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Studies of Congenital Transmission of a Leukemia Virus in Mice.* (27045)

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Gross reported in 1951(1,2) and in more detail later(3-5) that some of the extracts and filtrates prepared from leukemic tissues of AK strain mice induced lymphocytic leukemia in certain recipient mice, particularly in the C3H/Bi and C3Hf/Bi strains. Experiments were described(2, 6, 7, 8) of an attempt to study the "natural" transmission of the Gross virus in C3H mice. The concept was developed that the virus isolated from AK leukemic mice was responsible for the high frequency of lymphocytic neoplasms within that strain, and that the virus was transmitted through the germ lines resulting in successive generations of leukemic AK mice with similar frequencies and latent periods (See 7). It had to be assumed also, but this was not discussed in considering the concept, that the paternal line was as effective a means of transmission of virus as the maternal line(9). The results of the early "natural" transmission studies were equivocal(2). The frequencies of leukemia among the uninoculated offspring from virus-inoculated parents were suggestive of transmission of virus in some matings(7) but not in others, the latent period was long and too few mice were observed beyond one generation.

Recently, Gross has reported on certain aspects of the so-called "vertical" transmission of the Gross virus using a more highly potent, serially passaged virus(10).

All C3Hf/Bi offspring born to virus-inoculated females developed leukemia and average age at leukemic death was similar to that of the inoculated parents. None of the offspring born to non-inoculated females mated with virus-inoculated males had developed leukemia up to 9 months of age. The experiments were not designed to determine the manner of transmission of virus through the maternal line.

The isolation and concentration of a leukemogenic virus from transplantable Sarcoma 37(11,12) presented the opportunity to study certain epidemiologic aspects of this neoplastic disease in mice, including that of natural transmission of the virus. This report presents the results, preliminary in some aspects, of the fate of offspring in successive generations, born to parenterally-infected parents.

Materials and methods. Virus. The preparation designated LT(V) 96 used throughout these studies was prepared as follows: A pool of leukemic lymph nodes and spleens from

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virus-inoculated BALB/cAn mice served as source material. The tissues were extracted and the particulate fraction concentrated by differential centrifugation. The details of the operative procedures have been published(11). Final concentration of the virus material was 0.5 g equivalents per milliliter. A 1.0 g equiv. per ml concentrate is equal to 1 ml of final suspension for each gram of pooled tissue processed. Aliquots of the virus preparation were quickly frozen and stored at the temperature of a dry ice chest until used.

The mice which ranged in age from less than 1 day to 21 days were inoculated subcutaneously with 0.1 ml of a 10^{-1} dilution of the virus. The diluent employed was 0.05 M.pH 6.8, sodium citrate buffer containing 1.0 mg of crystalline hyaluronidase per 100 ml of medium. Exact times of inoculation are given in the individual experiments. All members of a litter were inoculated and were returned to their mothers where they remained until weaning at 4 weeks of age. Successive generations of uninoculated mice (G_1, G_2, etc.) were usually caged 3 females to 1 male.

Successive generations of control mice were maintained at the same time and were derived from non-inoculated litter mates of the mothers supplying the experimental mice.

The results of a bioassay of this virus preparation in newborn BALB/c mice, the standard reference strain, are presented in Table 1 for comparative purposes.

TABLE I. Bioassay of virus concentrate in newborn BALB/c mice.

Log_{10} Dilution	No. leukemic/ No. inoculated	Incidence of leukemia (%)	Avg Age at leukemic death (Mo)
−1	14/14	100	3
−2	18/18	100	4
−3	17/17	100	4
−4	5/5	100	4½

