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Can LCM Virus Cause Lymphomatosis in Mice?

By

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It was reported in 1941 (17) that lymphomatosis frequently occurs in mice infected *in utero* or neonatally with the virus of lymphocytic choriomeningitis (LCM). Such animals are tolerant towards LCM virus. Their cells do not show a defensive reaction against this agent (22) and specific antibodies are not demonstrable in their blood and organs (24, 7, 19). Consequently, infectious virus persists in the organs of such mice for life and continues to multiply in their susceptible cells at about the same rate as in the embryonic stage (16, 21) without causing symptoms suggestive of a viral infection.

In the experiments to be presented in this paper an attempt was made to study the conditions under which lymphomatosis arises in such animals and to answer the question whether it is caused by the chronic LCM infection or by a latent leukemia agent present in many stocks of white mice. For literature the reader is referred to recent monographs (1, 5, 7a, 14).

Materials and Methods

Mice: The ancestors of our stock III of white mice, from which all animals used in the present study originated, were kindly furnished by the Cancer Research Institute in Philadelphia, Pa., in 1955. As far as we know, the mice of this stock are not inbred. They are suitable for experiments with LCM virus and behave towards this agent much like animals of the Princeton colony from which LCM virus was isolated in 1934. This statement particularly regards the persistence of virus at high titer following intrauterine infection.

In an effort to obtain genetically pure lines of mice, brother-to-sister mating has been practiced for a number of generations (see Table 3). This work was started by the late Dr. *K. Gehring* and continued by Dr. *U. Haakh* and Mrs. *C. Härtkorn*. The breeding animals were selected randomly and not with regard to tumor incidence in close relatives.

LCM-infected mice: Chronic LCM infection with strain W (18) was established in a small subcolony of stock III in 1958 and has persisted with remarkable regularity ever since. The mice of this infected colony have been random-bred, close inbreeding being carefully avoided.

Five lines of inbred mice (Nos. 1, 6, 7, 10, and 11) were derived from this colony by brother-to-sister mating in successive generations. The parents of the F_1 generation were litter mates which, like all animals in the infected stock, had become infected congenitally with LCM virus (18). Lines 1 and 6 originated from successive litters of the same parents. Infectivity tests carried out from time to time with blood from mice of these 5 lines invariably gave positive results. We have not encountered a single litter which did not become infected *in utero*. It is therefore safe to assume that all LCM-infected mice used in the present experiments remained virus carriers for the entire period of observation.

Experimental

Incidence of spontaneous lymphatic leukemia in stock III

Data on the natural incidence of tumors in stock III were obtained from experiments on the mechanism of immunity of mice to EEE (eastern equine encephylitis) virus (25, 23) in which large numbers of immunized

Table 1. Frequency of spontaneous lymphatic leukemia in EEE-immune mice from stock III

Sex	No. of animals observed for life	Cases of lymphatic leukemia or lymphomatosis	Incidence (per cent)	Average tumor age (days)
gonadectomized males	241	66	27.4	545
females	445	70	15.7	478
total	686	136	19.8	510

mice were observed up to the time when they showed diseases of old age menacing their lives. The majority of these diseases were tumorous conditions (25). In order to prevent losses from fighting, the males were gonadectomized at the age of about 4 weeks. This operation is known to effect an increase in the incidence of lymphatic leukemia (10, 11).

The frequency of spontaneous lymphatic leukemia in orchidectomized males and in females is evident from Table 1. The disease occurred more frequently in castrated males than in females with a total incidence of about 20 per cent and an average tumor age of approximately 17 months. It is likely that these figures come close to those for normal mice of this strain, since it is very improbable that the EEE infection influenced the genesis of lymphatic leukemia to any great extent. This is indicated by the fact that the frequency of the disease was about the same in mice immunized with live virus as in those treated with non-infectious formalinized vaccine.

