Long-Term Effects of Virus Infection on Behavior and Aging in Mice[1] (34760)

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During the course of long-term experiments on the ability of certain viruses to cause late onset neurological disease, changes in behavior and longevity were noted. It seems possible that these incidental observations may be indicative of more widespread, subtle, long-term consequences of virus infection, deserving of greater attention. Although these results can only be regarded as preliminary, the experimental period has lasted for over 3 years; a brief report appears justified.

Materials and Methods. Coxsackie virus strains were stock reference isolates of the laboratory; lymphocytic choriomeningitis (LCM) virus was the same UBC strain as used in previous experiments (1). Inoculations of newborn Albany strain mice were by the intracerebral (ic) route unless otherwise stated, using a volume of 0.02 ml. Mice were kept initially in groups of 20 in large metal cages with food and water *ad libitum*. As numbers decreased they were transferred to smaller cages.

Mortality rates of survivors. The cumula-*upon late mortality.* A series of experiments was performed in which groups of approximately 80 newborn mice were each inoculated with one of the Coxsackie viruses A-1 to 24 and B-1 to 6. The numbers of immediate survivors are shown in Table I (column 4), the remaining mice had 100% mortality. The additional data in Table I will be referred to later.

Mortality rates of survivors. The cumulative mortality of those mice surviving the acute illness following neonatal inoculation was expressed graphically and the time for 50% mortality was measured. The 50% mortality time of combined male and female mice varied between 23 and 30 months in those groups which contained 20 or more total mice, whereas the control animals (115 mice) reached 50% mortality in only 19 months. There was no correlation between the acute mortality and final (chronic) mortality, but all virus inoculation survivors showed extended life spans relative to the uninoculated controls of the experiments. Examination of the early mortality of the controls showed this to be due to the males among which early death from fighting was common. When the mortality was analyzed by sex a clear difference emerged (see Table I, column 5). The control males showed a much earlier 50% mortality time than females (11 versus 31); however this difference was obliterated in the case of most of the groups of survivors which generally showed little difference between male and female mortality. This change was evaluated by subtracting the appropriate control 50% mortality value from each experimental group for both males and females (Table I, column 6). Averages of these differences gave an overall increase of male 50% mortality time of 13.5 months as a result of surviving neonatal Coxsackie virus infection. Female mice showed a decrease of 4.7 months.

Virus effects upon weight. The weight curves of the Coxsackie virus survivors are shown in Fig. 1a,b,c. Most of the weight curves follow the same general pattern, however the point at which weight begins to be lost varies with different viruses. The shape of the curves was the same for both male and female mice. In the case of Coxsackie A-9 there was a particularly rapid initial growth rate

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TABLE I. Mortality and Weight Changes of Mice Inoculated at Birth with Coxsackie Virus.

Virus	% Acute (28 days) mortality	Sex	No. of mice studied	50% Mortality time (months)	50% Mortality change relative to controls[a]	Age at wt change
A-2[b]	91	F	4	24	—7	20
		M	3	—	—	19
A-3	59	F	16	27.5	—3.5	21
		M	16	3.5[c]	—	—
A-4[b]	96	F	3	—	—	24
		M	0[c]	—	—	—
A-5[d]	7	F	13	30	—1	25
		M	15	30	19	23
A-6	67	F	16	27	—4	24
		M	10	25.5	14.5	21
A-8	44	F	16	26	—5	21
		M	24	21.5	10.5	21
A-9[b]	92	F	4	22	—9	17
		M	2	19	8	15
A-20	94	F	31	23.5	—7.5	27
		M	0[e]	—	—	—
A-21	54	F	18	27	—4	22
		M	14	28	17	22
B-3	88	F	29	27	—4	19
		M	0[e]	—	—	—
B-5	1.4	F	35	26.5	—4.5	22
		M	36	24.5	13.5	27
B-6	11.0	F	36	29	—2	25
		M	32	23	12	18
Control		F	48	31	—	22
		M	67	11	—	19

[a] 50% mortality of infected — 50% mortality of controls (months).

[b] Results not significant owing to small numbers.

[c] Males killed owing to severe lacerations due to fighting.

[d] 2-week-old mice were used with this virus since mortality was 100% with all younger aged mice.

[e] Males accidentally discarded.

followed by early and relatively rapid weight loss. Table I shows that this same virus group exhibited early mortality. The long-surviving animals were noticeably more placid than controls and the males appeared to be less irritable and less aggressive. The weight curves (Fig. 1a,b,c) showed that after a steady rise, weight increase ceased and the weight loss began at varying ages. The time when weight gain changed to loss was determined by inspection and is shown by small arrows on Fig. 1a,b,c. These values are expressed in Table I, column 7.

In view of the association between weight loss and mortality, the data in columns 5 and 7 of Table I were expressed as a graph (Fig. 2) relating age at 50% mortality to the time of inversion of the weight curve (the commencement of weight loss). As shown in Fig. 2, most of the points fall close to a straight line indicating a correlation between beginning of weight loss and 50% mortality time.