Mice. Four inbred strains of mice were used, principally, in this study: C3Hf/Lw, C3Hf/Gs, C3Hf/Fg, and RFM. All C3H strains were foster-nursed following removal by Caesarean section and did not carry the Bittner milk agent. The origin and characteristics of the C3Hf/Lw strain have been described(9,13). C3Hf/Gs mice were received from Dr. Ludwik Gross, through Dr. Ray Bryan of this Institute. Brother x sister inbreeding was continued from a single pair and pedigreed mice from our F_6 to F_8 were used. Spontaneously occurring neoplasms of reticular tissues are not common in either strain. A frequency of approximately 10% has been observed in our laboratory but the leukemias appear usually beyond 1 year of age. Although some are derived from cells of the lymphoid series, the thymus appears to play no part in the genesis of these. The C3Hf/Fg strain is a high-leukemic strain and the origin and characteristics of this strain have been described(9,14). A high frequency of different morphologic forms of leukemia has been observed throughout our F_1 to F_{14} breeding generations. The mean age at death from leukemia is approximately 14 months and rarely are the neoplasms observed in females before 7 months and in males before 9 months. Numerous attempts to isolate and concentrate a cell-free material from leukemic tissues of C3Hf/Fg mice have been unsuccessful(9).

RFM strain mice were originally obtained from the Jackson Memorial Laboratory in 1959 and inbreeding has been continued since that time. An occasional lymphocytic or granulocytic neoplasm has been observed in our colony but the strain is a low-leukemic strain. The incidence and latent period of the disease for RFM mice have been published by Upton and colleagues(15).

Results. The response of several strains of mice, not heretofore studied, to virus preparation LT(V) 96 is shown in Table II. A low frequency in C57BL mice observed previously(12) was observed here in another subline, C57BL/Ka. In addition, high leukemic C58 mice, related in origin to C57(16), and NH mice, particularly the latter, indicate a degree of resistance to the Moloney virus. The mean age at leukemic death also indicates a lower susceptibility in the Lw subline of C3H compared with the other 2 C3H sublines studied. It is clear under the conditions employed here that all of the strains studied show a lower susceptibility than BALB/c mice, the standard reference strain (Table 1).

Transmission Studies in C3Hf/Gs Strain Mice (Maternal Line Series N10). The untreated offspring of 2 virus-inoculated C3Hf/Gs litters (N10 and N13) have been observed

TABLE II. Induction of Leukemia in Inbred Strains of Mice by Moloney Virus Preparation LT(V)96.

Strain of Mouse	No. Litters Inoculated	Time of Virus Inoculation (days)	No. Leukemic / No. Mice	Age at Leukemic Death (Mo)	Transmission to Offspring via Mother[1]
C3Hf/Lw	2	7, 7	4, 4 – – 4 4	7, 7½	+
C3Hf/Gs	3	13, 8, 1	4, 3, 5 – – – 5 3 5	5, 3½, 4½	+
C3Hf/Fg	5	5, <1, 2, 14, 7	2, 7, 4, 0, 3 – – – – – 2 7 4 7 4	6, 3½, 5½, –, 4½	+
RFM	4	7, <1, 4, 1	3, 1, 3, 2 – – – – 3 1 3 3	4, 5, 4½, 3½	–
C58	4	6, 2, 5, 5	0, 1, 1, 0 – – – – 4 5 9 5	–, 5½, 4, –	–
C57BL/Ka	3	5, 10, 1	0, 1, 1 – – – 9 6 6	–, 4½, 6	?
AKR/Lw	4	20, 4, 5, 7	0, 7, 3, 2 – – – – 6 7 3 2	–, 4, 4, 5	?
NH	5	5, 21, 11, 2, 6	0, 0, 0, 0, 0 – – – – – 4 4 9 3 5	—	–

[1] At least one subsequent generation, including several litters, observed for each strain.

over a period of several successive generations. In the N-10 series a litter of 5 mice born to female 45820 received virus through a subcutaneous inoculation at 13 days of age. Two females developed leukemia at 3 and 3½ months of age and 2 of 3 males at 6 and 7 months of age. A litter of 3 was obtained from one of the virus-inoculated females and a litter of 5 from the second female, both mated with the virus-inoculated male which eventually developed leukemia at 6 months of age. All 8 untreated offspring (5 females and 3 males) born to the 2 inoculated females developed leukemia. Age at leukemic deaths ranged from 3½ to 7½ months and was similar to that for the virus-inoculated parents. Fig. 1 gives a detailed representation of the fate of the original virus-inoculated litter (G_0) and subsequent generations of untreated C3Hf/Gs mice born to infected† (but untreated) mothers. It may be seen that the majority of offspring developed leukemia in the G_2 generation, 5 litters from 5 different untreated G_1 females and in the G_3 generation, 5 litters from 5 different untreated G_2 females. Average age at leukemic death has remained essentially the same throughout the 4 generations. Three litters in the G_1 to G_3 series suckled mothers which became leukemic during the suckling period and 2 litters were born to the uninoculated (but infected) mothers mated with non-inoculated ("normal") C3Hf/Gs fathers. The frequency and latent period of the disease was essentially similar in these mice to others in the series.

