that AbPB caused greater increases in inferior vena caval pressure than femoral venous pressure thus impairing circulation through the limb. On the other hand, the diaphragm which is displaced headwards prevents a rise in thoracic venous pressure and allows blood to circulate essentially unimpeded.

Summary. Anesthetized dogs were subjected to 3-21 mm Hg CPPB or AbPB. Carotid and femoral arterial blood flow and mean carotid pressure decreased inversely proportional to increasing CPPB while femoral pressure remained essentially unaltered. Thus vascular resistance was significantly elevated only in the femoral circulation. In general, since all levels of AbPB were without effect except for the slight depression in femoral flow, the data suggest that elevations in abdominal pressure which occur during CPPB do not play a significant role in the restoration of circulatory parameters.

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Influence of Foster-Nursing on Virus-Induced and Spontaneous Leukemia in Mice. (27871)

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Preliminary results have been reported of a study of the congenital transmission of the leukemogenic virus of Moloney (MLV) in certain inbred strains of mice(1). Transmission of virus occurred through the maternal line in 3 C3H sublines, 2 low-leukemic (Gs and Lw) and one high-leukemic (Fg). Offspring born to non-infected females mated with MLV-infected males, however, did not develop a higher frequency or earlier disease than controls observed concomitantly. The most effective route of transfer in the maternal line was through the mother's milk. Transmission of MLV during the prenatal period was found to be relatively ineffective.

The present report is an extension of the

transmission experiments, particularly of the effect of foster-nursing on transmission of MLV and the comparative effect of successive foster-nursings, combined with successive thymectomies, on the frequency of spontaneous leukemia in a high-leukemic strain, AKR/Lw.

Materials and methods. The virus preparation used was that designated LT(V)96 and has been described(1). Entire litters of the 3 strains, discussed here, C3Hf/Gs, C3Hf/Lw and C3Hf/Fg (free from MTA) were inoculated subcutaneously with 0.1 ml of a 10^{-1} dilution. A 1.0 g equivalent per ml concentrate of the virus preparation used was equal to 1 ml of final suspension of each gram of pooled leukemic tissue processed. 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. Mice receiving virus were 1 to 13 days of age. Mice remained with their mothers until weaning at 4 weeks of age. These inoculated mice are referred to as the G_0 generation. Successive litters of non-inoculated mice of each of the 3 C3H strains were obtained by matings of sibs. These are referred to as G_1, G_2, etc. These litters were "infected" and obtained virus from their mothers. The frequency of leukemia and mean age at death have been obtained through at least 7 successive generations, *i.e.,* through G_6 for several lines of each of the three C3H strains. Most members of successive litters (G_1 through G_6) obtained from originally virus-inoculated mothers (G_0) became leukemic. High-leukemic lines were established and maintained without apparent loss of virulence of MLV(2). Females from the several generations were used as foster nurses.

The origins and characteristics of the 3 sublines of C3H mice used: C3Hf/Lw, C3Hf/Gs and C3Hf/Fg have been described (1). All mice were from our pedigreed colonies. The frequency of reticular neoplasms, principally lymphocytic leukemia, ranges from 10%-20% in the *Lw* and *Gs* sublines depending upon the samples observed throughout life; whereas, a high frequency and of different morphologic forms of leukemia (50% in males and 80% in females) has been determined, from F1 through F17, in the C3Hf/Fg strain. These neoplasms in the *Fg* subline occur late in life, 14 months average age at leukemic death, and are rarely observed before 7 months in females and 9 months in males. All attempts to isolate and concentrate a cell-free material from leukemic tissues of the *Fg* strain have been unsuccessful(3).

Diagnoses, where possible, were made from histologic preparations. The majority of leukemias observed here were lymphocytic, chiefly of thymic origin, but other forms make their appearance. These are mentioned in the specific experiments.

Foster-nursings were done in a manner to insure that offspring did not obtain their own mother's milk. Litters were allowed to drop through a raised platform constructed of ½″ × ½″ mesh hardware cloth inserted in the bottom of a plastic cage. Many of the deliveries were observed and in no case was there a question of offspring suckling their own mothers. The efficacy of the method has been demonstrated in reciprocal foster-nursing studies demonstrating transfer of antibodies to polyoma virus(4).

