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# "Vertical" Transmission of Passage A Leukemic Virus from Inoculated C3H Mice to their Untreated Offspring.* (26545)

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When the initial experiments on cell-free transmission of mouse leukemia were reported (1,2), the problem of natural transmission of the virus was also considered. It was known at that time that foster-nursing of mice born to mothers of high-leukemic strains C58(3) or Ak(4,5) by female mice of low-leukemic lines, did not prevent development of leukemia in the foster-nursed animals. Incidence of leukemia in descendants of high-leukemic strains was essentially undiminished even though such mice had been removed from the womb of their Ak mother by Caesarean section(6) and were then nursed by a low-leukemic-line foster mother. It was apparent that the leukemic agent was not transmitted through the milk, unlike the mouse mammary carcinoma virus(7). Transmission occurred most probably directly through the embryos(1).

The natural transmission of the leukemic virus from injected parents to their non-treated offspring was demonstrated in a series of experiments in which newborn C3H mice were inoculated with cell-free Ak leukemic extracts; after these mice had reached sexual maturity, they were mated and their untreated offspring observed for development of leukemia. Incidence of leukemia in the $F_1$ generation was significant in some experiments(2), but relatively low in others(8), since the leukemic filtrates initially employed had a low content of infective virus. After a highly potent strain of leukemic virus (passage A) was developed(9) it appeared of interest to repeat these experiments. Preliminary results of this study are reported here.

*Materials and Methods.* The 23rd to 26th cell-free serial passage A of the leukemic virus strain, initially derived from spontaneous Ak leukemia, was employed in this study. At this passage level, the potency of this virus is sufficiently high to induce an incidence of leukemia of over 95%, after a latency of less than 3 months, following inoculation into newborn or suckling mice of either C3H/Bi, or C57 Brown/cd inbred lines. Filtrates (Selas 02) of 20% concentration prepared in the usual manner(9) from C3H donor mice with passage A virus-induced leukemia, were inoculated intraperitoneally (0.2 to 0.3 ml). All animals were either of strain C3H(f)/Bi (free from the mammary carcinoma virus), or of the C57 Brown/cd inbred line. Incidence of spontaneous leukemia in our laboratory in mice of either strain has not exceeded, during the past several years, 0.5 to 1% in animals beyond 12 months of age.

*Experimental. Development of leukemia in non-treated offspring of both virus-injected C3H(f) parents.* In this series of experiments, both males and females of the C3H strain were inoculated, within a few days after birth, with passage A leukemic filtrate. After these mice reached sexual maturity, they were mated; litters were born and

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raised before the virus-injected parents developed, and died from, leukemia. (Several of the inoculated couples developed leukemia before they had an opportunity to raise a litter; such animals were not included in this report).

In the first experiment (LV 398), a 3-day-old C3H(f) litter was inoculated with passage A filtrate. Three weeks later, one male and one female from this litter were mated. About 2 months later, a litter of 2 females and 3 males was born to the inoculated parents. Both virus-injected C3H(f) parents developed leukemia at 3½ months of age. All 5 non-inoculated offspring also died from leukemia at ages varying from 4 to 5 months. All leukemias were of the usually observed, lymphatic form.

In a second similar experiment (LV 399), a 3-day-old C3H(f) litter was inoculated with passage A leukemic filtrate. Three weeks later, one male and one female from the inoculated litter were mated. About 6 weeks later, a litter consisting of 4 males was born to the inoculated parents. Both virus-injected C3H(f) parents died from leukemia at the age of 3 and 4 months. Of the 4 non-injected offspring, 3 developed leukemia at 5 months, and the fourth animal at 8½ months of age.

*Development of leukemia in non-treated offspring of virus-injected C3H(f) mothers, and non-inoculated C3H(f) fathers.* In the first experiment of this series (LV 400), part of a C3H(f) litter was inoculated, when less than 1 day old, with the leukemic filtrate; the non-inoculated mice had their tails tied and cut, for identification. Three weeks later, one of the virus-injected females from this litter was mated to a non-injected brother. About 6 weeks later, a litter of 2 females and 3 males was born to this couple. The virus-injected mother developed leukemia at 3 months of age. Of 5 non-inoculated offspring, 3 developed leukemia when 4½ to 5 months old, and the 2 remaining mice at 6 and 8 months of age. The non-inoculated father remained healthy.

