Serial Cell-Free Passage in Rats of the Mouse Leukemia Virus. Effect of Thymectomy.* (28216)

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The mouse leukemia virus(1) isolated from spontaneous Ak mouse leukemia and then passed by serial filtrate inoculations ("passage A") through newborn C3H mice(2) is also pathogenic for rats. When inoculated into newborn or suckling rats of the Sprague-Dawley or Osborne-Mendel strains, it induces a high incidence of leukemia(3).

This report deals with results of serial cell-free passage of this virus through suckling rats, the forms of leukemia induced with the mouse leukemia virus in the rat, and the effect of thymectomy on susceptibility of the rat to the virus.

Materials and methods. Animals. From a nucleus of randomly bred Sprague-Dawley rats received in June, 1960, from the Animal Production Unit, Nat. Inst. of Health, Bethesda, Md., a small colony of rats has been raised in our laboratory by brother-to-sister mating(3). In addition, from 2 litters of rats of the Osborne-Mendel strain obtained from the same source in February, 1961, a separate colony of rats has been also raised in our laboratory by brother-to-sister mating.

C3H(f) mice used in experiments reported here were of the Bittner subline of strain C3H, free from the mammary carcinoma agent by foster nursing, raised in our laboratory by brother-to-sister mating from a nucleus originally obtained in 1944 from Dr. John J. Bittner.

Source of leukemic virus, and preparation of filtrates. The mouse leukemia virus isolated from spontaneous Ak mouse leukemia and then passed serially, by filtrate inoculation, through newborn C3H mice(2) was used. Serial passages 29 through 32 harvested from pooled thymic tumors and spleens from several C3H(f) mice with passage-A-virus-induced leukemia, were used as the initial source of the virus. In the subsequent experiments, leukemic rats with virus-induced leukemia were used for preparation of extracts; usually one, occasionally 2, leukemic rats were donors of pooled tissues from which the extracts were prepared in the usual manner(2). Fragments of tissues were removed aseptically from thymic and mesenteric tumors and from spleens of leukemic rat donors; the fragments were weighed, cut with scissors, and ground by hand in a mortar with sufficient quantity of chilled physiological saline solution to obtain a 20% concentration. Following centrifugation at 0°C at 3,000 rpm for 15 minutes, then at 9,500 rpm for 5 minutes, the second supernate was passed through Selas, porosity 02, filter candles, and immediately used for inoculation of 1 to 5 day old rats of either the Sprague-Dawley (S-D), or less frequently of the Osborne-Mendel (O-M) strain. All rats employed in our experiments were of Sprague-Dawley strain unless otherwise specified. In some experiments 1 to 5 day old C3H(f) mice were also inoculated. Filtrates were inoculated intraperitoneally, 0.5 ml each for rats, and 0.3 ml each for mice. Animals were weaned when 3 weeks old, marked individually, separated by sexes, and kept for observation.

We have observed thus far only a limited number of untreated rats of either of the strains. We have noticed an occasional development of spontaneous mammary carcinoma in old female rats, but not spontaneous development of either leukemia or lymphosarcomas. Two out of 27 breeding Sprague-Dawley female rats, observed for periods of time exceeding 7 months, developed spontaneous mammary carcinomas; none developed leukemia. In another group of rats consisting of 28 virgin females and 8 males of the Sprague-Dawley strain, otherwise not treated, but born to virus-injected parents,

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TABLE I. Serial Passage of the Leukemic Virus in Rats.

Serial passage in rats No.*	Strain of rats inoc.†	No. of rats inoc.	No. dev. leuk.	Leuk. inc. (%)	Avg age leuk. dev. (mo)
1	S–D	93	52	56	4.2
	O–M	18	16	89	3
2	S–D	72	67	93	3.3
	O–M	6	6	100	3.1
3–4	S–D	22	22	100	2.5
	O–M	37	37	100	2.4
5–6	S–D	57	56	98	2.8
	O–M	3	3	100	2.7
7–8	S–D	79	79	100	3.1
	O–M	—	—	—	—

* Virus harvested from C3H(f) mice with passage A virus-induced leukemia. First passage, mouse-to-rat; all subsequent passages, rat-to-rat. Figure in this column indicates serial number of successive passages in rats.

† Rats of either Sprague-Dawley (S–D), or Osborne-Mendel (O–M) strains, inoculated (0.5 ml i.p. each) when 1 to 5 days old.

4 female rats developed mammary carcinomas at ages varying from 13 to 16 months; none developed leukemia; they were observed until they reached 16 months of age.

Our experience with non-treated rats of the Osborne-Mendel strain has been limited to a small group of animals observed for periods of time varying from 5 to 10 months. Out of 13 breeding females and 13 males of that strain, one female developed mammary carcinoma at the age of 3½ months. None developed leukemia.

