proteins showed appreciable radioactivity. From the hydrolysate of the radioactive proteins cystine and methionine were separated on chromatograms, and eluted. Both cystine and methionine contained radioactive sulfur in appreciable amounts.

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Relative Resistance of Newborn Mice to Poliomyelitis Virus.* (17694)

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The present study was begun with the hope that propagation of the Lansing strain of poliomyelitis virus in newborn mice might yield material with higher virus titers. It appeared probable that this might be achieved, because repeated observations in this laboratory(1) indicated that when the mouse-adapted Hawaii dengue virus, which also produces flaccid paralysis in intracerebrally inoculated mice, is injected in suckling mice less than one week old, the infected brain suspensions usually have virus titers, which are approximately 100 times greater than those obtained from brains of mice inoculated at 2 weeks of age or older. However, a preliminary test carried out with the Lansing virus suggested that the newborn mice may be more resistant that the older animals. Since this observation was not in accord with the recorded properties of all other neurotropic viruses in mice(2-3,4), as systematic study was carried out on larger numbers of animals to elucidate this unexpected behavior of poliomyelitis virus.

Methods. The albino mice used in these tests were obtained from a Cincinnati dealer, who could not be certain of their genetic origin or composition except that they were probably "Swiss" mice. Pregnant females obtained from the dealer several days before expected delivery of young, were placed in separate boxes and the birth of new litters was noted twice daily. Mice were not used on the day of birth. All the tests were carried out during the months of June, July and August, 1948, when the temperature in the animal quarters was not infrequently 90 to 100°F. Following inoculation the suckling mice were returned to their mothers, and each family was observed separately. The virus consisted of a large lot of 10% centrifuged mouse brain suspension in saline, aliquots of which were distributed in sealed glass ampules which were maintained in the frozen state in a "dry ice" storage box; it represented the 203rd mouse brain passage and had an LD_{50} of $1 \times 10^{-3.7}$ for the 0.03 ml dose. Since only 0.01 ml was inoculated intracerebrally in the suckling mice, the dose for the older mice, used for control in each test, was also 0.01 ml. The inoculations were carried out without anaesthesia.

Results. When 0.01 ml of the 10^{-1} virus suspension, containing approximately 170 LD_{50} (based on titration in mature mice using 0.03 ml doses), was inoculated intracerebrally in mice of different ages, the significant difference between the newborn and older mice was in the length of the incubation period (Table I) and in the survival time after onset of paralysis (Table II). With regard to prolongation of the incubation period, there was no apparent difference between suckling mice tested 1, 2 or 3 days after birth, it was still definite but progressively less marked in those tested at 4 to 7 days, while at 15 days after

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TABLE I.
Incubation Periods in Mice of Different Ages Inoculated with Lansing Virus.

Age of mice inoculated days	No. of mice in group	% paralyzed or dead on indicated day after intracerebral inoculation of 0.01 ml of 10^{-1} virus																													% remained well
		2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	31	32	33	34	35	44	
1	52	0	0	0	0	0	0	4	13	19	35	38	50	63	65	75	81	85	85	87	87	87	87	87	88	90	—	—	92	—	—	—	7.7
2	87	0	0	0	0	3	5	8	11	16	23	41	53	60	68	72	75	78	79	79	84	86	—	—	—	—	—	—	87	89	—	—	11.5
3	50	0	0	0	0	0	0	0	4	6	12	24	46	64	72	78	82	90	90	92	—	—	—	—	—	—	94	—	—	—	—	96	4.0
4	39	0	0	8	8	10	10	15	21	26	36	59	72	82	87	90	90	90	92	—	—	—	—	—	—	—	—	—	—	—	—	—	7.7
6 and 7	40	0	0	7	7	10	15	27	40	45	65	72	80	80	90	90	90	92	95	—	—	—	—	—	—	—	—	—	—	—	—	—	5.0
15 (suckling)	38	0	0	16	29	42	55	66	74	79	84	87	89	92	95	—	—	—	—	—	—	—	—	97	—	—	—	—	—	—	—	—	2.6
15 (weaned) (litter mates)	28	0	0	25	43	57	75	82	86	89	—	—	93	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	7.1
21-35	157	0	3	16	32	48	54	64	72	78	85	87	90	90	92	94	94	95	—	—	—	—	—	96	—	—	—	97	—	—	—	—	2.6

TABLE II.
Survival Time of Mice Developing Paralysis During First 14 Days After Birth.