Infected mothers used in the reciprocal foster-nursing experiments were obtained from the following sublines: *C3Hf/Lw.* A litter of 3 females (G_0) received MLV at 7 days of age. These were mated with a stock male at sexual maturity. All 3 became leukemic at an average age of 4 months. Six successive generations (G_1-G_6) of untreated descendants have now been obtained with a high frequency of lymphocytic neoplasms(2). *C3Hf/Gs.* A litter of 2 females and 3 males was inoculated subcutaneously with MLV at 13 days of age and all became leukemic at an average age of 5¾ months, the females at 3½ months. Six succeeding generations of untreated offspring (G_1-G_6) have exhibited a high incidence of leukemia, principally lymphocytic. *C3Hf/Fg.* A litter of 4 C3Hf/Fg mice (2♀ and 2♂) received MLV parenterally at 7 days of age and 3 died of lymphocytic neoplasms before 6 months of age. Seven succeeding generations obtained from the original mating of one of the females with a potentially leukemic brother

TABLE I. Leukemia in Reciprocally Foster-Nursed Mice of C3H Sublines.

	Offspring from normal females foster-nursed to infected nurses					Offspring from infected females foster-nursed to normal nurses			
Strain	Litter No.*	Fate† of nurse	No. of offspring fostered	No. leukemic	Avg age at leukemic death, mo (range)	Litter No.	No. of offspring fostered	No. leukemic	Ages at leukemic death, mo
C3Hf/Lw	1	N-12	4	3	7½ (5½-10)	1A	5	1	8
	2	L- 3½	6	6	6 (3½-8)	2B‖	2	0	—
	3	L- 4	5	5	6 (4-9)				
	4	L- 6	3	3	6 (5-6½)				
	5	L- 4	4	3	5 (4½-5½)	5A	3	0	
	6	L- 7	3	3	5½ (4½-6)				
C3Hf/Gs	7	L- 4½‡	8	5	7½ (5½-9)	7A	2	0	
						7B	2	0	
	8	L- 5½‡	6	6	6 (5-7½)	8A	4	0	
	9	L- 7½‡	4	4	5 (3½-7½)	9A	4	2	5, 5
	10	L- 7	4	2	3½ (3-4½)	10A	5	0	—
	11	L- 5½‡	5	4§	8 (5-10)	11A	5§	3	5, 11½, 13
	12	L- 4½	6	3§	7 (5½-9)	12B	4	1	9½
	13	L- 7½	4	4§	7½ (7-8)	13A	3§	0	—
	14	L- 3½	6	6	4½ (3½-6)	14A	4	0	—
	15	L- 6	2	2	8 (5-11)	15B	4	0	—
C3Hf/Fg	16	N-13	5	5§	6½ (5-7)	16A	4§	0	—
	17	L- 4½	8	7§	6 (4½-7)				
	18	L- 5½	8	4§	5 (3½-6)				

Survivors among both groups are presently 15 to 18 mo of age. Those litters born on the same morning or same evening were interchanged. These reciprocally foster-nursed litters are designated in the table as 1 and 1A, 5 and 5A, etc.

* Litters #1, 2, 3, 10 and 18 were foster-nursed upon mothers which received MLV parenterally at birth (G0) and litters #1A and 10A were born to such mothers. All other litters were born to or foster-nursed upon "infected" mothers of the G1 or G2 generations, that is 1 or 2 generations removed from the original virus-inoculated litter.

† L = Foster nurse leukemic; N = Non-leukemic. Number = age at death in months.

‡ "Infected" nurses which fostered litters #7, 8, 9 and 11 (from normal females) and bore litters #7A, 8A, 9A and 11A were leukemic or preleukemic; all others were non-leukemic and signs of leukemia did not develop until 1½ to 5 mo later.

§ C3Hf/Lw litters used to suckle C3Hf/Gs and C3Hf/Fg "infected" nurses and C3Hf/Lw mothers used to foster C3Hf/Gs and C3Hf/Fg litters born to "infected" mothers.

‖ Litter 2B born to "infected" mother which became leukemic at 4 mo; litter 7B born to mother which became leukemic at 8 mo; litter 12B born to "infected" mother which died non-leukemic at 12 mo; litter 15B born to "infected" female which became leukemic at 4 mo.

show a high frequency of lymphocytic leukemia of thymic origin, leukemic deaths occurring before 7 months, the time at which spontaneous neoplasms of reticular origin make their appearance in this subline.