Of interest is the observation that none of 4 mice of a second litter born to an infected female of the G_1 generation has developed leukemia in 10 months following removal by Caesarean section and foster nursing on a C3Hf/Lw mother. A subsequent generation of 6 mice also remains leukemia-free. All 4 of

† The term "infected" will be used, for convenience, to mean those mice obtaining virus through their mothers. Virus-inoculated litters are designated G_0 and successive litters, obtained by matings of sibs, as G_1, G_2, etc. "Normal" refers to those mice having no apparent contact with virus.

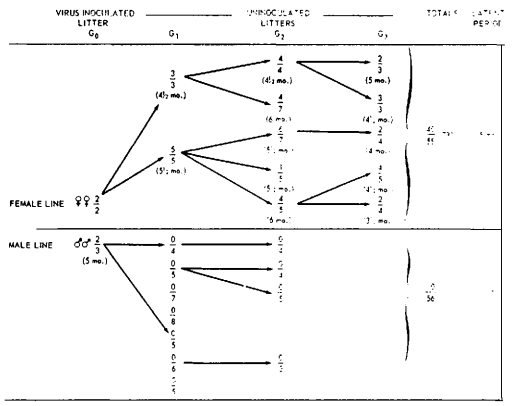

FIG. 1. Litter of 5 (2 females, 3 males) C3Hf/Gs mice, born to female 45899 (F90+6) inbred generation, inoculated at 13 days of age with 0.1 ml preparation LT(V)96 subcutaneously, (G_0). The 2 females were autopsied leukemic at 3 and 3½ months of age. Two of the males were leukemic at 6 and 7 months of age. Single litters (G_1) were obtained from each of the 2 potentially leukemic females No. 45925 and 45926, mated with one of the potentially leukemic males No. 45929. All subsequent litters in the female line were obtained by matings of potentially leukemic females with their potentially leukemic male litter mates (Female line). To establish the male line, potentially leukemic males (G_0) No. 45928 and 45929 were mated at 6 weeks of age with uninoculated C3Hf/Gs females of the same inbred family. Eight G_1 litters were produced as shown and these produced the 4 G_2 litters as shown. All of these offspring are now 8 to 10 months of age. Numbers above line refer to leukemic mice; below line, to total no. of mice in litter surviving to appearance of first leukemics within each litter. Latent period is average age at leukemic death in months.

the first litter which suckled their own mother became leukemic. A reciprocal foster-nursing of a litter of 6 C3Hf/Lw mice was accomplished using an infected C3Hf/Gs mother of the G_2 generation. Four of 6 in this litter have developed leukemia at 5 months of age.

Paternal Line. Each of the 2 virus-inoculated males of the N-10 series was mated with 3 uninoculated ("normal") C3Hf/Gs females of the same subfamily. Three litters (G_1) were fathered by the inoculated male which became leukemic at 5 months and 4 litters by the inoculated male which became leukemic at 7 months. Four G_2 litters were born to 4 different G_1 mothers mated with their litter mates. No leukemias have appeared among the 56 offspring obtained, now 8 to 10 months of age.

Series N-13. Three of 4 *(Series N-13)* C3Hf/Gs mice survived following inoculation of virus preparation LT(V) 96 at 8 days of age. These were born to female 45819 a litter mate to female 45820. All 3 (G_0), a female and 2 males developed leukemia at 3½, 3½ and 4 months.