The age distribution of the cases of lymphatic leukemia in either sex is recorded in Table 2 (group 1). Included in this table is a second group of EEE-immune mice observed for a limited period of time only. It can be seen that only one case of the disease occurred in animals less than 200 days old, the bulk of the cases appearing much later. The first

Table 2. Age distribution of cases of lymphatic leukemia and lymphomatosis in EEE-immune mice from stock III

Group No.	Age span (days)	Gonadectomized males		Females	
		No.*	Percentage	No.*	Percentage
1 (observed for life)	0–100	0/241	0	0/445	0
	101–200	0/241	0	0/445	0
	201–300	1/235	0.4	7/434	1.6
	301–400	7/227	3.1	10/402	2.5
	401–500	13/215	6.0	20/356	5.6
	501–600	25/181	13.8	25/267	9.4
	601–900	20/117	17.1	8/104	7.7
2 (not observed for life)	0–100			0/437	0
	101–200			1/437 (192 days)	0.2
	201–300			9/392	2.3
	301–400			3/156	1.9

* Cases of leukemia / mice alive at start of period indicated in column 2.

evidence of the disease was obtained either from enlargement of the palpable peripheral and mesenteric lymph nodes and of the spleen or, more often, from labored respiration as a consequence of thymic lymphosarcomas.

The pathological picture presented by mice on which a diagnosis of lymphatic leukemia or lymphomatosis was made, was not uniform.

The great majority of the animals showed thymic lymphomas and strongly enlarged lymph nodes and spleens, the spleen being purple or bluish in color and of soft consistency. Marked swelling of the liver was common and the kidneys frequently showed lymphomatous areas. The

lymph nodes in the abdominal cavity were almost always involved, the mesenteric nodes frequently reaching enormous dimensions. Occasionally, serous exudation was noted in the thoracic and abdominal cavities. Fully developed cases always showed a leukemic blood picture with moderate to enormous increase of the number of lymphocytes. Some mice presented thymic lymphosarcomas only without marked changes in other organs or in the blood. In general, the disease progressed rather rapidly after it had been recognized and ended fatally in animals which were not killed at an advanced stage. This picture, including the mice showing fast-growing thymic lymphomas only, was designated as type I.

Much less frequently, another picture (type II) was seen, which was characterized by a much lower degree of malignancy. Such mice showed very marked swelling of some peripheral lymph nodes in the absence of striking changes of the thymus, the spleen and the liver. The slight to moderate enlargement of the spleen was obviously due to an increase in size and number of the Malpighian bodies. The consistency of this organ and its color were almost normal, indicating that the spleen pulp was not heavily involved in this process in contrast to type I. The abdominal lymph nodes, especially the mesenteric ones, were only moderately enlarged or not swollen at all, and there usually was no evidence of leukemia. The designation "lymphomatosis" is therefore more appropriate for this condition than that of "lymphatic leukemia". The progress of the disease was slow compared with that in type I mice. The ratio of type I and type II cases was approximately 11 : 1.

In a small number of mice individual organs, such as the spleen or the kidneys, were exclusively involved.

Influence of linebreeding on incidence of spontaneous lymphatic leukemia

Unfortunately, no records are available on the incidence of lymphatic leukemia in LCM-free mice of the early generations of the inbred lines derived from stock III. The cases of the disease observed in some later generations (F_{10} to F_{15}) are listed in Table 3. The males were not gonadectomized in this experiment because they showed no tendency to fight. For special reasons, the period of observation had to be limited to approximately 7 months.

As Table 3 shows, lymphatic leukemia of type I appeared in line F only with an incidence of 13 per cent in generations F_{11} and F_{12}. The 7 cases observed comprised 1 case of highly malignant lymphosarcoma of the thymus without any other gross changes and with normal blood picture. The remaining 6 cases were of type I with lymphocyte counts ranging from 25,000 to 346,000. In addition, 2 questionable early cases of type II were noted among the 19 males of the F_{11} generation. In these two animals, the inguinal and axillary lymph nodes were slightly swollen

Table 3. Incidence of spontaneous lymphatic leukemia in LCM-free inbred lines derived from stock III*