Normal, non-infected mothers and their litters obtained from our pedigreed colonies of the 3 C3H strains and of C57Bl/Ka were used in the reciprocal foster nursings.

Litters born of virus-inoculated (G_0) or of virus-infected (G_1-G_6) mothers (those infected through the maternal line) were foster-nursed on "normal" non-inoculated mothers and in many cases where possible, the reciprocal foster-nursings were done. Entire litters were foster-nursed except in Series II where litter size was purposely reduced.

Results. Series I foster-nursings. The results of the reciprocal foster-nursings, normal litters fostered upon "infected" mothers and litters obtained from "infected" females fostered upon normal mothers, show clearly that the most effective route of transmission was through the mother's milk. Eighty-two per cent of mice (75 of 91) which presumably received virus only through milk developed leukemia. Infected females of all 3 sublines were capable of transmitting virus (Table I). In this series the infected mothers (G_0) and the litters from them used for foster-nursings upon normal females were themselves inoculated with MLV or were one or 2 generations removed from the original virus inoculated litter (G_1 and G_2).

Two of the mothers of the C3Hf/Gs subline, those nursing litters Nos. 9 and 11, had signs of leukemia while nursing and 2 others, those nursing litters Nos. 7 and 8, became leukemic within 2 weeks following nursing. All other infected mothers used as foster-nurses, however, were healthy at the time of nursing and did not present signs of leukemia until 1½ to 5 months later.

Among the leukemias appearing in this group, litters from normal mothers foster-nursed upon "infected" females, all were generalized lymphocytic neoplasms except 3, which were granulocytic; one of these appeared at 9 months in litter No. 7, and 2 at 7 and 7½ months respectively in litter No. 8, all from the C3Hf/Gs subline.

Of the 51 mice from 14 litters born to infected mothers (G_0 to G_2) but foster-nursed upon normal mothers, obtained from the pedigreed colony, only 7 have developed leukemia within a 15- to 18-month period of observation. Interestingly, 5 leukemic mice were among those born to infected mothers which showed signs of leukemia 2 weeks after nursing (see litters 9A and 11A). Two of these, however, at 11½ and 13 months, were generalized granulocytic leukemias and may indeed represent the spontaneous disease. Such neoplasms have been observed among C3Hf/Gs mice in our pedigreed colony and in mice beyond 12 months of age. Another leukemic mouse of the C3H/Lw strain presented a reticulum cell sarcoma, type A at 8 months of age (see litter 1A).

It should be pointed out that although the majority of reticular neoplasms appearing among C3H mice receiving MLV are lymphocytic leukemias, primarily thymic in origin, or thymic lymphosarcomas, they are not exclusively of the lymphocytic form. For example, among 92 C3Hf/Gs leukemics from our high-leukemic lines histologic and hematologic study showed that 9 or approximately 10% were reticulum cell sarcomas, 1 of type A and 4 of type B (see 5), and 4 were granulocytic neoplasms. These other morphologic forms do not necessarily appear in older mice.

Establishment of high- and low-leukemic lines through foster-nursings. Several high-

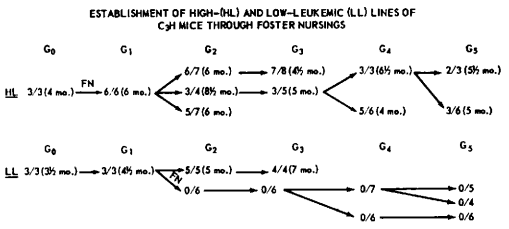

FIG. 1. FN = foster nursing. G_0 litters received MLV parenterally; G_1 through G_5 litters received virus from "infected" mothers or were foster-nursed. Numerator = No. leukemic and denominator = No. in litter. Numbers in parentheses = mean age in mo at death from leukemia. C3Hf/Lw strain mice used in high-leukemic line (HL); C3Hf/Gs mice, in low-leukemic line (LL).

(HL) and low-leukemic (LL) lines have been derived simply by foster-nursings. Two of these are shown in Fig. 1.

A litter of 6 C3Hf/Lw mice born to a non-infected mother from the pedigreed colony was foster-nursed immediately after birth to a C3Hf/Lw mother inoculated at 7 days of age with MLV. The infected mother was 2½ months old at time of fostering. She eventually became leukemic, along with her 2 litter mates, at 4 months of age. All 6 fostered mice (G_1) developed lymphocytic neoplasms at 3½ to 8 months. Three of the 5 females bore litters (G_2) from which subsequent generations were produced. A high frequency and low mean age at death from the disease have been observed through 5 successive generations.