A first litter of 3 females was born to virus-inoculated female mated to one of the inoculated males. All 3 (G_1) females, which were uninoculated, developed leukemia at 3½, 4 and 7 months. A litter was obtained from each of these 3 females (prior to developing leukemia), mated with a male from the same subfamily but having no contact with virus. The fate of the members of these 3 litters (G_2) was as follows: 2 out of a litter of 4, 5 of 5 and 3 of 4 died leukemic at 3½ to 7 months of age, average age at leukemic death being 5 months. A later generation (G_3) was obtained from one of the infected (but untreated) females of a G_2 litter. This female died from leukemia at 3½ months of age. Four of 6 of her litter have died leukemic at 3½ to 5 months. The controls for the N-13 series consisted of successive generations through G_4 of uninoculated litters born to C3Hf/Gs female 45818 a sister of the source animals for the experimental series. No leukemias have been observed among 4 litters totalling 25 mice, presently 8 to 10 months of age.

Transmission in other strains of mice. The pattern of congenital transmission of Moloney virus in the *Fg* (high leukemic) and *Lw* (low leukemic) sublines of C3H strain mice is similar to that observed in C3Hf/Gs mice. Transfer of virus through females resulting in lymphocytic neoplasms in the offspring was observed through the G_3 generation, following introduction parenterally at G_0. (Table III).

In contrast, although RFM mice are highly susceptible to this leukemia virus, transmission was observed only through the first generation.

All 4 offspring developed thymic neoplasms and died at 4 months of age, but all had suckled a leukemic mother. Eleven litters (62 mice) of the succeeding G_2 to G_4 generations have remained non-leukemic through 10 months. Attempts were made to detect the presence of virus by foster-nursings of susceptible C3Hf/Gs upon RFM mothers in the G_2 to G_4 generations. These mice also have

TABLE III. Transmission of Leukemia Virus (Moloney) through the Maternal Line of C3Hf/Fg, C3Hf/Lw and RFM Mice.

	No. of Litters	Age of mice at time of infection	No. Mice Leukemic / No. in Litter	Avg Age in Mo at Leukemic Death
C3Hf/FG				
Virus-inoculated litter (G_0)	1	7 days	3[1] / 4	6
Subsequent non-inoculated litters				
G_1	1	—	3[1] / 5	5
G_2	2	—	3, 1 / 3 4	5, 6
G_3	3	—	3, 2, 3 / 9 6 8	4, 4½, 4½
C3Hf/Lw				
Virus-inoculated litter (G_0)	1	7 days	3 / 3	4
Subsequent non-inoculated litters				
G_1	3	—	1, 6, 1 / 3 6 2	7, 6, 6
G_2	4	—	2, 4, 4, 4 / 3 7 7 6	5, 5, 4, 5
G_3	2	—	1, 2 / 3 4	4½, 5
RFM				
Virus-inoculated litter (G_0)	1	1 day	3 / 3	2½
Subsequent non-inoculated litters				
G_1	1	—	4[2] / 4	4
G_2	1		0[3] / 3	—
G_3	5	—	0, 0, 0,[3] 0, 0[3] / 3 10 14 11 16	—
G_4	5	—	0, 0, 0,[3] 0, 0 / 7 5 8 6 6	—

[1] See text for studies of transmission through paternal line, using infected males of these litters.
[2] This litter suckled a leukemic mother.
[3] Foster-nursings of highly susceptible C3Hf/Gs mice upon mothers of these litters did not reveal presence of virus.

remained non-leukemic.

All of the lymphocytic neoplasms appearing in C3Hf/Fg mice, listed in Table III, were thymic in origin; many were thymic lymphosarcomas. The gross and histologic patterns of the virus-induced disease were unlike those observed in the spontaneous disease of this subline in which the thymus apparently is not essential(9). The first spontaneous leukemia among the control mice of this series was observed at 8 months of age.

In several instances C3Hf/Lw and C3Hf/Fg

females of the G_1 and G_2 generations (receiving virus from their mothers) were mated with uninoculated males from the breeding colony. The frequency of leukemia among their offspring was similar to that among offspring born to infected mothers mated with infected males.