Line	Generation	Males**			Females			Total type I	Average tumor age (days)
		No.	definite cases of type I	? early cases of type II	No.	definite cases of type I	? early cases of type II	(definite cases only)	
A	F_{13}	5	0	0	7	0	2	0	
	F_{14}				2	0	0		
E	F_{13}	12	0	0	2	0	0	0	
	F_{14}	6	0	0	19	0	1		
	F_{15}				6	0	0		
F	F_{10}	3	0	0	6	0	0	7/54 (13 p. c.)	202
	F_{11}	19	2	2	12	2	0		
	F_{12}				14	3	0		
H	F_{11}				3	0	0	0	
	F_{12}				12	0	0		
L	F_{12}	17	0	0	18	0	0	0	
	F_{13}				14	0	0		
	F_{14}				1	0	0		
M	F_{11}	10	0	0	14	0	0	0	
	F_{12}				8	0	0		

* Mice observed for about 7 months. ** Not gonadectomized.

(lentil size). Other questionable early cases of type II were found in lines A (2 females) and E (1 female). In view of their doubtful significance, they were not counted as positive.

There can be little doubt, however, about the significance of the leukemia incidence in line F if one considers the age distribution of spontaneous cases in non-inbred mice as recorded in Table 2. It appears as if inbreeding had at least reduced the average tumor age.

Attempt at cell-free transmission of leukemia from line F to non-inbred newborn litters of stock III

In an experiment designed to transmit the leukemia virus (3) presumably present in mice of line F to newborn normal litters of stock III, 10 litters, less than 16 hours old, were inoculated intranasally and intracerebrally with organ extract from a line F mouse with severe lymphatic

Table 4. Inoculation of newborn litters from stock III with organ extract from a leukemic mouse of line F (Table III)

Litter No.	No. and sex of newborn mice inoculated		Incidence of lymphatic leukemia*				Age at which type I cases were recognized (days)
			Type I		Questionable early cases of type II		
	M**	F	M	F	M	F	
1	1	2	0	0	0	0	
2	1	4	0	0	1	0	
3	2	4	0	0	0	0	
4	1	3	0	2	0	0	151; 162
5	2	5	1	2	0	0	107; 88; 88
6	3	5	0	0	0	0	
7	2	5	0	0	1	0	
8	1	6	0	0	0	0	
9	3	5	0	0	0	0	
10	3	4	1	0	0	0	88

* Time of observation: 5½ months.
** Males gonadectomized at age of 4 weeks.

leukemia. The extract was prepared from the deep-frozen spleen, liver, lymph nodes and brain and cleared by centrifugation. The suckling mice were inoculated in ether anesthesia and the males orchidectomized at the age of 4 weeks. The period of observation was 5½ months.

As can be seen in Table 4, six mice originating from 3 different litters (Nos. 4, 5 and 10) showed type I leukemia at the age of 88 to 162 days. In 5 animals the disease progressed rapidly. They were sacrificed when showing pumping respiration and other severe symptoms and presented the usual marked lesions with lymphocyte counts ranging from 30,800

to 89,600. The sixth animal was sacrificed at the beginning stage of the disease, when it showed slightly labored respiration but no enlargement of the palpable lymph nodes. An extensive thymic lymphosarcoma, moderate enlargement of the spleen, which had the usual purple color and soft consistency, and slight swelling of the mesenteric lymph nodes were noted at autopsy. The white cell count was 30,800, the cells consisting of lymphocytes and smudge cells exclusively. This case, in combination with other ones not recorded here, suggests that a tumorous change of the thymus often, but not always, initiates the type I form of lymphatic leukemia and supports the observation of other investigators that the thymus plays an important part in the pathogenesis of this disease (9, 5).

Again, 2 mice from 2 other litters (Nos. 2 and 7) showed slight enlargement of some peripheral lymph nodes in the absence of any other gross changes.

Even though the incidence of leukemia in the inoculated mice was as low as 10 per cent, the result appears significant in view of the difficulties encountered by others in the cell-free transmission of the disease from spontaneous cases (5). The number of positive cases might have increased if the animals had been observed for a longer period of time.

There is a slight possibility, however, that the inoculation of newborn mice merely effected an acceleration of the onset of the disease in mice which otherwise would have developed leukemia much later. This alternative has to be considered in view of the findings of *Rudali, Duplan* and *Latarjet* (12, 13), who observed such an acceleration in newborn Ak mice injected with cell-free tumor extracts from Ak donors.

