Response of Infant and Adult Mice to Lymphocytic Choriomeningitis Virus Infection. (19036)

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Immature animals are more susceptible than adults to many viral infections(1-10). The Coxsackie viruses are one extreme expression of this general rule(11) to which the Lansing strain of poliomyelitis virus is an exception(12,13). As the present report shows, lymphocytic choriomeningitis appears to be a second exception.

Unsuitability of infant mice for isolation of virus. The original observation was made while recovering strains of lymphocytic choriomeningitis virus (LCM) from a specimen of human cerebrospinal fluid and from a number of house mice. All these samples proved to be infectious for adult mice and the cerebrospinal fluid was infectious as well for guinea pigs. The cerebrospinal fluid was inoculated intracerebrally into two 200-400-g guinea pigs, five 4-week-old mice, and eleven 1- to 3-day-old mice The animals were of the Albany standard strains. The dose was 0.1 ml for the guinea pigs, 0.03 ml for the older mice, and 0.01 ml for the infant mice.

Only 1 of the 11 infant mice died (Table I). It was found on the 10th day and was partially eaten when discovered. All of the adult mice died within 9 days and both guinea pigs within 15 days. Both groups had exhibited characteristic signs of lymphocytic choriomeningitis and microscopic examination revealed histologic changes consistent with that diagnosis. The infectivity of brain suspensions of the guinea pigs and adult mice was neutralized by sera from monkeys hyperimmunized with LCM virus strain 4707.* Similar results were obtained with organ suspensions from the 5 house mice. The brains were ground separately; the liver and spleen of each were pooled before grinding. Ten per cent (by weight) suspensions were made. The diluent was 10% physiologic saline-nutrient broth solution containing 1000 units of penicillin and 1000 μg of streptomycin per milliliter. Dose and route of inoculation were the same as used with the cerebrospinal fluid. Of the 73 infant mice so treated, 7 died during the period when mature mice characteristically manifest signs of choriomeningitis. Six others died after a month or more. In contrast, 44 of the 45 adult mice died within 8 days, 38 following signs of LCM infection.

The agents isolated from each of the tissue suspensions were identified as LCM viruses by neutralization tests with hyperimmune monkey serum. Thus of 50 adult mice injected with samples from the various sources for primary isolation of LCM virus, 43 died or showed typical signs. Of 84 infant mice injected with the same specimens only 14 died, 6 of them approximately a month after

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* Lymphocytic choriomeningitis virus strain 4707 was isolated in Raleigh, N. C., by Mrs. Margaret Douglass Umphlett and was identified in the Grasslands Hospital Laboratory, Valhalla, N. Y., by Dr. Gilbert Dalldorf.

TABLE I. Primary Isolation of Lymphocytic Choriomeningitis Virus in Adult and Infant Mice.

Inoculum	Adult mice* Total No.	Day of death	No. survivors	Infant mice† Total No.	Day of death	No. survivors
Human cerebrospinal fluid	5	9 9 (9)‡(9) (9)	0	11	10	10
House mouse						
No. 1 Brain	5	1 1 1 3 (7)	0	5		5
Liver and spleen	5	(7) (7) (7) (7)	1	11	6 6 35	8
No. 2 Brain	5	(7) 7 7 7 7	0	8	13	7
Liver and spleen	5	(7) (7) (7) (7) 8	0	12	6 6 35	9
No. 3 Brain	5	4 4 (7) 7 7	0	7	8	6
Liver and spleen	5	(7) (7) (7) (7) 7	0	6	8	5
No. 4 Brain	5	7 7 7 7 7	0	7		7
Liver and spleen	5	(7) (7) (7) (7) 8	0	9	32 35 35 38	5
No. 5 Liver and spleen	5	(7) (7) (7) (7) 7	0	8		8
Total	50		1 = 2%	84		70 = 83%

* 4 wk old. Dose, .03 ml, inoc. intracer.
† 1 to 3 days old. Dose, .01 ml, inoc. intracer.
‡ Number in parentheses signifies animal killed after typical convulsions.