Studies of Transmission through the Paternal Line. One of the males of a litter of 4 C3Hf/Fg mice (Table 2), inoculated at 7 days with Moloney virus (G_0) developed generalized lymphocytic leukemia with thymic involvement at 4 months of age. Prior to the appearance of the disease, this male was mated with 3 untreated C3Hf/Fg females of the same subfamily and 3 litters (G_1) resulted. Two litters representing G_2 were obtained by mating G_1 litter mates. All of these offspring have remained free from leukemia through 8 months.

Two C3Hf/Fg males of the G_1 generation which received virus as a result of suckling their mother were also mated with untreated C3Hf/Fg females. One of these males became leukemic at 6 months, the other has remained non-leukemic. The resulting 4 litters of the G_2 generation and 2 litters of the G_3 generation are non-leukemic after 8 months.

A litter of 7 C3Hf/Fg mice was born to female 26753. All members of the litter received Moloney virus, preparation LT(V)96 subcutaneously when less than 24 hours old. The 4 females and 3 males developed leukemia, with thymic involvement at 3 to 4 months (average age at death, 3½ months). A litter of 2 males (G_1) was obtained from one of the females and both died leukemic at 3 and 4 months.

These males had suckled a virus-inoculated mother. Three litters of a G_2 generation were obtained, before death of these males, through matings of uninoculated C3Hf/Fg females with the infected males. An additional 7 litters representing the G_3 and G_4 generations, totalling 26 mice, were obtained from matings of litter mates. No leukemias have been observed through the 8 month period preceding spontaneous occurrence of leukemia within this subline.

One of the males of the litter of 7 C3Hf/Fg mice which received Moloney virus parenterally at less than 24 hours of age, was mated to an untreated C3Hf/Fg female and produced a litter of 6 offspring prior to developing leukemia at 4½ months of age. Sib matings within this litter and subsequent litters provided an additional 8 litters (44 mice), constituting the G_1 to G_2 generations. This group has remained non-leukemic through the 8 month observation period.

Reciprocal Foster-Nursing Experiments. To determine the route of transmission of virus from the mother to her offspring, reciprocal foster nursings were carried out. Transmission could occur before birth through extra-embryonic membranes to the embryos, after birth through the mother's milk or both before and after birth. It is unlikely that a regular transmission of Moloney virus occurs through gametogenesis and fertilization since the evidence at present indicates that transfer does not occur through the paternal line.

Foster-nursings were accomplished using C3H mothers of 3 different sublines. The litters were allowed to drop through a raised platform constructed of ½″ x ½″ mesh hardware cloth inserted in the bottom of a regular plastic cage. Many of the deliveries were observed and since there was no question of the possibility of litters suckling their own mothers, the adopted procedure was to isolate near-term females at night and collect the young 12 to 15 hours later, or isolate in the morning and collect the litters for foster-nursing at some time during the day. The efficacy of this method of reciprocal foster-nursing has been demonstrated in studies of the transfer of antibodies to polyoma virus(17).

Litters born of virus-inoculated (G_0) or of virus infected mothers (G_1-G_3), that is, infected through the maternal line, were foster nursed on "normal" non-inoculated mothers and *vice versa*. Entire litters were foster nursed.

Two separate experiments rule out the possibility of spread of infection through mouse to mouse contact. Virus-inoculated and non-inoculated litter mates were allowed to remain together from birth through a period which has now reached 9 months. In 3 litters, 6 of 9 virus-inoculated C3Hf/Lw mice became leukemic whereas 11 of the untreated litter mates remain nonleukemic. Four C3Hf/Gs mothers,

TABLE IV. Lymphocytic Neoplasms in Reciprocally Foster-Nursed Mice of 3 C3H Sublines.