Whatever the correct explanation may be, the presence of a leukemia virus in stock III can hardly be doubted if one considers the results of more recent etiological studies on mouse leukemia, particularly those of *Gross* (5). From a pathological point of view, the lymphatic leukemia occurring in stock III is strikingly similar to that in Ak mice, for which the viral etiology has been proved (5).

Incidence of lymphomatosis in inbred lines of mice derived from the LCM-infected colony

Cases of lymphomatosis have not been observed in the small LCM-infected colony ever since its establishment in 1958. This colony rarely comprised more than 40 breeding mice and their progeny, which was weaned at the age of 3 to 4 weeks. As a rule, 4 females and 1 male were mated in one cage and the young left in the cage up to the time of weaning. Fertility was high in spite of the chronic LCM infection. Breeders were replaced by young animals taken from different breeding cages when they had reached the age of 5 to 6 months. Occasionally, breeding animals were kept for longer periods in order to have old tolerant mice available

Table 5. Incidence of lymphomatosis (type II) in LCM-infected inbred mice of lines 1, 6, 7, and 10

Line	Generation	No. of mice and sex*	Cases of lymphomatosis***				Tumor age (days) (definite cases only)
			Stage of disease recorded at autopsy				
			Advanced	Medium	Early		
					Definite	Suggestive	
1	F_3	1 F	0	0	0	0	
	F_4	5 F	0	0	0	0	
	F_6	1 M, 4 F	0	0	0	0	
	F_7	1 M, 5 F	0	0	0	0	
	F_8	2 M	0	0	0	0	
6	F_4	5 F	0	0	0	0	
	F_5	7 M, 8 F	0	0	0	0	
	F_6	2 M	0	0	0	0	
		9 F	0	0	1**	2	182
	F_7	9 M	0	0	0	0	
		6 F	0	0	0	1	
	F_8	5 M	0	0	1	0	176
		6 F	0	0	2	1	164; 164
7	F_4	9 F	0	0	0	0	
	F_5	1 F	0	0	0	0	
10	F_3	7 F	0	0	0	0	
	F_4	6 F	0	0	0	0	
	F_5	6 F	0	0	1	1	182
	F_6	1 M	0	0	0	0	
		5 F	0	0	1	1	182
	F_7	9 M	0	0	0	1	
		13 F	0	0	0	0	
	F_8	4 M, 3 F	0	0	0	0	

* Males not gonadectomized. ** Thymic lymphosarcoma. *** Time of observation: 6 months.

Table 6. Incidence of lymphomatosis (type II) in LCM-infected inbred mice of line 11

Generation	No. of mice and sex*	Cases of lymphomatosis**				Percentage	Average tumor age (days)
		Stage of disease recorded at autopsy					
		Advanced	Medium	Early			
				Definite	Suggestive	(Definite cases only)	
F_2	1 F	1	0	0	0		184
F_3	16 F	10	4	0	0	87	142
F_4	3 M	0	0	2	0	67	182
	12 F	2	3	4	0	75	159
F_5	37 M	0	1	8	1	24	150
	28 F	1	15	7	1	82	136
F_6	32 M	0	1	2	2	9	184
	28 F	3	3	11	3	61	161
F_7	56 M	0	2	11	7	23	163
	46 F	1	18	6	2	54	138
F_8	26 M	0	0	4	1	15	150
	20 F	1	6	6	1	65	130

		Males	Females
F_5-F_8	Total incidence	28/151 — 18.5 p. c.	78/122 — 63.9 p. c.
	Average tumor age	159 days	141 days

* Males not gonadectomized. ** Time of observation: 6 months.

for experimental purposes. Lymphomatosis did not appear in such animals either.

The picture changed when inbreeding by brother-to-sister mating was practiced. Of the five inbred lines mentioned above, 4 lines (Nos. 1, 6, 7, and 10) remained free from lymphomatosis for at least 4 successive generations, that is, the disease did not become evident during an observation period of 6 months. In later generations, few cases of the disease with a low degree of malignancy appeared after relatively long incubation periods (see Table 5).