Experimental. Serial passage of leukemic virus from rat to rat. In the first series of experiments, filtrates prepared from leukemic C3H(f) mouse donors were inoculated into newborn Sprague-Dawley or Osborne-Mendel rats; 56 to 89% of these animals developed leukemia (Table I). The leukemic virus could be readily recovered from leukemic rat donors, and passed serially, by filtrate inoculations, into newborn rats. In each successive passage, filtrate was prepared from leukemic rat donors of the preceding virus passage. After 2 successive passages through rats, the virus induced leukemia in practically all inoculated animals after a relatively short latency of less than 3 months (Table I). At the time of writing the virus is in its 9th and 10th serial rat passage.

Induction of leukemia in mice with passage A virus, following its serial transfer through rats. The virus retained its pathogenic potency for mice after several serial rat passages. Even after 3 to 7 serial passages through rats, virus harvested from leukemic rat donors and inoculated into newborn C3H(f) mice induced an incidence of leukemia exceeding 95% (Table II).

Symptoms and signs of leukemia developing in rats following inoculation of the passage A virus. Early recognition of development of leukemia in inoculated rats was not always easy. Examination of peripheral (tail) blood was diagnostic for leukemia in only 52 out of 96 leukemic rats examined (54%). Quite frequently leukemic animals developed only large thymic and possibly also other visceral tumors, but did not necessarily have significant changes in their peripheral blood morphology. Gradually we have learned to suspect, and in more advanced cases to recognize, leukemia in rats on the basis of certain clinical features. Among the more characteristic signs of disease was enlargement of spleen which could be detected by gentle palpation. Labored breathing was indicative of a mediastinal, *i.e.*, thymic tumor. In certain advanced cases characteristic pin-point haemorrhages were present, particularly apparent around the eyelids. Quite frequently tails of leukemic rats were relatively cold. This curious, but not too reliable, feature was probably due to circulatory difficulty. Gradually, leukemic rats developed muscular weakness, and in some instances, paralysis of one or two limbs; the hind limbs appeared more frequently involved. In more advanced phases there was a marked loss of weight. Ruffled

TABLE II. Induction of Leukemia in Mice* with Passage A Virus Following Its Serial Transfer Through Rats.

Passage through rats	No. of mice inoc.	No. dev. leuk.	Leuk. inc. (%)	Avg age leuk. dev. (mo)
1†	69	60	87	4.9
2‡	21	20	95	3.3
3§	32	31	97	3.6

* C3H(f) mice inoculated when 1 to 5 days old.

† Mouse × rat × mouse.

‡ Mouse × rat × rat × mouse.

§ Virus passed through 3 to 7 serial passages in rats, then inoculated into mice.

fur in the leukemic rats was also quite characteristic, and contrasted sharply with the smooth, shiny fur of healthy animals. We have not observed in leukemic rats significantly enlarged inguinal or axillary lymph nodes.

There was as a rule no difficulty in recognizing leukemia when the rat died or was sacrificed. Most of the leukemic rats had large thymic tumors; in some animals disease was limited to that organ. Most of leukemic rats had also large spleens and livers, and large mesenteric tumors. In a few animals the spleens, in some instances the livers, were unusually large, occupying a considerable part of the abdominal cavity. Enlarged lymph nodes were often found in the submaxillary region. In some leukemic rats we observed in the subcutaneous tissue, particularly in sternal, axillary, abdominal or dorsal area, gelatinous-like soft swellings, which on microscopic examination had the structure of a myxoma.

In some instances only, and then with considerable reservation, was it possible to suspect the probable form of leukemia by macroscopic examination. Presence of a very large thymic tumor with concurrent absence of any other significant pathology, was suggestive of aleukemic lymphatic disease; such rats usually had no enlargement of spleen or liver. Subcutaneous petechial haemorrhages accompanying generalized leukemia were frequently indicative of an acute stem-cell form of the disease. However, stem-cell leukemia was also observed without concurrent haemorrhagic manifestations. Generalized lymphatic and myelogenous leukemia frequently presented a similar picture to the unarmed eye; only microscopic examination of blood, bone marrow, and tissue sections revealed the specific form of the disease. Bone marrow was involved in most, but not in all, cases. Some rats in which disease was limited to a large thymic tumor had an apparently normal bone marrow; these, however, presented a relatively small minority.

All forms of leukemia observed were progressive. From the time leukemia was recognized in such animals, without exception they died within one to 2 weeks.