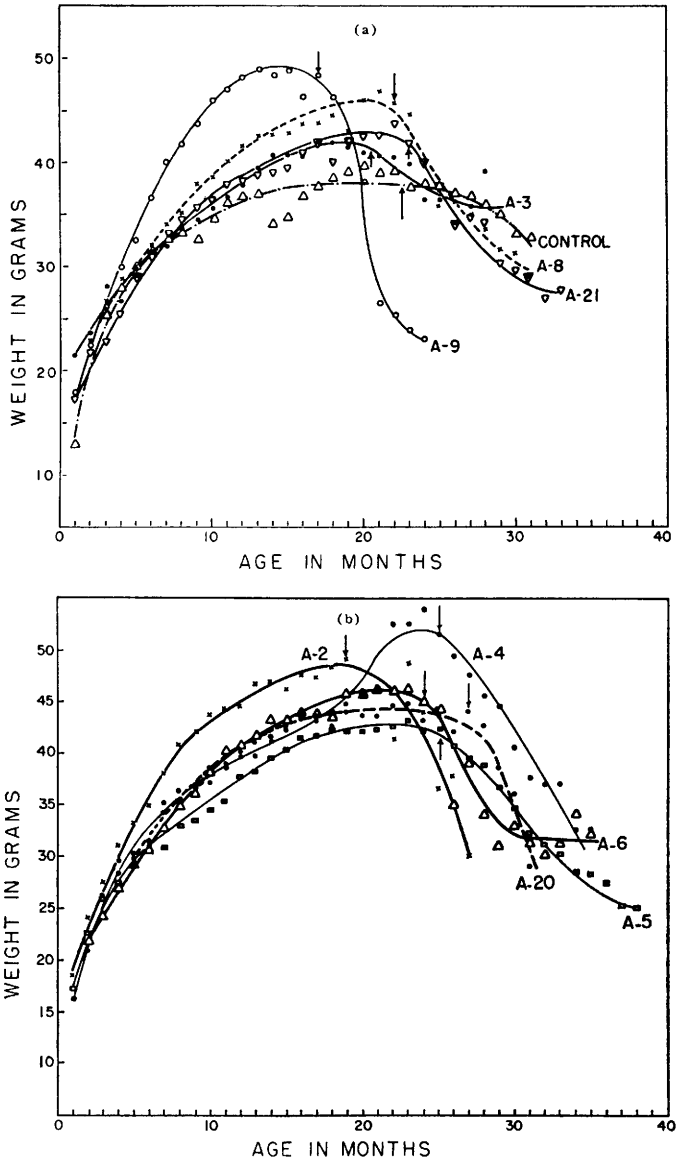
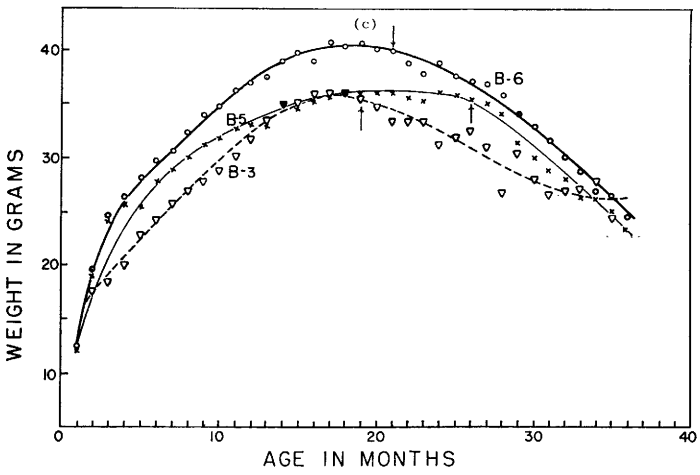


FIG. 1a, b, c. Weight curves of animals surviving neonatal inoculation with Coxsackie viruses. Arrows show points of inversion of the slope of the curves used for Table I and Fig. 2.

Two of the virus groups (male B-5 and female A-20) are considerably displaced from the line, but the male control group was the farthest point from the line in the whole series. An additional point was placed on Fig. 2 from an experiment with a group of female mice neonatally inoculated with LCM virus. This additional point falls very close to the line on Fig. 2, supporting the conclusion that the 50% mortality time is related to the time of commencement of weight loss, and that these factors can be varied as a result of virus infection in early life.

Other long-term effects. The mice surviving neonatal Coxsackie virus infection also showed other signs of disease in certain groups. The B-3 virus survivors exhibited a phase of apparent nervousness and irritability from the second through the fifth month following infection, but this phase was not



permanent. Several of them developed cataracts and became blind at about the 14th month. A proportion of the A-9 group became paralyzed in one rear limb a few months prior to death.