Age at time of inoculation days	Time after birth paralysis appeared days	No. mice in group	% of mice surviving indicated No. of days after paralysis first observed									Avg survival time,* days,
			1	2	3	4	5	6	7	8	9	
1-6	14 or less	71	18	21	20	14	10	7	6	1	3	3.44
1-6	15 or more	92	64	24	8	3	1	0	0	0	0	1.53
21-35	23 " "	100	76	20	0	2	1	1	0	0	0	1.35

* Only mice in whom paralysis was observed prior to death are included in this analysis. This does not affect the average survival time of the suckling mice paralyzed within 14 days after birth, because only 2 of the total number were found dead without previously exhibiting paralysis, but exaggerates the average survival time of the others, because in approximately 20 to 30% the interval between onset of paralysis or other CNS signs and death is too short to be observed when the examinations are made only once a day.

birth the distribution of incubation periods among 38 suckling mice was practically identical with that found among 157 weaned mice, 3 to 5 weeks of age (Table I). To test the effect of premature weaning, 28 mice were removed from their mothers at 15 days after birth and tested simultaneously with their litter mates which were left with their mothers. The prematurely weaned mice received bread and water until they were big enough to chew pellets and reach the water bottles. The mice weaned at 15 days of age succumbed more rapidly than their suckling litter mates and the older weaned mice (Table I), but the number used in the test is not large enough

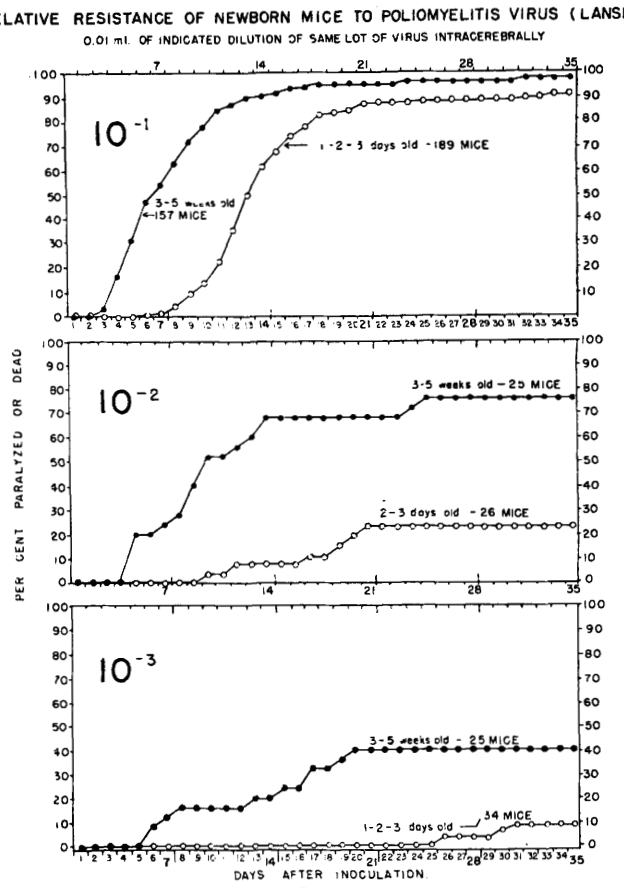

FIG. 1.

to permit significant deductions. With regard to prolongation of survival time after onset of paralysis, it was noted that the determining factor was not the age of the mouse at the time of inoculation but rather its age at the time paralysis first appeared. Inspection of the protocols indicated that the critical period was around 14 days after birth. Mice becoming paralyzed during the first 14 days of life generally (61% of 71 mice) survived 3 or more days, and survival times of 5 to 9 days were not unusual (27%). However, when the incubation periods were prolonged beyond 15 days of age the survival time pattern among the remaining litter mates approached that observed among the older weaned animals (Table II).

When the inoculum was reduced to 0.01 ml of the 10^{-2} and 10^{-8} dilutions of virus, the difference was not only in the prolongation of the incubation period but also in the larger number of survivors without paralysis among the 1- to 3-day-old suckling mice Fig. 1). These mice were observed for 70 days after inoculation without any change in the results shown in Fig. 1 for the first 35 days. Calculation of the LD_{50} on the basis of the data for the 0.01 ml dose, shown in Fig. 1, yields a value of $10^{-1.67}$ for the 1- to 3-day-old mice and a minimum estimated value of $1 \times 10^{-2.64}$ for the 3- to 5-week-old mice; it should be recalled, however, that a complete titration of this virus preparation in older mice, including the 10^{-4} and 10^{-5} doses, yielded an LD_{50} of $1 \times 10^{-3.7}$ for the 0.03 ml dose, with an estimated LD_{50} of $1 \times 10^{-3.2}$ for the 0.01 ml dose. Thus, the minimal virus inoculum required to produce the disease was 10 to 30

TABLE III.
Level of Virus Multiplication in Suckling and Older Mice at Onset of Paralysis.

