the average for the entire period was 48 ± 10.55 colonies from 10^{-6} dilution of the 1 mg/ml BCG vaccine. A significant deviation from this mean occurred only during one period.

Viability of stored vaccine is shown in Table II. Approximately 50% of the BCG elements remained viable in the 3-week-old vaccine, 30% in the vaccine 6 weeks old, and 2% survived for one year.

TABLE I. Viability of BCG Vaccine Freshly Prepared from 10-11-Day-Old Sauton Culture.

Statistical analysis of number of colonies cultured on Löwenstein's egg medium from 10^{-6} suspension of 1 mg/ml BCG vaccine diluted in physiological saline solution (120 cultures in each group).

Weekly lots of BCG vaccine, wk	No. of colonies	Probability	
		t	*P*
1 to 12	45 ± 5.39*	—	—
13 to 24	44 ± 6.25	.162	.87
25 to 36	52 ± 11.49	1.715	.10
37 to 48	47 ± 7.49	.735	.47
49 to 60	44 ± 12.09	.245	.82
61 to 72	46 ± 9.39	.294	.77
73 to 84	38 ± 7.00	2.450	.03
85 to 96	47 ± 8.78	.612	.55
97 to 108	37 ± 6.33	2.695	.02
109 to 120	38 ± 6.32	2.694	.02
121 to 132	48 ± 8.71	.906	.38
133 to 144	43 ± 3.88	.808	.42
145 to 156	63 ± 24.40	2.205	.03
157 to 168	42 ± 8.25	.612	.55
169 to 180	61 ± 23.20	2.180	.04
181 to 192	68 ± 21.03	*3.330*	*<.01*
193 to 204	57 ± 9.17	2.709	.02

* Mean ± stand. dev. of the mean.
Significant deviation italicized.

TABLE II. Viability of BCG Vaccine Stored at 2-4°C Up to 3½ Years.

Vaccine Lot No.	Storage time	Colonies per ml of dilution of 1 mg/ml vaccine	% survival rate	Growth of BCG vaccine diluted in Dubos' medium (pos. a.f.b. smear)
A-90	Fresh	49 × 10^6	—	10^{-7}
"	1 day	45 "	92	10^{-7}
"	2 days	41 "	84	10^{-7}
"	3 "	46 "	92	10^{-7}
"	4 "	37 "	76	10^{-6}
"	5 "	41 "	84	10^{-7}
"	6 "	40 "	82	10^{-6}
"	7 "	38 "	78	10^{-6}
"	2 wk	26 "	53	10^{-5}
"	3 "	24 "	49	10^{-5}
"	4 "	19 "	39	10^{-5}
B-90	6 "	15 "	31	10^{-5}
A-87	3 mo.	9 "	18	10^{-5}
A-81	6 "	6 "	12	10^{-5}
A-75	9 "	12 × 10^5	2.5	10^{-4}
A-68	1 yr	9 "	1.8	10^{-4}
A-41	2 "	4 × 10^2	0.0008	10^{-1}
A-15	3 "	0	—	0
A-2	3½ "	0	—	0

Summary. Comparison of 204 consecutive weekly lots of BCG vaccine prepared under standardized conditions were found to be satisfactorily uniform in the number of viable organisms and clumps of organisms they contained. The survival rate of BCG vaccine stored at 2-4°C has been determined.

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Oral Administration of Coxsackie Viruses to Newborn and Adult Mice.* (18475)

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Chimpanzees and cynomologus monkeys are readily infected by oral administration of Coxsackie or C virus, although with no apparent illness(1,2). In both species a virus carrier state is produced followed by the development of neutralizing antibodies to the infecting strain of virus. The present study is concerned with the susceptibility of newborn and adult mice, when tested for oral infection.

Virus strains. The following C virus strains were employed in the form of suspensions of

* Aided by a grant from the National Foundation for Infantile Paralysis.

1. Melnick, J. L., *Bull. N. Y. Acad. Med.*, 1950, v26, 342, and unpublished data.

2. Melnick, J. L., and Ledinko, N., *J. Immunol.*, 1949, v64, 101.

skinned, eviscerated carcasses of infected infant mice: Conn.-5, Texas-1, Easton-2, Easton-14, and High Point (Hi-Pt). They were isolated in this laboratory and their properties have been described(1,3). *Susceptibility of newborn mice.* Groups of 8 newborn mice were fed virus by means of a 0.25 ml syringe and a blunted 26 gauge needle which was inserted into the mouth, care being taken not to injure the buccal membrane. Each mouse was given 0.01 ml of virus but in many instances not all of the suspension was consumed. Mice were observed daily for a period of 2 weeks and those mice which survived at the end of this time were bled from the heart, the blood being used for neutralization tests. *Susceptibility of adult mice.* Small pieces of bread on petri plates were soaked with 1.5 ml of a 20 per cent suspension of Hi-Pt virus; these plates were then set in cages, each one containing 2 mice. This procedure was repeated on the following day and when all the virus had been consumed, the mice were reassembled into one cage. At the end of 2 weeks, the experiment was terminated and the animals bled from the heart.