TABLE II. Results of Challenge* with Established Strain of Lymphocytic Choriomeningitis Virus (49189) in Infant Mice† Surviving Inoculation of Infected Tissues.

Original inoculum	Reinoculation— No. days after primary inoc.	Reinoculated mice — Total No.	Day of death	No. survivors	Control mice‡ Total No.	Day of death	No. survivors
House mouse							
No. 1 Brain	42	5		5	5	1 1 6 6 6	0
Liver and spleen	42	8	6	7			
No. 2 Brain	42	7	1	6			
Liver and spleen	42	9		9			
No. 3 Brain	47	6		6	10	1 6 6 6 6	0
Liver and spleen	47	5		5		6 7 7 7 9	
No. 4 Brain	43	7	(6)§	6			
Liver and spleen	43	5	1	4			
No. 5 Liver and spleen	43	8	1	7			
Total		60		55 = 91.6%	15		0

* Challenge dose is .3 ml by intracer. route of .1 % suspension of virus strain 49189.
† 1 to 3 days old at time of first inoculation.
‡ Same age as test mice.
§ No. in parentheses signifies animal killed after typical convulsions.

inoculation.

Challenge of infant survivors. The surviving infant mice were tested for active immunity from 42 to 47 days after the original inoculation. They were then inoculated intracerebrally with 0.03 ml of an 0.1% suspension of an established strain of lymphocytic choriomeningitis virus (49189).† Normal mice of approximately the same age were similarly inoculated and served as controls. All but 2 of the previously inoculated mice were resistant, while all the control mice died (Table II).

Relative resistance of infant mice to second passage strains. Second passages of 4 of the strains isolated from house mice were made in parallel in infant and adult mice. The inoculum was brain, and in one instance, pooled liver and spleen, all from first passage adult mice. The conditions of the experiment were otherwise identical with those of the first. The results duplicated those obtained

† This is the original strain of lymphocytic choriomeningitis virus (designated E371) which was received in 1949 from Dr. Charles Armstrong, National Institutes of Health, Bethesda, Md. It had been through 371 mouse passages in his laboratory and 9 mouse passages in this laboratory.

TABLE III. Response in Adult and Infant Mice to an Established Strain of Lymphocytic Choriomeningitis virus (49189).

	Adult mice*								Infant mice†							
Date of test	Total No.	Virus dilution	Day of death					No. survivors	Mean survival time (days)‡	Total No.	Virus dilution	Day of death				No. survivors	Mean survival time (days)‡
11/14/50	5	10^{-3}	(6)§	(6)	(6)	(6)	(7)	0	6.2	5	10^{-3}	8	10	13	13	13	0	11.4
11/30	5	10^{-3}	(6)	7	7	7	7	0	6.8									
5/11/51-A	9	10^{-2}	(6) 7	(6) 7	(6) 7	6 7	6	0	6.4	8	10^{-2}	10 10	10 10	10	10	10	1	10
	10	10^{-4}	5 (7)	7 (7)	(7) 10	(7) 10	(7) 10	0	7.7	8	10^{-4}	10	10	10	12	12	3	10.8
5/11/51-B										8	10^{-2}	10	10	10	10	10	3	10
										8	10^{-4}	10 11	11	11	11	11	2	10.8
Total	29							0	6.7	37							9 = 24.3%	10.6

* 4 weeks old. Dose .03 ml, inoc. intracer.
† 1 to 3 days old. Dose .01 ml, inoc. intracer.
‡ Mean survival time computed only for mice that died.
§ No. in parentheses signifies animal killed after typical convulsions.

previously. Of a total of 41 infant mice inoculated, 3 died 2 weeks later, 2 more died at the end of a month, and the rest survived. When challenged by the intracerebral route with mouse-adapted strain 49189, all but 3 proved immune. Of the 35 adult mice, however, 31 died within 8 days, and one more on the 13th day. None of the 3 adult survivors was immune to LCM strain 49189.

Relative susceptibility of infant mice to a mouse-adapted strain. Adult and infant mice were inoculated into the brain with 0.03 and 0.01 ml, respectively, of dilutions varying from 1% to 0.01% of the mouse-adapted LCM strain 49189. None of the mice in the adult group survived (Table III). The mean survival time was 6.7 days. Nine (24.3%) of the infant mice survived. The mean survival time was 10.6 days.