Age at inoculation, days	Days after inoculation paralyzed	No. mice	Titration of suspension of spinal cords and brain stems: No. of mice succumbed in 35 days after inoculation with indicated dilution 10-1	10-2	10-3	10-4	10-5	LD_{50}
2	11	4	10/10	10/10	7/10	1/10	0/10	3.3
28-35	10-11	4	10/10	8/10	6/10	3/10	1/10	3.3

TABLE IV.
Effect of Intracerebral Injection of Lansing Virus on Suckling and Mature Mice of "C-57 Black" Strain 0.01 ml of 10^{-1} Virus.

Mouse No.	3 days old, Days after inoculation: Paralyzed	Dead	5 days old, Days after inoculation: Paralyzed	Dead	4-6 weeks old, Days after inoculation: Paralyzed	Dead
1	8	11	3	11	4	6
2	8	13	3	11	5	6
3	9	14	3	11	—	6
4	—	10	9	12	6	7
5	10	12	—	12	—	7
6	10	13	16	18	7	9
7	10	14			—	8
8	11	15			9	10
9	14	16			9	11
10					10	11
11					11	12
12					11	12
13					—	12
14					13	14
15					16	17
16					42	43
17					—	survived 60
Mean survival time after onset of paralysis	3.5 days		5.8 days		1.2 days	

times greater for the 1 to 3-day than for the 21- to 35-day-old mice.

Level of Virus Multiplication at Onset of Paralysis in Suckling and Older Mice. The available data did not indicate whether the extent to which the virus had to multiply for the production of paralysis was greater or smaller in sucklings than in older mice. Accordingly (4 mice, which were inoculated with 0.01 ml of 10^{-1} virus at 2 days of age, were sacrificed at the first appearance of paralysis 13 days after birth, and a pool of their spinal cords and brain stems was titered for virus content. On the same day were also sacrificed 4 moribund mice, which were 4 to 5 weeks old at the time of inoculation with the same dose of virus; 3 of these also had an incubation period of 11 days and one of 10 days, and a pool of their spinal cords and brain stems was titered simultaneously with that obtained from the suckling mice. The results shown in Table III indicate that at onset of paralysis, the level of virus multiplication was the same in suckling and older mice. Thus the greater survival time after onset of paralysis would appear to depend on factors other than the level of viral multiplication in the spinal cord and brain stem.

Behavior of Lansing Virus in Sucklings of Other Genetic Strains of Mice. Small numbers of "W-Swiss" suckling mice, derived from mothers bred in this laboratory or from "CFW" pregnant females sent by Carworth Farms, exhibited the same type of resistance as the albino mice used in the tests described above. The results obtained with "C-57

TABLE V.
Immunity of Mice Surviving Previous Intracerebral Inoculation of Lansing Virus.
Challenge Dose = 100 LD_{50} Intracerebrally.

Previous inoculation	No. tested	% paralyzed or dead on indicated day				% resistant
		7	14	21	32	
During 1st week of life	24	62.5	62.5	75.0	75.0	25.0
After 2nd " " "	12	33.3	33.3	41.7	50.0	50.0
Total	36	52.8	52.8	63.9	66.7	33.3
None—mothers of above	43	60.0	81.4	88.4	90.7	9.3
" —new "old" controls	24	71.0	96.0	100.0	—	0

Black" mice, bred in this laboratory from Jackson Memorial stock, are shown in Table IV. Although the number of mice is too small for a significant comparison of incubation periods, the difference in survival time after onset of paralysis is very marked and similar to that observed in the suckling and older albino mice.