The situation was quite different with line 11 (see Table 6), in which a female of the F_2 generation (mother of the F_3 generation) showed signs of lymphomatosis at the age of 6 months. The condition was recorded as a severe case of type II at autopsy. Since such rapid appearance of the disease as a consequence of linebreeding was not anticipated, the animals of the F_1 and F_2 generations were not kept for observation. It is therefore not possible to determine in which generation the change occurred. Of 16 females of the F_3 generation 14 developed rather severe lymphomatosis of type II. The average tumor age was 142 days. The incidence among males and females in the F_4 to F_8 generations is registered in Table 6. In general, the disease was considerably more frequent in females than in non-gonadectomized males. The same is true for mice infected with lymphatic leukemia (5).

Malignancy was relatively high in the F_3 generation. Of the 14 females which developed lymphomatosis, 5 animals succumbed to the disease 10 to 27 days after its discovery. Others would have died if they had not been sacrificed for tissue. Nevertheless, the autopsy picture was different from that of the highly malignant cases of type I. Unfortunately, it has not been possible to make a thorough histological investigation of the lesions and their genesis, which might have led to a clearer distinction of the fundamental changes present in malignant cases of types I and II. The malignancy of the disease decreased considerably in generations F_4 and F_5 and then appeared to remain at a constant level.

The dominant lesion of the disease, as it presented itself in generations F_5 to F_8 of line 11, was moderate to extremely strong swelling of all or some peripheral lymph nodes and moderate to marked enlargement of the spleen. Sometimes, the lymph node swelling was one-sided concerning, for instance, the left axillary, subscapular and inguinal lymph nodes. In several cases, one submaxillary or one inguinal lymph node only was enlarged to the size of a large pea or a bean. It is not known why the lymph nodes in the thoracic and abdominal cavities were only rarely involved. The same applies to the thymus and the liver. In the kidneys, however, grayish areas consisting of lymphomatous tissue were occasionally seen. Leukemic blood pictures were rare. It is therefore appropriate to speak of lymphomatosis rather than lymphatic leukemia.

As column 6 in Table 6 shows, there were 18 suggestive cases of beginning lymphomatosis. Since such mice could not be reliably differentiated from those showing slight but non-malignant swelling of the lymph nodes and spleen as a consequence of chronic LCM infection, they were not counted as positive. It is quite likely, however, that a high percentage of them would have become positive if they had been observed for a longer period of time. The animals listed as "definite" early cases in column 5

showed lymph node swelling to such a degree as it had never been observed in LCM-infected tolerant mice without lymphomatosis.

Females of line 11 which had themselves not developed lymphomatosis up to the age of 6 months nevertheless transmitted the disease to their offspring. The incidence of lymphomatosis was 9 per cent (4/36) in their male descendants and 61 per cent (17/28) in the females. The corresponding figures for comparable mothers which later did show lymphomatosis were 24 per cent (14/59) and 64 per cent (27/42), respectively. The different frequency in male descendants from positive and negative mothers does not appear significant, however, because similar differences were observed in males descending from mixed batches of females in different generations (compare percentages for males from generations F_5 to F_8 in column 7 of Table 6).

Attempt to transmit a lymphomatosis agent from line 11 to newborn litters from lines 1, 6, and 10 and from the infected colony

The high frequency of lymphomatosis in line 11 raised the question of whether LCM virus was the cause of this condition or whether it was brought about by a leukemia virus present in line 11 only and provoked to high activity by linebreeding. An attempt was therefore made to infect newborn litters from lines 1, 6, and 10 and from the infected stock, in which the incidence of lymphomatosis with a short incubation period was very low or zero, with organ extracts from relatively malignant cases of lymphomatosis in line 11.

Twenty per cent extracts in Pyl's isotonic buffer (8.28 g NaCl and 1.187 g $Na_2HPO_4 \cdot 2\,H_2O$ in 1000 ml H_2O) were prepared as needed from the deep-frozen spleens, livers, lymph nodes and brains of positive mice of line 11 and cleared by centrifugation. The cleared extracts were shown to contain active LCM virus by intracerebral inoculation of mice.

