INFLUENCE OF AGE FACTORS ON SUSCEPTIBILITY OF MICE TO RABIES VIRUS

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The literature on rabies contains scattered statements that young animals are more susceptible than old ones but presents no systematic study of the extent of such differences (1, 2). During the course of our work on rabies infection in mice it became evident that age factors influence susceptibility markedly (3). Hence a quantitative study was undertaken of the response of mice of different ages to strains of both fixed and street virus. Some of these findings have been reported in abstract elsewhere (4); full details of the observations will now be described.

Materials and Methods

W-Swiss mice (3) of known age and weight were employed throughout. Most of the comparative tests were made on 7 to 9 day old mice, 20 day and 60 day old mice. The 7 to 9 day old mice remained with their mothers during the test until they were 21 days old. All mice were maintained on a dried milk diet and their weights were low,—5 gm. for the 7 to 9 day mice, 10 gm. for the 20 day mice, and 16 gm. or more for the 60 day mice. Although this low weight factor may have influenced slightly the degree of susceptibility observed in the mice in the following tests, it was presumably operating alike on all age groups and hence could hardly have accounted for the differences between groups to be described.

Tests were carried out with "street," "fixed," and "Pasteur" virus. Street virus was obtained through the courtesy of the New York City and New Jersey State Boards of Health. Negri body-containing bits of sterile dog Ammon's horn were received from these laboratories and passed intracerebrally into mice before each experiment. Mouse-fixed strains also came originally from rabid dogs in New York. These strains have been passed repeatedly through mice intracerebrally. Finally, the Pasteur strain, kindly sent by Dr. P. Lépine of the Pasteur Institute, has been maintained in our laboratory by serial mouse passage. Virus for each test was prepared by removing the brain from a mouse prostrate following intracerebral injection of virus and triturating it in sterile 10 per cent horse serum plus distilled water. The material was further diluted to form a 10 per cent suspension and centrifuged at 1,500 R.P.M. for 5 minutes. The supernatant, designated as a 1:10 suspension of brain virus, was then further diluted according to the requirements of the experiment.

Batches of mice of different ages were inoculated with a single strain of virus by the intracerebral, intramuscular, subcutaneous, or intraperitoneal routes. For the intra-

cerebral test, 0.03 cc. of virus in tenfold dilutions was used and for the subcutaneous and intraperitoneal tests, 0.5 cc. was usually given in tenfold dilutions. For the intramuscular test, 0.01 cc. was inoculated into the lower third portion of the gastrocnemius muscle in fourfold dilutions. From four to six mice were injected with each dilution of virus by each route.

Experiments with Fixed Virus

7 to 9 day mice proved more susceptible to intracerebral or intramuscular injections of fixed virus than 20 day or older mice.

TABLE I

Virulence of Rabies Fixed Virus in W-Swiss Mice

Virus		Mice		Mortality of mice following injection													
				Dilutions injected intracerebrally (0.03 cc.)								Dilutions injected intramuscularly (0.01 cc.)					
Strain	No. of passages in mice	Age	Weight	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}	10^{-10}	1:20	1:80	1:320	1:1,280	1:5,120	1:20,480
		days	*gm.*														
Pasteur	57	7	4–5	—	—	4/4*	4/4	3/4	2/3	2/4†	0/4	4/4	4/4	4/4	4/4	3/4	4/4
		20	8.5	—	—	2/4	4/4	0/4	0/4	—	—	4/4	4/4	4/4	3/4	1/4	0/4
		60	15	—	—	4/4	2/4	1/4	0/4	—	—	4/4	3/4	1/4	0/4	0/4	—
	62	7	4.5	—	—	4/4	2/4	1/3	0/4	1/4	0/4	—	—	—	—	—	—
		100	17	—	3/3	4/4	2/4	1/4	0/4	—	—	—	—	—	—	—	—
	66	7	4	—	—	4/4	4/4	4/4	4/4	2/4	1/4	—	—	—	—	—	—
		80	20	—	3/3	3/3	2/3	3/3	0/3	—	—	—	—	—	—	—	—
R_1	241	9	4	—	—	4/4	4/4	1/4	3/4	0/4	0/4	—	—	—	—	—	—
		95	20	—	4/4	4/4	0/4	0/4	0/4	—	—	—	—	—	—	—	—

— = dilution not tested.

* 4/4 = 4 mice out of 4 inoculated died of rabies.

† The underlined dilution signifies the titration end point, 1 M.L.D.