A low-leukemic subline (LL) was established as follows: A litter of 3 C3Hf/Gs strain mice (1♀ and 2♂) received MLV subcutaneously at 8 days of age. All developed lymphocytic leukemia at an average age of 3½ months. Female #40 produced a litter of 3 females and these mated to a stock male produced leukemic offspring. One female #162, prior to developing leukemia at 7 months of age bore 2 litters. The first litter (G_2) suckled infected ♀162 and all 5 developed lymphocytic neoplasms at 3 to 7 months of age. All members of a subsequent litter of 4 (G_3) born to one of these females also became leukemic at 7 months of age. A second litter of 5 mice, born to ♀162, did not receive any milk from their infected mother and were foster-nursed immediately upon a normal non-infected C3Hf/Lw strain mother.

This litter and 5 subsequent litters of C3Hf/Gs mice representing G_2 through G_5 have remained leukemia-free. The observation period is now 14 months for the oldest and 8 months for the youngest litters.

Series II foster-nursings. A second group of foster-nursings was done using C57Bl/Ka strain mothers as nurses. Litters to be foster-nursed were obtained from "infected" females of the 3 C3H strains representing later generations of established high-leukemic lines (G_3 to G_5). Pertinent information concerning each of the mothers providing litters is given under remarks in Table II. Litter size was purposely reduced and litters fostered upon C57Bl/Ka mothers did not obtain milk from their own "infected" mothers. The reciprocal foster-nursings were not done routinely.

No leukemic offspring have been obtained in a 10- to 12-month period of observation among 14 litters, totalling 48 offspring, born to these potentially high-leukemic females but nursed upon C57Bl/Ka mothers. Fifty-three offspring born to some of these foster-nursed C3H litters also have remained free of leukemia.

It is of interest that 2 of the C3H mothers providing litters for foster-nursings were either leukemic or preleukemic at time of delivery of their litters (females 1066 and 986 in Table II). Both presented signs of leukemia within 2 weeks of delivery of their litters.

Two litters (not shown in Table II) born to C3Hf/Gs infected mothers were allowed to remain from 24 to 48 hours with their mothers before transfer to C57Bl/Ka foster nurses. One of 4 and 2 of 8 members of these litters developed leukemia before 8 months of age. It is clear therefore that ingestion of a small amount of milk, obtained from the mother within a 24-48 hour period following birth, is sufficient to transfer effective MLV.

Foster-nursing combined with thymectomy in AKR mice. The transmission of leukemia from generation to generation in certain high-leukemic strains of mice, *viz.*, AKR, C58, F, and C3Hf/Fg, follows the pattern expected from chromosomal transmission(2, 6,7,8). Extra-chromosomal transmission, similar to the Bittner milk agent (MTA) has not been observed in these high-leukemic strains. Numerous attempts to influence the frequency and expression of the disease through foster-nursings upon low-leukemic strains, or reciprocally, of establishing leukemia in low-leukemic strains through foster-nursing upon mothers of high-leukemic strains have not been successful. If a close integration exists between a hypothetical virus and the genetic material of host cells in these long-established high-leukemic strains, it was felt that successive foster-nursings, combined with total thymectomy, could conceivably affect this pattern.

A litter of 7 AKR/Lw strain mice was born to ♀45474 (F51). These mice did not receive their mother's milk and were foster-nursed immediately upon a low-leukemic C3Hf/Lw mother. Thymectomy of the litter was performed at 1 month of age. This litter is referred to as G_1 (F52) in Table III.

The mother of this litter ♀45474 and 3 litter mates developed leukemia at 10, 8½, 9 and 8 months, respectively. One of her sisters provided a litter from which successive generations of AKR mice were obtained, F52 through F58, and which constitute controls.

Successive litters were then obtained in the experimental group, G_2 through G_6, which were similarly foster-nursed and thymectomized.