In a second experiment of the same series (LV 415), part of a 3-day-old C3H(f) litter was inoculated with leukemic passage A filtrate. Three weeks later, one of the inoculated females of this litter was mated to one of her non-injected brothers. Six weeks later, a litter of 2 males was born to this couple. The virus-injected mother developed leukemia at 3 months of age. Both non-injected offspring developed leukemia at 4½ and 5 months of age. The non-injected father remained healthy.

*Observation of offspring born to non-injected mothers, and virus-injected fathers, of the C3H(f) strain.* The third series of experiments was similar to the second series, except that the prospective fathers, instead of mothers, were inoculated with the leukemic virus. Three, less than 5-day-old, C3H(f) litters were split; a part of each litter was then inoculated with passage A filtrate. Three weeks later, a virus-injected male from each litter was mated to his non-injected sister.

In the first experiment of this series (LV 401), 2 successive litters, a total of 3 females and 6 males, were born to a non-inoculated mother and a virus-injected father. None of the 9 offspring has developed leukemia, although they are already over 9 months old. The virus-injected father died from leukemia at the age of 4½ months. The non-injected mother remained healthy.

In a second experiment (LV 402), 3 successive litters, a total of 5 females and 2 males, were born to a non-inoculated C3H(f) mother, and a virus-injected C3H(f) father. The inoculated father died from leukemia at 3½ months of age. None of the 7 offspring has developed leukemia, although they already are over 9 months old. The non-injected mother also remained healthy.

In a third experiment of this series (LV 403), 4 successive litters, a total of 5 females and 10 males, were born to a non-inoculated C3H(f) mother, and a virus-injected C3H(f) father. None of the 15 offspring has developed leukemia, although they are now more than 9 months old. The virus-injected father died from leukemia at 4 months of age. The non-injected mother remained healthy.

*Failure of offspring of virus-injected C57 Brown/cd parents to develop leukemia.* New-

born, suckling, and young adult mice of the C57 Brown cd strain are as susceptible to the leukemogenic action of the passage A virus as are mice of the C3H/Bi strain(10,11). It was therefore of interest to determine whether in mice of this strain also, offspring of inoculated parents would develop leukemia. Newborn and suckling, less than 7-day-old, C57 Brown/cd litters were inoculated with passage A filtrate. After the inoculated mice reached sexual maturity, they were mated to their own littermates. In the first 2 experiments, both parents were inoculated. A litter of 3 mice was born to the first couple, and a litter of 2 mice to the second couple. Only 1 developed leukemia at 7½ months of age; the remaining 4 mice are still in good health, although they are now over 9 months old. All 4 inoculated parents died from leukemia at 3½ to 4½ months of age.

In a second group, consisting of 2 couples, a total of 19 offspring (11 females and 8 males) was born to virus-injected C57 Brown cd mothers, and non-injected C57 Brown/cd fathers. None of the 19 offspring (4 litters) has developed leukemia, although they are now more than 9 months old. Both virus-injected C57 Brown cd mothers died from leukemia at 4 and 4½ months of age. The non-injected fathers remained healthy.

In an additional series consisting of 3 C57 Brown/cd couples, non-injected C57 Brown cd females were mated to their virus-injected brothers. None of the 40 offspring (6 litters) born to such parents has thus far developed leukemia; they are now more than 9 months old. All 3 virus-injected C57 Brown cd fathers developed leukemia at 3 to 3½ months of age. The 3 non-injected C57 Brown/cd mothers remained healthy.

*Discussion.* The high incidence of leukemia in successive generations of mice of certain inbred strains, such as C58 or Ak was at first explained by genetic factors(3,12). Transmission of a leukemic factor through milk of nursing females to their offspring was considered, but was promptly excluded by foster-nursing experiments(3,4,5).

When the viral nature of mouse leukemia was demonstrated(1,2), natural transmission of the leukemic agent from one generation to another was again considered. Development of leukemia in offspring of high-leukemic inbred strains, such as Ak, could not be prevented even if the embryos were removed at term by Caesarean section from the womb of their Ak mother, and transmitted for foster nursing to female mice of a low-leukemic strain(8); moreover, Ak mice born from transferred Ak ova also developed leukemia (13). That the leukemic virus is present in the developing embryo was demonstrated in direct bio-assay experiments in which extracts prepared from normal, healthy, Ak(1, 2,8), or C58(14), embryos, induced leukemia following inoculation into newborn C3H mice.