Transmission of resistance to offspring. Fifteen adult female mice, 2 animals per cage, were fed 10% Hi-Pt virus as indicated above. When these mice had consumed the virus, they were distributed among 3 cages for mating, each cage housing 5 females and one male. The 3 colonies of mice were observed daily for the appearance of the young. Litters born to these mice were separated together with their mothers from the colony of mice and placed in individual cages. The newborn mice were then challenged by intraperitoneal inoculation of 10 to 100 LD_{50} doses of Hi-Pt virus within 48 hours of birth. Following inoculation they were nursed by their own mothers. Adult female mice were also inoculated with Hi-Pt virus by the intraperitoneal and subcutaneous routes, and their offspring also challenged according to the procedure already described(4). *Transmission of C virus from inoculated to uninoculated mice.* Two groups of 4 newborn mice (less than 24 hours old) were inoculated subcutaneously with Hi-Pt virus diluted 10^{-2}. Four uninoculated mice of the same age were placed in the box with each of the 2 inoculated groups. This was also done with Easton-2 and Easton-14 viruses, except that there were 8 mice in each group inoculated with 10^{-5} and 10^{-6} dilutions respectively. The mice were observed for 3 weeks following which they were bled for neutralization tests.

Neutralization tests. These tests were carried out as previously described(3), using 10-fold serial dilutions of serum and a constant amount of virus (100 ID_{50} doses). The serum-virus mixtures were incubated at room temperature for one hour and then inoculated intraperitoneally into newborn mice. The endpoint was considered as being that serum dilution which conferred complete protection.

Results. Susceptibility of newborn mice. That newborn mice are susceptible to the oral administration of 5 different strains of C virus belonging to 4 different immunological types(4) is evident from the results given in Table I. Newborn mice contract the paralytic disease when fed virus diluted at least to 10^{-3} concentration of infected carcass. From the neutralization tests carried out with the blood of those mice surviving oral inoculation, it is apparent that not all of them became infected. The mice surviving oral administration of Conn.-5 virus in a concentration of 10^{-3} and 10^{-5}, showed neutralizing antibody titers of 1:100 and 1:10 respectively, whereas the blood of those mice surviving oral inoculation with 10^{-1} concentration did not respond with the development of antibodies. None of the survivors given Texas-1 virus had neutralizing antibodies in their blood. They were detected in two other instances (Easton-2 and Ohio-1). It is quite possible that mice which survived the apparent exposure to the more concentrated virus (10^{-1}) and which failed to develop antibodies may not have swallowed the virus given them.

Susceptibility of adult mice. As indicated in Table I, feeding Hi-Pt virus to adult

3. Melnick, J. L., and Ledinko, N., *J. Exp. Med.*, 1950, v95, 463.

4. Melnick, J. L., Clarke, N. A., and Kraft, L. M., *J. Exp. Med.*, 1950, v92, 449.

TABLE I. Response of Newborn (A) and Adult Mice (B) to Oral Administration of C Viruses.

Virus fed	Dilution	Fate of mice*	Neutral. antibody response of mice surviving after 2 wk. Dilution of serum giving complete protection
		A. Newborn mice	
Conn.-5	10^{-1}	8/13(7)	0
	10^{-3}	5/13(4)	1:100
	10^{-5}	1/12(0)	1:10
Texas-1	10^{-1}	12/17(8)	0
	10^{-3}	7/15(5)	0
	10^{-5}	0/16	0
Easton-2	10^{-1}	4/13(4)	1:10
	10^{-3}	1/16(1)	0
	10^{-5}	0/6	0
Ohio-1	10^{-1}	5/6(3)	nd
	10^{-3}	2/14(2)	1:10
	10^{-5}	1/16(1)	0
Hi-Pt	10^{-2}	6/9(5)	nd
	10^{-4}	2/8(0)	nd
	10^{-5}	0/4	nd
		B. Adult mice (lactating females)	
Hi-Pt	10^{-1}	0/25	0

nd = not done.

* The numerator indicates number of infected mice. The number in parentheses indicates the number of mice with observable paralysis.

TABLE II. Susceptibility of Mice Born of Mothers Fed or Inoculated Parenterally with High Point Virus.

Susceptibility of infant mice to 10-100 ID_{50} doses (mothers fed virus)	Susceptibility of infant mice to 100-1000 ID_{50} doses (mothers inoculated parenterally with virus)
34/39 (22)	8/74 (5)

lactating females did not induce signs of infection in these animals; the absence of infection is further indicated by their failure to develop neutralizing antibodies to this virus. The explanation offered above for the failure of newborn mice to develop antibodies after being fed virus does not obtain in this instance since all of the virus given the adults was definitely consumed. In addition to these animals we have observed many adult female mice who cannibalized their infected young without effect on the mothers. *Susceptibility of mice born of mothers fed or inoculated parenterally with virus.* Mice born of mothers fed virus were highly susceptible, since, as shown in Table II, 87 per cent of such mice succumbed to intraperitoneal inoculation with 10 to 100 ID_{50} doses of virus. This result is correlated with the fact that none of the mothers themselves became ill or developed antibodies. In contrast to the absence of resistance in the young born of mothers *fed* virus, those mice born of mothers *inoculated* parenterally with virus did possess a considerable degree of resistance to the challenge dose of virus. As indicated in Table II, about 90% of these newborn mice withstood the larger challenge dose.