Thus, Table I shows the results of four experiments. In the first, in which the 57th mouse passage of the Pasteur strain was employed, the intracerebral injections were fatal to 50 per cent or more of the 7 day mice through the 10^{-9} dilution, as contrasted with the 10^{-6} dilution in the 20 and 60 day mice,—a one thousandfold difference in minimum lethal dose. In the following test, in which the 62nd passage Pasteur strain was used, the least fatal dose for both 7 and 100 day mice was the 10^{-6} dilution. In the next test, using the 66th passage of the Pasteur strain, the least fatal dose for the 7 day mice was the 10^{-9} dilution, as contrasted with 10^{-7} for

the 80 day mice,—a one hundredfold difference. And in the last test with the R_1 fixed strain, 241st mouse passage, the end points were the 10^{-8} and 10^{-5} dilutions, respectively, a one thousandfold difference. Finally, in one intramuscular test, the M.L.D. for the 20 day mice was 16 times, and for the 60 day mice 256 times that for the 7 day mice.

TABLE II

Virulence of Rabies Virus R_1, Passage 197, in 60 and 20 Day Old W-Swiss Mice

Age of mice	Intracerebral test					Amount of resistance in mouse units*
	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}	
days						
20	6/6†	5/6	4/6‡	2/6	—	1
60	6/6	5/6	1/6	0/6	—	10
	Intramuscular test					
	1:80	1:320	1:1,280	1:5,120	1:20,480	
20	4/4	4/4	4/4	4/4	0/4	1,627
60	3/3	3/4	0/4	0/4	0/4	26,041
	Subcutaneous test					
	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	
20	2/2	5/5	5/5	5/5	2/4	1,670
60	2/2	5/5	0/5	1/5	0/4	1,666,670
	Intraperitoneal test					
		10^{-2}	10^{-3}	10^{-4}	10^{-5}	
20		4/4	3/4	3/4	0/4	16,670
60		1/4	1/4	0/4	0/4	1,666,670

— = dilution not tested.

* Mouse unit is taken as the maximum intracerebral dose resisted by 51 per cent or more of 20 day old W-Swiss mice.

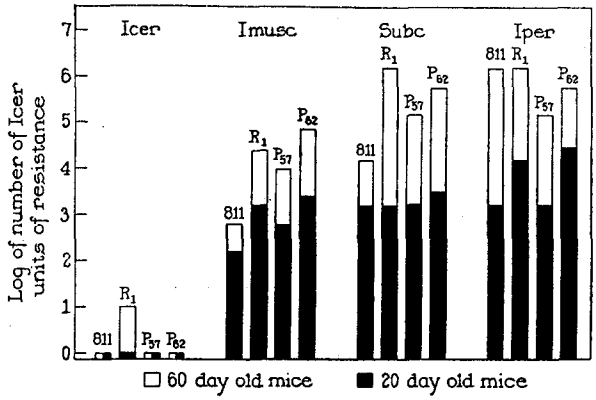
† 6/6 = 6 mice dead of rabies of 6 injected.

‡ The underlined dilution signifies the titration end point, 1 M.L.D.

20 and 60 day mice were compared in several tests, since these ages are generally those selected for tests of rabies vaccines and for determination of the presence of rabies virus. They showed relatively little difference in their response to intracerebral injection but marked differences following intramuscular, subcutaneous, and intraperitoneal injections.

Table II shows the results of an experiment in which batches of 20 and 60 day mice were tested by various portals with a single suspension of fixed

virus. The intracerebral titre of virus in the 20 day old mice was 0.03 cc. of the 10^{-7} dilution and in the 60 day old mice 0.03 cc. of the 10^{-6} dilution,—a tenfold difference. If a unit of resistance is taken as the maximum intracerebral dose of virus tolerated by 51 per cent or more of the youngest mice, then the 20 day old mice resisted one and the 60 day old mice ten units. Following intramuscular injection, the titre in the 20 day mice was 0.01 cc. of the 1:5,120 and in the 60 day mice 0.01 cc. of the 1:320 dilution. The difference is sixteenfold and, according to calculation, the 20 day old mice resisted 1,627 intracerebral units of resistance and the 60 day mice 26,041 units. Similarly, following subcutaneous injection, the titres were 10^{-5} and 10^{-2},—a one thousandfold difference,—or 1,670 and 1,666,670 intra-



TEXT-FIG. 1. Comparative resistance of 60 and 20 day old mice injected by various routes with different strains of fixed rabies virus.

cerebral units of resistance respectively. Following intraperitoneal injection, the titres were 10^{-4} and 10^{-2} respectively,—a one hundredfold difference,—or 16,670 and 1,666,670 units respectively.