The virus is probably transmitted from parents to offspring directly through the fertilized ovum; it is possible, however, that the virus could pass through the placenta in the pregnant, virus-carrying, female, and infect the embryo.

The term "vertical transmission" was suggested to designate the natural passage of a pathogenic virus from one generation to another(2). Actual transmission of the virus from virus-injected parents to their offspring could be demonstrated. When newborn mice of strain C3H were inoculated with Ak leukemic extracts, and when such virus-injected mice were subsequently mated, some of their non-treated offspring developed leukemia(1, 2,8). However, in previous studies, a leukemic virus of low potency was employed, and probably for that reason incidence of leukemia developing in offspring of the inoculated C3H parents was relatively low; furthermore, the disease usually appeared late. In the present study, a considerably more potent leukemic virus was employed for inoculation of the prospective parents. Incidence of leukemia in the offspring of the inoculated parents was high, and latency period relatively short.

Curiously, inoculation with the leukemic virus of the prospective mothers only, had in our present experiments as much influence on incidence of leukemia developing in their offspring, as had the inoculation with the virus of both prospective parents. When the prospective fathers only were inoculated, and later mated to non-injected females, no leu-

kemia has appeared in their offspring; additional observations are needed, nevertheless, to clarify this point. The animals in this series, now only about 9 months old, should be observed for a longer period. In our previous similar studies (Gross, unpublished) we have observed occasional development of leukemia in offspring of non-injected mothers and virus-injected fathers of the C3H strain; however, incidence of leukemia in such offspring was low, and latency prolonged. It was apparent nevertheless, that under proper experimental conditions the leukemic virus could be transmitted from one generation to another not necessarily through the mother, but also through a virus-carrying father.

It has been observed(3,15,16) that incidence of leukemia in $F_1$ generation of hybrids was higher, at least in some experiments, when the mother was of the high-leukemic-line, such as Ak or C58, and the father of a low-leukemic strain, such as C3H, Rf, or StoLi. If the experimental conditions were reversed, *i.e.,* father was of the high-leukemic strain, and the mother of a low-leukemic line, the $F_1$ generation of hybrids had a lower incidence of leukemia. In other experiments, however, incidence of leukemia in $F_1$ generation of hybrids was approximately similar when either the mother or the father were of a high-leukemic strain(3,12,15,16).

Mice of the C57 Brown/cd strain are highly susceptible to the leukemogenic action of passage A virus. When suckling C57 Brown/cd mice were inoculated with the passage A virus, practically 100% of them developed leukemia after a latency of about 3 months(11; also unpublished); yet most of the offspring of virus-injected C57 Brown/cd mice did not develop leukemia in our experiments, even when both parents had been inoculated with the passage A virus. Offspring of such virus-injected C57 Brown/cd mice, still in good health, are now more than 9 months old; additional observations are needed to determine whether some of them will not eventually develop leukemia. It appears, however, that mice of the C57 Brown/cd strain, though highly susceptible to the leukemogenic action of the passage A virus, do not transmit this virus to their offspring as readily as do mice of the C3H strain. Susceptibility to the oncogenic action of a virus is thus distinct from the ability of the host to transmit the inoculated virus naturally to its offspring.

*Summary.* 1. C3H(f) mice inoculated within a few days after birth with passage A leukemic filtrates, were subsequently mated. 2. When both females and males selected for mating received inoculation of the passage A virus, or when inoculated females were mated to non-inoculated males, all offspring born to such parents developed leukemia at 4½ to 7 months of age. 3. When, however, non-inoculated females were mated to virus-injected males, 31 non-treated offspring have remained thus far in good health, although they have already reached 8 to 9 months of age. 4. Similar experiments were performed employing mice of the C57 Brown/cd strain, highly susceptible to leukemogenic action of passage A virus. 5. Of the 64 non-treated C57 Brown/cd offspring, now 9 months old, only 1 has thus far developed leukemia when either a) both parents, b) mothers only, or c) fathers only had been injected with the passage A virus.

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