Sixty AKR mice representing G_1 through G_6, successive generations of foster-nursing and thymectomy failed to develop leukemia within a 12-month period of observation (Table III). Non-fostered and non-thymectomized AKR mice (F56) born to females of the G_4 generation (4 successive foster-nursings and thymectomies) and those born to females of the G_5 and G_6 generations (F57 and F58), however exhibited a frequency of the disease and age at leukemic death similar to that observed in the control series of mice. It is clear therefore that foster-nursing and thymectomy through successive generations did not influence the pattern of transmissibility of the leukemic potentialities in AKR mice.

TABLE II. Influence of Foster-Nursing, with C57Bl/Ka Mothers, on the Frequency of Leukemia in Offspring from Infected Mothers of High-Leukemic C3H Sublines.

Donor mother No.	C3H subline	Generation	Leukemia* in mother (age at death) (mo)	No. in litter fostered	No. leukemic	No. raised in subsequent generations	Remarks
978	Lw	4th†	+, 8	2	0‡	—	2nd litter of 4 born to 978 were all leukemic at 6½ mo. All litter mates of 978 were leukemic.
1065	"	"	+, 7½	3	0	5, 6, and 2 in G5; 5 and 8 in G6	2 of 3 offspring of a prior litter of 1065 leukemic at 5½ mo. All litter mates of 1065 and 1066 leukemic at 6 mo.
1066	"	"	+, 4§	2	0	—	
884	"	3rd	—, 11½	3	0	8 in G4	5 of 9 litter mates of 884 leukemic at 6 mo.
930	"	"	—, 12	3	0	5 in G4 4 in G5	4 of 6 litter mates of 930 leukemic at 6½ mo.
994	"	"	+, 7½	3	0	3 in G4	In prior litter of 994, 6 of 8 leukemic at 5½ mo.
932	"	"	—, 12	4	0	—	4 of 5 litter mates of 932 leukemic at 6 mo.
1101	"	4th	+, 4½	5	0	—	4 of 7 litter mates including 1101 and 1102 leukemic at 6 mo.
1102	"	"	+, 9	6	0		
1139	"	5th	+, 5½	3	0	—	8 offspring of a prior litter leukemic at 5 mo; all litter mates of 1139 leukemic.
547	Gs	3rd	+, 5½	3	0	—	In prior litter of 547, 3 of 4 leukemic at 4½ mo. All litter mates of 547 also leukemic.
986	"	5th	+, 4§	4	0	—	All litter mates of 986 leukemic at 5½ mo; 5 of 5 in 2nd litter of 986 leukemic at 6 mo.
1097	"	"	—, 9	5	0	7 in G6	
1107	Fg	"	+, 4½	2	0	—	4 of 5 litter mates of 1107 leukemic at 5 mo.

* All leukemias were of the lymphocytic form; + = leukemic, — = nonleukemic.

† These donor mothers were from later generations of the "high-leukemic" lines. G4, for example, is 4 generations removed from original (G0) MLV-inoculated litter. The litter provided by this mother is therefore G5.

‡ Litter of 8 C57Bl/Ka strain foster-nursed to 978; 4 were leukemic at 7, 8, 9, and 10 mo respectively.

§ Preleukemic or leukemic at birth of litter; these mothers developed signs of lymphocytic neoplasms 1 and 2 wk later.

Discussion. The results reported earlier (1) and extended here show clearly that maternal transmission of a leukemogenic virus (MLV) through successive generations occurs principally through mother's milk. "Low-leukemic" strains may be transformed to "high-leukemic" strains and *vice versa* simply by foster-nursings. This was shown in the present study using different C3H strains of mice and presumably is true for other strains susceptible to MLV.

Virus-infected C3H females, either those inoculated during the neonatal period (G_0) or receiving MLV from their mothers (G_1 through G_5) transmitted virus to their fostered litters prior to development of leukemia (from 1½ to 5 months prior to developing signs of disease). Two mothers which died non-leukemic at 12 and 13 months transmitted MLV to their fostered litters. It has also been observed that females from "high-leukemic" lines of each of the C3H

TABLE III. Influence of Combined Foster-Nursing and Thymectomy, through Successive Generations, on Spontaneous Leukemia in AKR Mice.