The extracts were injected intracerebrally and intranasally and, sometimes, intraperitoneally into suckling mice less than 16 hours old from the sources indicated in Table 7, column 1. Newborn mice were used because *Gross* (2) had found them to be more susceptible to infection with his leukemia virus than older mice. Before inoculation, the litters were divided into two halves. One half was inoculated and left with the mother. The other half was not injected and transferred to a foster mother from stock No. II of the Institute, in which lymphatic leukemia had not been observed among the breeding animals in the course of more than 5 years. The foster mothers invariably became infected with LCM virus by contact with the infected baby mice as shown by the cerebral immunity which they all acquired. In order to lower the resistance to lymphomatosis, all males were gonadectomized when they had reached the age of 4 weeks. The animals were observed for 6 months.

The details of the experiment are given in Table 7 which shows that the result was essentially negative. There is no evidence suggesting that a lymphomatosis agent was transmitted by the inoculations to the

suckling mice which, as expected, did not react to LCM virus on account of their very solid interference immunity (21). A few cases of lymphomatosis occurred with about equal frequency among the inoculated mice and the controls. They were to be anticipated in view of the fact that the disease had occasionally appeared in animals from later genera-

Table 7. Inoculation of newborn litters from lines 1, 6, and 10 and from infected stock III with organ extracts from mice of line 11 showing severe lymphomatosis

Litters*			Incidence of lymphomatosis**					
			Inoculated mice			Foster-nursed controls		
Line	Generation	No.	No. and sex	Definite cases	Tumor age (days)	No. and sex	Definite cases	Tumor age (days)
1	F_7 and F_8	4	10 M	0		6 M	0	
			10 F	0 (1 ?)		8 F	1 thymic. sarc. 1 of type II	114 175
6	dtto.	5	10 M	2 of type II	166; 169	7 M	0 (1 ?)	
			10 F	0		11 F	1 of type II	166
10	dtto.	6	9 M	0		10 M	0	
			15 F	0		12 F	0	
stock III	?	4	6 M	0		7 M	0 (1 ?)	
			9 F	0 (1 ?)		8 F	0	

* Males gonadectomized at age of 4 weeks.

** Time of observation: 6 months.

tions of lines 6 (closely related genetically to line 1) and 10 (see Table 5). The same applies to the few questionable cases of lymphomatosis recorded. A single case of thymic lymphosarcoma was found among the controls from line 1.

Discussion

Theoretically, it would not surprise for the following reasons if LCM virus actually did cause lymphomatosis in naturally infected mice (virus carriers):

a) the virus is acquired very early in life (16, 6, 18) and is not destructive for embryonic cells (8, 22) or lymph node cells from adult mice (22);

b) the infection persists for life because the virus is tolerated by the infected cells (22) and specific antibodies are not formed (for lit. see 20);

c) LCM virus appears to promote the growth of lymph node cells from tolerant mice in tissue cultures (22);

d) the virus likewise has a stimulating effect on lymphatic tissue *in vivo*, which is most marked in the lymph nodes following subcutaneous infection of adult mice (22) and in the spleen following intravenous infection (15);

e) a blood picture reminding of lymphatic leukemia is not infrequently seen in mice infected intravenously with LCM virus (15);

f) there is evidence in favour of the transmission of LCM virus from generation to generation by way of the female genital cells (18).

It was pointed out previously (17) that, in order to cause malignant growth of lymphoid cells, the virus appeared to require an accessory factor. It did not seem probable at that time that a hereditary factor was involved because the observations were made on random-bred mice. However, the experiments described above leave no doubt that linebreeding is of great importance (for lit. on genetic studies see 5). LCM virus was transmitted from generation to generation with equal regularity in lines 1, 6, 7, 10, and 11, but a high incidence of lymphomatosis was recorded in line 11 only.

A similar result was obtained from linebreeding with mice from the LCM-free stock in which lymphatic leukemia, closely resembling that in AkR mice, is endemic. Cases of lymphatic leukemia with a short incubation period were recorded in line F but not in lines A, E, H, L, and M. It is therefore obvious that the transmission from generation to generation of lymphomatosis in LCM-infected mice and that of lymphatic leukemia in LCM-free mice follow similar patterns.