Strain	Offspring from "Normal" females Foster-nursed to "Infected" Mothers				Offspring from "Infected" females Foster-nursed to "Normal" Mothers			
	Litter No.	No. of Offspring	No. Leukemic	Avg Age at Leukemic Death Mo	Litter No.	No. of Offspring	No. Leukemic	Avg Age at Leukemic Death Mo
C3H/Lw	1	4	2	3½	—	—	—	—
	2	3[1]	2	5	—	—	—	—
	3	6	6[2]	6	—	—	—	—
	4	4	1	1½	4A	5	0	—
	5	2	1	7	5A	2[4]	1	7
	6	5	4	5½	—	—	—	—
C3Hf/Gs	1	2	1[3]	5	1	5	0	—
	2	6	1	3½	2	4	0	—
	3	6[1]	4	5	3	5	0	—
	4	2[1]	2	5	—	—	—	—
	5	6	0	—	5A	5	1[4]	4
	6	6	4	5	6A	4	0	—
	7	4	3	4½	7A	4	2	5
C3Hf/Fg	1	6	2	4½	1A	4	0	—
	2	8[1]	3	5	—	—	—	—

Survivors among the group of animals in this table are presently 7 to 9 months of age. Litters born to "normal" and "infected" mothers on same morning or evening were interchanged. These are designated in table as 4 and 4A, 5 and 5A, etc.

[1] These litters were foster-nursed upon infected mothers of G_1 or G_2 generation, i.e., those mothers 1 or 2 generations removed from the original virus-inoculated litter. All others were foster-nursed upon mothers which received virus parenterally at birth.

[2] This litter of 6, all of which became leukemic, was foster-nursed upon a virus-inoculated C3Hf/Lw female. The foster-nurse died leukemic at 3½ months of age. Two litters of a succeeding generation were born to 2 of 6 of these foster-nursed mice; 4 of a litter of 7 and 3 of a litter of 7 died leukemic at average ages of 5 and 4 months.

[3] Females of this litter which suckled a virus-inoculated mother, died of leukemia at 5 months of age. A litter of 3 males was born prior to the mother's developing leukemia. Two of 3 of this succeeding litter have died leukemic at 5½ months of age.

[4] These litters were born to leukemic mothers but did not obtain mother's milk before foster-nursing.

which suckled virus-inoculated litters, were allowed to deliver subsequent litters. None of 25 offspring has developed lymphocytic neoplasms. Moloney(19) has obtained evidence in BALB/c mice of the non-contagious nature of this virus.

It is clear from the results in Table IV that this leukemia virus is effectively transmitted through the mother's milk in all 3 C3H sublines studied. Further, it is clear that transmission is as effective by mothers infected originally through suckling as by mothers receiving virus parenterally. Apparently high-leukemic lines may be established by foster nursing of previously unexposed litters upon infected mothers. (See Litter #3, C3Hf/Lw strain and Litter #1, C3Hf/Gs strain).

The results are somewhat equivocal in those offspring born of "infected" mothers and foster-nursed upon "normal" mothers. In 2 instances in which leukemia appeared among the offspring, the mothers exhibited signs of leukemia before births of their litters. More data are desirable to determine if Moloney virus passes the placental or other extra-embryonic barriers. Ida and Moloney(20) have obtained preliminary data indicating such a transfer, however the conditions concerning route and time of inoculation, strain of mouse etc. were quite unlike those reported here.

Discussion. It is clear from the present results employing the highly leukemogenic virus of Moloney that transmission occurs through successive generations of the 3 different C3H strain sublines studied following introduction of the virus in neonatal mice. The most effective route is through the mother's milk. There is every indication that a "low-leukemic" strain may be transformed to a "high-leukemic" strain provided the strain is susceptible to the leukemogenic action of the virus

and is capable of transmission of it to the offspring through the maternal line. Infection through 4 successive generations of C3Hf/Gs mice was observed with frequencies and average age at death from the disease being quite similar. RFM mice, although highly susceptible to the leukemogenic action of Moloney virus are apparently not capable of transmitting it to offspring. C58 mice, with a lower susceptibility to Moloney virus, appear also to fall in this category. C57Br/cd mice were found by Gross(10) to be highly susceptible to Passage A strain of his leukemia virus but apparently were unable to transmit it to offspring. A similar failure of inoculated mothers of the AB strain to transfer myeloid leukemia virus to their offspring was reported(21).

Our findings are similar to those of Rubin *et al.*(18) concerning transmission of an avian leukosis virus to the extent that transmission of infection was not achieved through the paternal line.

It is apparent also that a "low-leukemic" line is established simply by foster-nursing mice born to infected mothers upon mothers having no previous contact with virus, providing the foster-nursed litter does not obtain milk from its infected mother. This was achieved in C3Hf/Lw mice.