The results of similar tests with four strains of fixed virus are summarized in Text-fig. 1. Taken together, they show that 20 and 60 day old mice, injected intracerebrally with fixed virus, show not more than tenfold differences in titration end points but that following intramuscular injection, the 20 day mice withstand 162 to 2,600 intracerebral units of resistance according to the strain tested, and the 60 day mice 4 to 32 times these amounts. Following subcutaneous injection, the 20 day mice resist 1,670 to 3,330 units, and the 60 day mice 10 to 1,000 times these amounts, according to the strain tested. 20 day mice, injected intraperitoneally, resist 1,670 to 33,330 intracerebral units and the 60 day mice 20 to 1,000 times these amounts. Finally, all mice, regardless of age or strain of virus em-

ployed, are more susceptible to the intramuscular than to the subcutaneous or intraperitoneal routes of injection.

Experiments with Street Virus

Similar tests were carried out with street virus following a single passage through mouse brain, with the result that no such conspicuous differences

TABLE III

Virulence of Rabies Street Virus R_{81} in 60 and 20 Day Old W-Swiss Mice

Age of mice	Intracerebral test					Amount of resistance in mouse units*
	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	
days						
20	—	3/4†,‡	1/4	0/4	0/4	1
60	4/4	4/4	0/4	0/4	0/4	1
	Intramuscular test					
	1:20	1:80	1:320	1:1,280	1:5,120	
20	4/4	4/4	2/4	2/4	0/4	6
60	2/4	3/4	3/4	2/4	1/4	6
	Subcutaneous test					
	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	
20	4/4	4/4	3/4	0/4	—	170
60	3/4	2/4	2/4	0/4	—	170
	Intraperitoneal test					
	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	
20	3/3	2/4	0/4	0/4	—	1,670
60	3/3	2/4	0/4	0/4	—	1,670

— = dilution not tested.

* Mouse unit is taken as the maximum intracerebral dose resisted by 51 per cent or more of 20 day old W-Swiss mice.

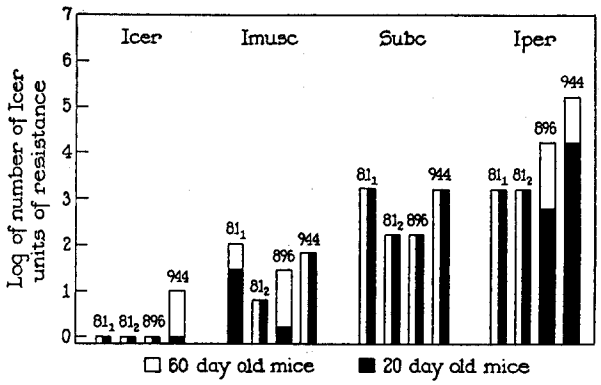
† 3/4 = 3 mice dead of rabies of 4 injected.

‡ The underlined dilution signifies the titration end point, 1 M.L.D.

were observed between the 20 and 60 day old mice in their resistance to peripheral injection.

Table III shows the results of a test with street virus similar to the one with fixed virus demonstrated in Table II. Street virus, injected intracerebrally, proved fatal through the 10^{-4} dilution to 20 and 60 day old mice alike; injected intramuscularly, through the 1:1,280 dilution to young and old alike; injected subcutaneously, through the 10^{-3} dilution, and intraperitoneally, through the 10^{-2} dilution in young and old alike.

The results of similar tests with four strains of street virus are summarized in Text-fig. 2. In general they show that 20 day mice do not differ materially from 60 day mice in their susceptibility to street virus. No extensive tests have been made on still younger or older mice but the indications from a few experiments are that the differences, although greater than those in the case of the 20 and 60 day mice, are still less marked than those with fixed virus under similar conditions.



TEXT-FIG. 2. Comparative resistance of 60 and 20 day old mice injected by various routes with different strains of street rabies virus.

DISCUSSION

Convincing evidence that the process of aging in the absence of exposure to infection may be associated with increasing resistance to the effects of peripheral inoculation with certain animal-passaged strains of neurotropic virus has been submitted by Olitsky, Sabin, and Cox (5). King (6), however, while confirming these observations, failed to note differences in susceptibility associated with age when freshly isolated strains of equine encephalomyelitis virus were employed. Our own experiments indicate that age differences in susceptibility to street virus are not especially noteworthy but do develop with fixed virus which has been passed artificially from animal to animal.

CONCLUSIONS

1. 7 to 9 day old mice are more susceptible than older mice to injections of fixed or street virus by any route.

2. 20 day old mice are more susceptible than 60 day old mice to peripheral but not intracerebral injection of fixed virus.

3. 20 day and 60 day old mice are equally susceptible to street virus.

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