Effects of persistent tolerant infection (PTI) with LCM virus upon responsiveness of mice to auditory and visual stimuli. The long-term effect of persistent LCM virus infection has been shown to be a gradual disease process involving chronic glomerulonephritis (1–3). Under these conditions blood and organ virus titers remain high for the lifetime of the infected animals which also appear to be more aggressive and irritable than uninoculated or LCM-immune controls. This behavioral aspect was further investigated during a study (4) of the effects of electric shock avoidance learning (ESAL) induced stress upon the animals. During the ESAL experiments it was observed that the PTI animals seemed more successful at avoiding stress than controls.. To test this impression 40 PTI mice (14–20 g) and 40 uninoculated controls of the same age were used to count which type of mice was most successful at avoiding the shock. Avoidance was achieved by the mice running to the opposite end of the cage after a warning light went on for 5 sec, prior to the shock. Five mice of each type were put into this ESAL cage together, none had been exposed to it before. The PTI mice had been marked with dye, and the first 5 mice to cross the cage after the stimulus were counted. The scores of the 2 groups in a typical experiment are shown in Table II. These results indicated that the PTI animals were first to respond 70% of the time versus 30% for comparable normal controls. Consistently similar results were obtained in 10 repeated tests using different animals each time. In 9 of the 10 cases the PTI mice scored higher than the controls ($p = 0.02$).

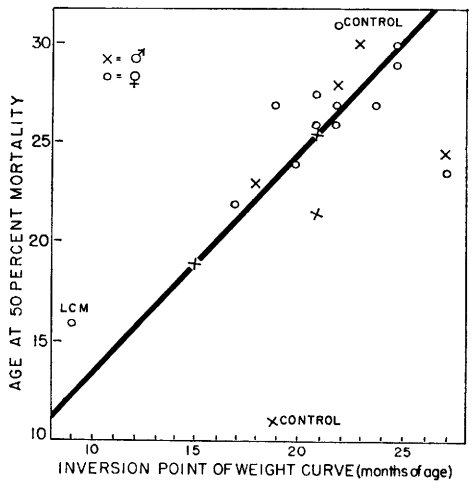


FIG. 2. Relationship between age at 50% mortality and inversion point of weight curve. Each point represents one group of virus infected or control mice.

Discussion. These results suggest that viruses may produce long lasting subtle changes in the young host, which result in differences in behavior and longevity. The effects of the Coxsackie viruses are apparently due to the long-term sequelae of changes produced by the virus during the acute infection.

TABLE II. Score of PTI and Control Mice, Counting the Proportion of the First Five Animals to Respond to a Warning Stimulus in the ESAL Box.

Five mice of each type were placed together in the box at the same time (one set was marked).

Test no.	Score of 5 mice	
	PTI	Control
1	3	2
2	4	1
3	3	2
4	3	2
5	3	2
6	5	—
7	4	1
8	3	2
Total	28	12

The LCM infection is, however, a persistent one, with continual virus multiplication. Neither of these types of effect is new, the long-term paralytic damage of poliovirus is classical, and in the slow virus diseases such as scrapie (5) or visna (6) long-continued virus multiplication ultimately results in tissue damage and death by unknown mechanisms. Virus-induced behavioral changes have been previously described; rabies infection classically causes extreme irritability and aggression in many mammalian species, and kuru is diagnosed by behavioral changes prior to clinical disease in both man (7) and the chimpanzee. It may also be very relevant that subacute sclerosing panenencephalitis (SSPE) also initially causes only behavioral changes, and that there is mounting evidence that measles virus may be the cause of this fatal human disease (8). Lactic dehydrogenase elevating virus produced a long-term infection of mice in which the only marked changes are elevation of certain serum enzymes (9). Recent results on the effect of exposure of newborn mice to sex hormones (10, 11) upon their subsequent aggressive behavior after maturity may be relevant to the apparent "feminizing" effect of neonatal Coxsackie virus described here. Very low levels of male sex hormone present in the neonatal male or female mice sensitize or trigger the nervous system of the animal to react with aggressive behavior to subsequent mature levels of male hormone (11). Conversely brief neonatal treatment of male mice with female hormone prevents this sensitization producing apparently normal male mice which do not behave aggressively after developing their mature levels of male hormone. It seems feasible that the "feminizing" results described here may be due to a similar mechanism, whereby neonatal viral infection inhibits the normal production of male hormone. Future studies should attempt to test this hypothesis, which is strenghtened by the ability of testicular tissue to support the growth of numerous viruses.

It is quite conceivable that other viral agents produce lesions at the biochemical or molecular level which ultimately cause sufficient damage to constitute overt disease. If such damage is undetected by observations or appropriate tests, the end results would be akin to premature aging. In this respect it might be experimentally profitable to consider one aspect of the problem of aging, by treating it as a potential slow virus disease.

Summary. Large groups of newborn mice were each inoculated with one of 30 different Coxsackie viruses. Weight changes and mortality were followed for a period of 3 years by which time all mice had died. Results showed that different viruses caused marked differences in weight change and mortality rate; these two variables seem to be directly related to each other. There was no correlation between early mortality following virus inoculation and subsequent chronic mortality. Groups of male mice surviving initial virus inoculation showed an absence of aggression compared with controls, with a shift in response to that of female mice. It was concluded that neonatal virus infection can affect behavior and/or weight change patterns; factors which appear to exert significant effects on the aging process.

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