Group	Inbred generation	Exp procedure	No. mice	No. leukemic	Age at death (mo)	Age at leukemic death (mo)
Exp	G1 (F52)	FN* & thymectomy	7	0	12†	—
	G2 (F53)	*Idem*	5	0	12	—
	G3 (F54)	"	4	0	12	—
	G4 (F55)	"	13	0	12	—
	G5 (F56)	"	17	0	12	—
	G5 (F56)	None	8 ♀♀	8	—	10.4
			4 ♂♂	4	—	9.6
	G6 (F57)	FN & thymectomy	14	0	12	—
	G6 (F57)	None	20 ♀♀	19	—	7.8
			9 ♂♂	5	—	9.1
	F58	"	18 ♀♀	16	—	9.1
			6 ♂♂	3	—	10.0
	Totals		46 ♀♀	43 (93.5%)		8.7
			19 ♂♂	12 (70.0%)		9.5
Controls	F52 through F58	None	59 ♀♀	55 (93.2%)		9.1
			26 ♂♂	22 (84.5%)		9.4

* Foster-nursing.
† All foster-nursed and thymectomized survivors were sacrificed, non-leukemic, at 12 mo.

strains are capable of transmitting virus regularly to their offspring whether or not they eventually become leukemic(2). For example, 7 females which eventually died non-leukemic beyond 12 months of age delivered and suckled 11 litters. The majority of the members of 10 of these litters (39 of 50) developed leukemia from 4 to 8 months of age.

The low frequency and sporadic occurrence of leukemia observed in mice born to MLV-infected mothers but foster-nursed by normal C3H females indicates a very inefficient transmission of virus during the prenatal period in all of the C3H strains studied. Complete prevention of leukemia in all 3 C3H strains, provided C57Bl foster-nurses were used, may indeed indicate the presence in C57Bl milk of an "inhibitor." Experiments are in progress to determine this. Preliminary studies of Ida and Moloney(9) and of Gross(10) relating to prenatal transfer of MLV and Passage A virus respectively have been reported indicating efficient prenatal transfer but these studies did not in fact deal with the "natural transmission" of virus. In both, high concentrations of virus were inoculated into the mothers which nursed young; in the latter study it was likely as pointed out that some of the foster-nursed mice received milk from their inoculated mothers since some litters were not removed immediately after birth.

It was not unexpected to find that foster-nursings and thymectomies in successive generations of high-leukemic AKR strain mice were ineffective in altering the potentiality to develop lymphocytic neoplasms. Evidence against an extra-chromosomal transfer of leukemic potentialities has been obtained from reciprocal foster-nursings using several high-leukemic strains(6,7,8,11), transfer of fertilized ova reciprocally between the high- (AKR) and low-leukemic (C3H strains)(12, 13) and the similarity in frequency and age at death from leukemia in F1 hybrid offspring obtained from reciprocal matings of high- and low-leukemic strains(6,7,8,11). The transmission of leukemia from generation to generation in the high-leukemic strains AKR, C58, F and C3Hf/Fg follows the pattern expected from chromosomal transmission. In contrast, the pattern of vertical transmission of MLV and apparently also of Passage A agent(10,14) follows that expected for an extra-chromosomal mechanism.

A recent report of Gross(10) suggests that transmission to C3Hf/Bi offspring of Passage A virus requires the mother to be leukemic or in the preleukemic stages of disease (leu-

kemia appearing 1 to several weeks later) in contrast to what is observed and reported here concerning transmission of MLV. If this is true, it may account for the failures in several laboratories(3,15,16,17) to achieve vertical transmission of Passage A virus. In our laboratory, for example, over 200 untreated C3Hf/Bi mice of the 1st, 2nd and 3rd generations from C3H parents inoculated with a potent leukemogenic Passage A material have been observed throughout a 15-month period. Only 10% have developed reticular neoplasms and the frequency and age at death are similar to controls. All Passage A-inoculated parents, on the other hand, (36 out of 36) developed lymphocytic neoplasms at 3 to 4 months of age.

The striking difference between the pattern of vertical transmission of the leukemogenic viruses, MLV and Passage A virus, on the one hand (extra-chromosomal) and the chromosomal pattern of transmission in high-leukemic strains of mice, needs to be explained in terms of mechanisms. It has been claimed but not substantiated that in high-leukemic strains of mice, and specifically AK and C58(18) " . . . the leukemic virus was therefore found to be transmissible from one generation to another through embryos, like the virus of chicken lymphomatosis"(10). This situation has not been duplicated using several strains of mice, and Passage A virus (isolated from AK leukemia) or MLV. If the genotype of a high-leukemic strain, for example AKR, is necessary for a mechanism of transmission from parent to offspring such as determining the state of the virus-host cell relationship or of allowing close "integration" of virus and genetic material, it should be possible to duplicate such a system through introduction of leukemogenic virus into high-leukemic strains. This appears not to have been accomplished with Passage A virus in AKR mice(17) nor with MLV in high-leukemic C3Hf/Fg mice, as revealed in the present study. An extra-chromosomal pattern of transmission was characteristic of the C3Hf/Fg strain as well as the 2 low-leukemic C3H strains.