Immunity of Mice Unaffected by Previous Intracerebral Inoculation. The purpose of this test was to determine whether the mice which were unaffected by an intracerebral inoculation of 10^{-1} Lansing virus during the first week of life may have experienced an inapparent infection leading to resistance to further inoculations of virus. Twenty-four such mice were reinoculated with approximately 100 LD_{50} of virus 35 to 80 days after the initial injection. Twelve unaffected, surviving mice, which received their first inoculation at 15 to 35 days of age, were reinoculated as a check on the results which might be obtained with the first group. The mothers of the above survivors and older mice, weighing approximately 30 g and not previously exposed to inoculated animals, served as controls for the activity of the virus in mice of more advanced age. The results shown in Table V indicate that 75% of the 24 mice, which resisted a primary inoculation during the first week of life, succumbed to the second inoculation of virus, strongly suggesting that the first inoculation was not followed by inapparent infection. The survival of 25% of these mice and of 50% of the 12 which had the primary inoculation at 15 to 35 days of age, probably is significant by comparison with the previously uninoculated controls. However, it may only mean that the survivors represent a selected group, which regardless of age require a larger inoculum to initiate infection. Since there was no evidence of inapparent infection in the newborn mice, it may be justifiable to conclude that larger amounts of poliomyelitis virus are required to initiate multiplication in their tissues than in those of adult mice.

Discussion. The present data for poliomyelitis virus may be compared with the intracerebral titrations of 9 other neurotropic viruses carried out simultaneously in 3-day and 28-day-old mice by Lennette and Koprowski(4). They found that the minimal amount of virus which had to be inoculated to produce the clinically apparent disease was either the same or smaller for the 3-day than for the 28-day-old mice. Casals(3) showed that approximately 100 times more rabies virus had to be injected intracerebrally to produce the disease in full grown than in 7-day-old mice. Incubation periods have also been found to be shorter in very young mice intracerebrally inoculated with other neurotropic viruses(2). Thus far, poliomyelitis virus would appear to be unique in its capacity to multiply more readily and rapidly in the nervous system of older mice. This would suggest that multiplication of poliomyelitis virus in mice may require some factor or metabolic pathway which is different from the other neurotropic viruses studied thus far. Pinto(5) found that while peripherally inoculated Lansing virus was equally without effect in suckling and older mice, it invaded the

5. Pinto, M. R., *Am. J. Hyg.*, 1948, v48, 361.

central nervous system more readily in suckling than in older cotton rats. Although it is possible that the virus may behave differently in newborn cotton rats and mice, it is also possible that invasiveness from peripheral sites and multiplication within the central nervous system may depend on different mechanisms. It is perhaps noteworthy, in this respect, that it is generally agreed that poliomyelitis infection in human beings more commonly produces severe clinical disease in older individuals than in very young children, past the age of possible protection by placentally transmitted antibody.

Summary. The Lansing strain of poliomyelitis virus, unlike other neurotropic viruses in mice, produced the disease more readily and rapidly in mature mice than in newborn animals. The relative resistance of the newborn manifested itself by prolonged incubation periods and survival times after onset of paralysis in those inoculated with larger amounts of virus (170 LD_{50}), and by a failure to develop the disease following the intracerebral inoculation of smaller amounts (2-17 LD_{50}). The prolongation in incubation period was most marked in mice inoculated at 1, 2 and 3 days of age, was still definite but progressively less marked in those tested at 4 to 7 days of age, while suckling mice inoculated at 15 days of age exhibited incubation periods almost identical in their distribution with those of weaned, 21 to 35-day-old mice. The amount of intracerebrally inoculated virus required to initiate infection in newborn mice 1 to 3 days of age was 10 to 30 times greater than in 21- to 35-day-old mice. Although the incubation period was markedly prolonged in the newborn mice, the level of virus multiplication attained at the onset of paralysis was the same in the suckling as in the older mice. Mice becoming paralyzed during the first 14 days of life survived much longer (61% for 3 to 9 days) than the litter mates whose incubation periods were prolonged beyond 15 days of age (12% for 3 to 5 days). There was no evidence of inapparent infection in mice inoculated during the first week of life or subsequently, since the majority of surviving mice succumbed to a second inoculation of virus 35 to 80 days later.

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Conditioning, Through Stress, of the Action of Corticoids on Lymphoid Organs.* (17695)

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In vitro experiments concerning the action of corticoids on lymphocytes are at present inconclusive. While numerous investigators have suggested that gluco-corticoids have cytotoxic effects on lymphoid cells *in vitro* (1,2,3,7), other workers have found that total cortical extracts did not show this lympholytic action(4,5). The recent experiments of Hechter and Johnson(6) can perhaps explain the different effects of corticoids *in vitro* and *in vivo* on lymphoid cells. These investigators have found that total cortical extract is an effective lympholytic agent *in vitro* only in the presence of homogenates of lymphoid tis-

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