Discussion. Traub(14,15) has shown that in mouse colonies infected with lymphocytic choriomeningitis virus the adult carrier rate is high but the death rate low. The disease is transmitted *in utero*. The young commonly survive, become immune without manifesting any signs of illness, but excrete virus for long periods of time. In this manner the infection persists without destroying the colony.

In this study it was found that mouse adaptation apparently may enhance the virulence of the agent beyond the ability of previously exposed infant mice to withstand it. The human disease, however, is thought usually to be due to contact with infected house mice(16). The virus is presumably only one passage removed from the wild state when recovered from patients. This might explain the observation that the strain isolated from human cerebrospinal fluid behaved like those obtained from the house mice. It is consonant with the findings that infant mice were relatively resistant to second as well as primary passage strains derived from the house mice. We know that the relative resistance of infant mice to LCM virus in these experiments was not due to humoral immunity passively acquired *in utero* from an immune mother since they came from normal stock.

The relative lack of susceptibility of infant mice to Lansing poliomyelitis virus(12,13) is interesting in view of the gradually increasing frequency and severity of infantile paralysis in older persons. In this respect the present

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trend in the epidemiology of poliomyelitis is comparable to the epizoology of lymphocytic choriomeningitis in mice. Mouse encephalomyelitis is also being explored along these lines.

Summary and conclusions. Freshly isolated strains of lymphocytic choriomeningitis virus induced inapparent infection and immunity in infant mice. A mouse-adapted strain, however, caused obvious signs of infection and a high mortality rate among infant mice. Adult mice were fully susceptible to both freshly isolated and mouse-adapted virus. These findings are in keeping with the epizoology of the disease in naturally infected mouse colonies.

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Changes in Adrenal and Pituitary Concentrations of I^{131} and P^{32} Following Thyroidectomy.* (19037)

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It has been reported that an increased concentration of radioiodine I^{131} occurs in the pituitary and the adrenals of rats subjected to prolonged propylthiouracil treatment(1). This study was undertaken to determine whether a similar response occurs in total thyroidectomy, and by the uptake of radiophosphorus P^{32} to determine if the rate of phosphate utilization is altered in those organs.

Methods and procedure. Twenty young male albino rats weighing between 60 and 100 g were randomly divided into an experimental (Group I) and a control group (Group II). The experimental group was thyroidectomized and maintained with the control animals on regular laboratory Rockland rat ration for a period of 70 days following thyroidectomy. Twenty fully mature male albino rats weighing between 250 and 300 g were similarly divided into an experimental (Group III) and a control group (Group IV). The experimental group was thyroidectomized and maintained with the control animals under identical food, water and housing conditions as the younger rats of Groups I and II, for the same period of time. Three hours before sacrifice, a randomly selected one-half of each group (Groups I-IV) was injected intraperitoneally with 200 microcuries of radioactive iodine I^{131} in 2.5 ml of normal saline. The other half of each group of animals was injected intraperitoneally with 100 microcuries of radioactive phosphorus P^{32} in 2.5 ml of normal saline. At sacrifice the adrenals, pituitary, thymus, a portion of muscle and the thyroids of the control groups (Groups II and IV) were removed, weighed and the entire adrenals, pituitary, muscle sample, thyroid and a weighed portion of thymus dissolved in 5 ml of concentrated HNO_3. The acid, after the tissue was digested, was then diluted with distilled water to a measured volume of 50 ml and dip-counted, by a method previously described(2). Aliquots of the injected solutions of I^{131} and P^{32} were prepared and dip-counted in an identical manner as the tissue specimens. All assays were expressed as the percentage of injected activity after allowing for the respective dilutions for assay. To determine the relative ability of a tissue to concentrate radiophosphorus or radioiodine, the percentage of the administered isotope found in an organ was divided by that organ's percentage of body weight. Where only a portion of muscle was assayed, the activity was determined on the basis of one gram and the percentage of body weight of one

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