It was felt that perhaps a period of "adaptation" of MLV virus to C3H mice was required in order to develop "high-leukemic" strains similar to AKR, in exhibiting a chromosomal-type of transmission of virus. It is clear, however, using litters from "infected" C3H mothers of the 6th generation (G_5) fostered upon C57Bl nurses that transmission after this period of time is still of an extra-chromosomal type (Table III).

The present results certainly tend to rule out a regular persistence and replication of virus in the cytoplasm of the ovum similar to the situation encountered with RIF (chicken lymphomatosis virus)(19) and the type of intimate association between viral genome and chromosomes in lysogenic bacteria which has been suggested as a mechanism for leukemogenesis in the mouse(20,21). In this connection, results of studies designed to test MLV transmission by the male are of interest(1,2). Fifty-six G_1 and G_2 offspring, obtained by crossing each of 3 infected C3Hf/Lw males (which eventually died leukemic at 5 and 6 months of age) with normal, non-infected C3Hf/Lw females showed a frequency of leukemia of 7% with a mean age at death from the disease at 15.5 months. Control C3Hf/Lw offspring (matings of normal $\times$ normal) had a similar frequency and mean age at death (3 leukemias out of 41, and these succumbed from leukemia at 15 months). Thus, there appears to be no effective transmission of MLV through the male line.

Summary. Females of 3 different C3H strains, low-leukemic C3Hf/Lw and C3Hf/Gs and high-leukemic C3Hf/Fg, were found capable of transmitting MLV to successive generations of offspring. The most effective mechanism of transmission was through mother's milk. "Infected" females transmitted MLV whether or not they eventually became leukemic. Reciprocal foster-nursing experiments revealed that transmission of virus could occur from mother to offspring during the prenatal period. A low frequency, later age at death from the disease and sporadic occurrence were observed in litters born to "infected" females but foster-nursed upon normal, non-inoculated mothers of the C3H

strains. In contrast, however, no leukemias have appeared to date in those offspring born to "infected" mothers but foster-nursed upon C57Bl/Ka mothers. Stable high-leukemic and low-leukemic lines of different C3H strains have been established simply through foster-nursings. Foster-nursing upon low-leukemic mothers combined with thymectomy through successive generations of high-leukemic AKR strain mice did not influence the frequency of or mean age at death from lymphocytic neoplasms. The pattern of transmission of leukemic potentialities in AKR, as well as in other established high-leukemic strains, *viz.*, C58, F, and C3Hf/Fg, is of a chromosomal type in contrast to the extra-chromosomal pattern of transmission of MLV and presumably of Gross Passage A virus. Further data are given concerning the inability of "infected" males to transmit MLV.

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Cytotoxicity of Steroids to Mammalian Cells in Tissue Culture. (27872)

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In an earlier report(1) we mentioned that growth of Earle's L cells was markedly inhibited when small amounts of progesterone, testosterone, or androstenedione were added to the medium. Further investigation showed that certain steroids were more toxic than others to this cell line. Our observations on the relationship of steroid structure to cytotoxicity to Earle's L cells (NCTC 929), Gey's adrenal cells (3G29), and Ehrlich ascites cells (Squibb #2) growing in suspension culture are summarized here.

Materials and methods. The 3 cell lines used were maintained in suspension culture using previously described technics(2,3,4). Waymouth's medium MB 752/1(5) supplemented with calf serum (10%, w/v) and 4000 cps Methocel® (0.03%, w/v) (Dow Chemical Co.) was used as basal medium. The media used for the adrenal cells and the Ehrlich ascites cells also contained Darvan® #2 (0.05%, w/v) (R. T. Vanderbilt Co.). Inoculum for the cytotoxicity tests was grown in shaken rubber-stoppered Erlenmeyer flasks placed on a shaker rotating at 150 rpm (1 inch throw) located in a 37°C