The results reported here stand in sharp contrast to the facts developed through studies of certain high-leukemic strains of mice such as AKR, C58, F and C3Hf/Fg. All attempts to transform high-leukemic to low-leukemic strains and *vice versa* have proved unsuccessful. Reciprocal foster-nursings(16,22,23,24,25), successive foster-nursings in AKR mice (24) and transfer of fertilized ova reciprocally between AKR and C3H strain mice(26,27) have been ineffectual. Further, reciprocal matings of high- and low-leukemic strains usually results in populations of F_1 hybrid mice having very similar frequencies and average ages at death from the disease(16,22,23,24,25,28). It is clear therefore that in certain high-leukemic lines the male contributes equally as well as the female to the potentiality of F_1 hybrids to develop leukemia.

The failure of male transmission of Moloney virus and apparently also the Gross virus(10) may be the result of loss of virus from spermatozoa with the shedding of cytoplasm, as suggested for an avian leukosis virus(18). Since precise and rapid *in vitro* and *in vivo* bioassay techcniques are not yet available for these viruses this question cannot presently be resolved.

Only a sporadic occurrence of lymphocytic leukemia was observed in those offspring obtained from virus-inoculated or "infected" mothers and foster-nursed upon mothers having no previous contact with virus. To obtain unequivocal evidence of passage of Moloney virus across extra embryonic membranes, it will be necessary in future experiments to use the strictest of precautions and resort to Caesarean sections. It is clear, however, from our data that Moloney virus is not regularly transmitted via germinal cells. Definitive evidence concerning the Gross virus has not yet been published.

A counterpart may exist to explain the occurrence and transmissibility of a leukemia virus in certain high leukemic strains of mice, particularly AKR, from which the Gross virus is recoverable (from leukemic mice) in an infectious state. The virus which produces CO_2 sensitivity in *Drosophila* may assume alternative states. The stabilized state resembling prophage, can mutate to a form which is unable to produce mature infective virus. The reverse mutation also occurs. This virus in the stabilized non-infectious form is transmitted regularly to succeeding generations through the gametes. A shift to an infectious state however leads to transmissibility by cell-free extracts wherein natural transmission from fly to fly is not observed, and infrequent transmission to progeny occurs(29).

Summary. The pattern of congenital transmission of a leukemia virus was studied in several inbred strains of mice varying in their susceptibilities to virus. Transmission of virus occurred through the maternal line in 3 C3H sublines, 2 low-leukemic and one high-leukemic. RFM mothers failed to transmit infection. There was no indication that parenterally-infected males, of any of the strains studied, transmitted virus to their offspring. Reciprocal foster-nursings of litters born to infected and non-infected mothers revealed that transfer through the mother's milk was the most effective route of transfer. Results of transmission during the prenatal period

were equivocal. Leukemic lines were established in C3Hf/Lw and C3Hf/Fg strains through inoculation of virus into prospective parents in the neonatal period. The frequencies and ages at death from lymphocytic leukemia through 4 generations of C3Hf/Gs mice revealed no loss of virulence of virus. Apparently, low-leukemic and high-leukemic lines may be established simply through foster-nursing of litters. There was no evidence to support the concept for transmission of virus through germinal cells. The results reported here concerning the pattern for congenital transmission of Moloney virus stand in sharp contrast to the patterns for transfer of potentialities to develop leukemia in the high-leukemic strains of mice, AKR, C58, F and C3Hf/Fg.

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Plaque Formation by Myxovirus Parainfluenza Type 2 in Grivet Monkey Kidney Cells. (27046)

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The plaque technique developed by Dulbecco(1) provides a method for relatively precise analysis of infectivity of virus preparations. This technique also lends itself to isolation of pure lines of virus. In many instances plaque morphology has been used to differentiate between virus strains and types(2,3). Deibel has reported plaque formation with parainfluenza type 3 virus in stable human amnion cells(4). Marston and Vaughan have reported a plaque method for parainfluenza type 3 virus assay wherein the plaques are located by the hemadsorption technique(5). Hsiung has reported that the plaque technique