age	Route of inoculation, dilution ^a	Course to 4 months pi
1	0 day	se, -1	Hind limbs paralyzed; development otherwise normal
2	0 day	se, -3	Left hind limb paralyzed; progressive deterioration; death day 74
3	0 day	se, -4	Right hind limb paralyzed; development otherwise normal
4	1 day	ip, -1	Left hind limb paralyzed; development otherwise normal
5	1 day	se, -2	Hind limbs paralyzed; progressive deterioration; death day 36
6	1 day	se, -3	Right hind limb paralyzed; progressive deterioration; death day 36
7	2 days	ip, -4	Left hind limb paralyzed; development otherwise normal
8	3 days	se, -1	Left hind limb paralyzed; development otherwise normal
9	3 days	se, -1	Right hind limb paralyzed; development otherwise normal

^a Exponent to the base 10.

TABLE V. CF Tests with Junin Virus Antigen and Serum (pooled by litter) of Healthy-Appearing Mice Surviving at 30 Days after ip or sc Inoculation of Junin Virus.

Age of mice (days)	ip Inoculation, dilution:				sc Inoculation, dilution:			
	-1 ^a	-2	-3	-4	-1	-2	-3	-4
0	NS ^b	16 ^c	4	0	16	4	64	32
1	8	16	16	0	4	16	8	0
2	16	8	0	0	8	8	4	0
3	16	4	4	0	16	16	4	16
4	64	8	8	0	4	4	8	0
6	8	4	4	4	0	4	4	0
8	8	0	0	0	4	0	0	0
10	4	8	0	0	0	0	0	0
12	0	0	0	0	4	0	0	0
14	8	0	0	0	0	0	0	0

^a Exponent to the base 10.

^b NS = no survivors.

^c Reciprocal of serum titer; 0 = no fixation at dilution 1:4, lowest used.

peripheral inoculation with paralytic sequelae.

That viral multiplication occurred in healthy-appearing mice surviving to 30 days seems a reasonable inference from the finding that such mice, even when inoculated with dilutions 10^{-3} and 10^{-4} , developed antibody titers of 1:16–1:64. Infection with Junin virus likewise seems proved in the nine mice surviving with paralytic sequelae, since their sera did not react in CF test with various rodent-associated viruses that cause paralysis in mouse colonies and did have titers of 1:16 to 1:64 with Junin antigen.

This same picture of paralyzed mice surviving peripheral but not ic inoculation has been observed in experiments in this laboratory with Portillo virus, like Junin a member of the Tacaribe group. This virus was isolated from, and is considered to be etiologically related to, an illness in a human infant

characterized by hemolytic anemia, renal failure, and neurologic sequelae (6).

Although repeated inoculation of a viral suspension is a generally followed procedure for the preparation of diagnostic immune sera in mice, with viruses of the Tacaribe group this method has resulted in widely cross-reactive sera (7). The present observations suggest that diagnostic sera of greater specificity in CF test might be prepared by a single inoculation of virus into infant mice.

Summary. Junin virus is more pathogenic for laboratory mice aged 0–14 days when inoculated intracerebrally (ic) than when inoculated intraperitoneally or subcutaneously; its pathogenicity by the latter two routes is about the same. Susceptibility to infection with the virus inoculated ic diminishes rapidly after age 6 days. In some instances, involving all three routes of inoculation, mice showed no signs of illness but produced complement-fixing antibodies. A number of mice inoculated ic became infected but recovered completely, and they, too, developed antibodies. In addition, antibodies were detected in the sera of some mice that survived peripheral inoculation with lasting paralytic sequelae.

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