This raises the question of whether or not both conditions may have been caused by the leukemia agent (3) undoubtedly present in stock III. The following facts, however, favor the interpretation that the chronic LCM infection was responsible for the appearance of lymphomatosis in line 11:

a) as outlined above, the two conditions were different anatomically and hematologically;

b) the incidence of lymphomatosis in line 11 up to the age of 6 months was much higher than that of lymphatic leukemia in line F or in our inbred AkR mice;

c) it has not been possible to transmit lymphomatosis from line 11 to mice of lines 1, 6, and 10 or to non-inbred animals from the infected stock, all possessing a solid interference immunity to LCM virus.

The agent causing lymphatic leukemia in stock III may be transmitted from generation to generation in the same manner as LCM virus. According to a recent preliminary communication by *Gross* (5, p. 200), his "passage A" leukemia virus is transmitted "vertically" to the offspring by the females, the males playing a minor role, if any. The mode of transmission of the agent thus appears to be similar to that reported before for murine LCM (18). There is indirect evidence suggesting that LCM virus passes from one generation to another by way of the oval cells and that spermatozoa are unimportant in this respect despite the fact that they are in an infectious environment in the male as well as in the female prior to conception.

No matter which etiology one assumes for the lymphomatosis occurring in line 11, the question remains to be answered why linebreeding had a different effect on the tumor frequency in different lines of mice. If one considers the LCM-free lines of inbred mice only (Table 3), the possibility exists that the ancestors of line F were the only ones infected with the leukemia agent. This would correspond to a leukemia incidence of about 17 per cent, which compares favorably with that of 20 per cent observed in non-inbred animals of stock III. However, the findings of other investigators indicate that, in a strain of mice with a low incidence of leukemia, the percentage of infected animals is much greater than that of the mice actually developing the disease, in other words, that there are many cases of latent infection with the respective oncogenic virus. It was shown that X-irradiation may increase the leukemia incidence in such mice from less than 0,5 per cent to 60 or even 90 per cent (lit. cited by *Gross*, 5). There is good evidence that a latent leukemia virus is activated by the ionizing irradiation (9, 5). It may be assumed that similar conditions were prevailing in stock III and that the infection with the leukemia virus was not confined to the ancestors of line F among the animals selected for linebreeding.

With regard to line 11, the possibility has been considered that the formation of lymphomas in a single or several lymph nodes might be a local phenomenon resulting from an interaction between LCM virus and the cells in which it persists and continuously multiplies, possibly, the primitive reticular cells and their descendants (22). This, however, would not explain the hereditary character of the condition. It is more likely that the change occurred in the oval cells, the mutation being due to an effect of the virus upon the locus controlling the growth of lymphatic tissue. There may be some sort of biochemical linkage between this locus, the stem cells of the lymphatic system and the virus under study. The same may apply to the virus causing lymphatic leukemia.

The agents causing spontaneous leukemia in lines F and 11 differ from *Gross'* leukemia virus in one respect: they did not hemagglutinate mouse erythrocytes when tested according to the method used by *Gross* (4).

Summary

Extending earlier observations concerning the occurrence of lymphomatosis in murine carriers of LCM virus [Zentralbl. Bakt. I Orig. 147, 16 (1941)], the effect of linebreeding on the frequency of this disease in LCM-free and LCM-infected mice, both originating from the same stock, was studied. The incidence of spontaneous lymphatic leukemia in this colony amounted to 20 per cent with an average tumor age of 17 months.

In the LCM-free mice, linebreeding by brother-to-sister mating in successive generations resulted in a low incidence of lymphatic leukemia with relatively short incubation periods in 1 out of 6 lines (line F).

The same procedure applied to LCM-infected (tolerant) mice caused a much higher incidence of lymphomatosis with short incubation periods in 1 out of 5 lines (line 11).

The disease in line F corresponded to the classical type of murine lymphatic leukemia as seen, for instance, in AkR mice, while that in line 11 was characterized by a much lower degree of malignancy and designated as lymphomatosis because of the fact that lymphomatous swelling of peripheral lymph nodes was the outstanding feature.

Arguments in favor of the possibility that LCM virus was the cause of lymphomatosis in line 11 are presented and the genesis of the tumorous conditions in lines F and 11 discussed.

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