Comparison of oral and subcutaneous inoculation. There is a striking difference in infectivity of C viruses depending upon the route of inoculation. The comparison given in Table III shows that the subcutaneous route of inoculation with each of the 5 C virus strains was about 10,000 times more effective in producing disease than the oral route. As may be seen, adult mice are not susceptible to either route of inoculation.

Transmission of C viruses from inoculated to uninoculated mice. The results in Table IV show that even direct contact between mice inoculated with virus and uninoculated

TABLE III. Comparison of Infectivity of C Viruses Administered Orally and Subcutaneously. (ID_{50}).

Virus	Subcutaneous	Oral
	A. Newborn mice	
Texas-1	$10^{-7.5}$	$10^{-3.1}$
Hi-Pt	$10^{-7.5}$	$10^{-3.5}$
Conn.-5	10^{-6}	$10^{-2.4}$
Ohio-1	$10^{-5.5}$	10^{-2}
Easton-2	10^{-5}	$10^{-0.7}$
	B. Adult mice (lactating females)	
Hi-Pt	0	0

TABLE IV. Fate of Uninoculated Mice in Direct Contact with Inoculated Mice.

	Inoculated mice		Uninoculated contact mice		
Virus	No. of mice	Incubation period (days)	No. of mice	No. with disease	No. with neutralizing antibodies
Hi-Pt	8	2	8	0	0
E-2	8	4–5	4	0	0
E-14	8	4–5	3	0	0

susceptible mice did not result in transmission of the disease. This is apparent by the absence of paralysis in the uninoculated mice and by the lack of development of neutralizing antibodies. In the course of work with these agents we have seen numerous examples in which uninoculated newborn mice have been kept with their inoculated littermates and only the latter developed disease.

Discussion. It is evident from the results obtained that young mice are susceptible to C viruses orally administered, whereas adult mice neither develop signs of disease nor produce antibodies following such exposure to virus. Von Magnus(5) has recently observed that newborn mice are also much more susceptible than adult mice to oral administration of the TO strain of mouse encephalomyelitis virus.

These results indicate that although one must consider the possibility of accidental contamination of newborn mice in the laboratory (in view of the finding of virus in the intestinal contents of infected mice), the concentrations of virus required to infect by the oral route are much higher than by other routes. This lessens the probability of accidental infections through this cause. Placing groups of uninoculated mice next to or even in with groups of infected mice has not resulted in infection of the uninoculated group.

The fact that some mice surviving oral infection possessed neutralizing antibodies in their blood and some did not deserves comment. The possibility exists that the latter mice were more resistant to the administered virus and that a variation in susceptibility exists among individuals of the same litter of newborn mice. On the other hand, the apparent resistances may be due merely to the technic employed and these animals may not have actually swallowed the virus. It appears more likely that with the more concentrated virus (10^{-1}), mice develop the apparent disease or are not at all infected.

Summary. (1) Newborn mice may be infected by oral administration of at least 5 different strains, representing 4 immunologically distinct Coxsackie, or C, viruses. Adult mice were resistant. (2) Newborn mice surviving oral administration may produce neutralizing antibodies. (3) Adult female mice fed C virus did not transmit neutralizing antibodies to their offspring, in contrast to females inoculated parenterally. (4) In a comparison of routes of inoculation of newborn mice it was found that higher titers may be reached by subcutaneous than by oral administration. The subcutaneous route proved to be about 10,000 times more sensitive than the oral route. (5) Direct contact between inoculated and uninoculated mice did not result in the transmission of infection.

5. von Magnus, H., *Acta Path. Microbiol. Scand.*, 1950, v27, 611.

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Implanted Electrodes for Stimulating or Recording from Deep-Lying Brain Structures.* (18476)

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Recent studies (1-5) of brain stem mechanisms and their influences upon the electrical activity of the cortex have suggested the desirability of recording cortical and dience-

* Aided by a grant from the Commonwealth Fund.

1. Lindsley, D. B., Bowden, J. W., and Magoun, H. W., *EEG Clin. Neurophysiol.*, 1949, v1, 475.

2. Lindsley, D. B., Schreiner, L. H., Knowles, W. B., and Magoun, H. W., *EEG Clin. Neurophysiol.*, 1950, v2, 483.

3. Moruzzi, G., and Magoun, H. W., *EEG Clin. Neurophysiol.*, 1949, v1, 455.

4. Morison, R. S., Finley, K. H., and Lothrop, G. M., *Am. J. Physiol.*, 1943, v139, 410.

5. Starzl, T. E., and Magoun, H. W., *J. Neurophysiol.*, 1951, in press.