Forms of leukemia induced in rats with passage A virus. Most inoculated rats developed either lymphatic or stem-cell leukemia; a few animals developed the myelogenous form. Most leukemic rats had a rapidly progressing anemia; the hemoglobin level was in some animals exceedingly low, in a few instances below 3 g per 100 ml of blood. It was our impression that the number of platelets was not appreciably diminished. Microscopic examination revealed in spleens, livers and kidneys of some animals an intense extramedullary hematopoiesis. Lymphosarcomas were very common. They could be readily recognized on microscopic examination in the spleens, livers, kidneys and other organs infiltrated with leukemic cells. Reticulum-cell sarcomas were also found, though less frequently, in lymph nodes and spleens, occasionally in livers. Of particular interest were Hodgkin's-like lesions found in lymph nodes and spleens of some animals. These lesions, similar to those described in mice by Dunn (4), consisted of foci of non-homogenous infiltrates containing lymphocytes, histiocytes, plasma-cells, eosinophils and multi-nucleated giant cells.†

In a group of 44 leukemic rats examined, 20 (45%) developed lymphatic leukemia; of these, 15 had the aleukemic form. Twenty-one rats (48%) developed stem-cell leukemia and 3 (7%) developed myelogenous leukemia (Table III). In this group peripheral white cell blood counts ranged from 5,800 to 1,456,000 per cu mm; in 12 rats the peripheral WBC exceeded 100,000 cu mm. Most leukemic rats in this group had lymphosarcomas in lymph nodes, spleen, liver and other organs, one had a reticulum-cell sarcoma, and one had a Hodgkin's-like lesion. Two leukemic rats had extensive, gelatinous-like subcutaneous swellings which had the microscopic morphology of myxomas.

Effect of thymectomy on susceptibility of rats to the leukemic virus. In this series of experiments, newborn or suckling, 1 to 3 days old, Sprague-Dawley rats were inocu-

† We are very much indebted to Dr. T. Ehrenrich, consultant in pathology, who kindly reviewed microscopic slides prepared from blood, bone marrow, and different organs of leukemic rats and mice.

TABLE III. Forms of Leukemia Developing in Rats Following Inoculation of Passage A Virus.*

No. of leuk. rats exam.	No. dev. lymphat. leuk.	Lymphat. leuk. inc. (%)	No. dev. myeloid leuk.	Myeloid leuk. inc. (%)	No. dev. stem-cell leuk.	Stem-cell leuk. inc. (%)
44	20	45	3†	7	21‡	48

* Virus employed for inoculation harvested from leukemic mice.
† One rat also had a reticulum-cell sarcoma in spleen.
‡ Two rats had a shift toward myeloid form, and one had a Hodgkin's-like lesion in the thymic tumor.

lated intraperitoneally with passage A virus harvested from leukemic rat donors. About one week after inoculation, each litter was split; approximately one-half of the litter received no further treatment, serving as a control group. Rats of the other half of the litter were thymectomized when approximately 10 days old.

Thymectomy(5) was performed aseptically under ether anesthesia. After skin incision, the upper part of sternum was bluntly exposed, and the upper part of the manubrium sterni split longitudinally (about 3 to 4 mm) with fine scissors, exactly in the mid line. The fascia covering mediastinum was then exposed, and gently lifted with an ophthalmic forceps; a small opening was made in the fascia to visualize the thymus. Both thymic lobes were then removed by vacuum suction, using a small glass tubing bent at right angle, and an electric suction pump. The wound was closed by 2 or 3 interrupted silk sutures involving only the skin. Immediate postoperative mortality did not exceed 10%. On autopsy of the leukemic animals in the thymectomized group, 12 rats were found to have moderately large thymic tumors. This suggested that their thymic lobes had not been completely extirpated. These rats were not included in tabulation of final results. In 13 other leukemic rats in this group, small tumor nodules could be found in the mediastinal cavity where the thymus lobes had been removed. It was difficult to determine whether these small tumor nodules developed from fragments of thymic lobes. Even though thymectomy was not considered entirely satisfactory in these animals, they were included in tabulation of data referring to thymectomized animals (Table IV).

Of 55 rats, 51 (93%) developed leukemia at an average age of 4 months. In the littermate control group, 71 rats inoculated with the passage A virus, but not thymectomized, all died from leukemia at an average age of 2.8 months (Table IV). Among these animals 6 had extensive myxomatous infiltration of the subcutaneous tissues and adjoining muscles, particularly around the neck, sternum and abdomen.

The forms of induced leukemia were studied in all rats in this experiment. A few of the leukemic rats died without previous blood studies; in such cases study of the form of leukemia was limited to microscopic sections of tissues. In Table V only those animals are included in which morphology of peripheral blood was studied; in practically

TABLE IV. Effect of Thymectomy on Susceptibility of Rats to Leukemogenic Action of Passage A Virus.*

	Thymectomized rats				Litter-mate controls			
Sex	No. of rats inoc.	No. dev. leuk.	Leuk. inc. (%)	Avg age leuk. dev. (mo)	No. of rats inoc.	No. dev. leuk.	Leuk. inc. (%)	Avg age leuk. dev. (mo)
♀	25	24	96	4.2	40	40	100	2.8
♂	30	27	90	3.7	31	31	100	2.8
Total	55	51†	93	4	71	71	100	2.8

* Virus harvested from leukemic rats, and inoculated i.p. into 1- to 3-days-old Sprague-Dawley rats. Thymectomy performed at age of 10 days.
† 2 Rats in this group died at 3½ and 6 mo of age without signs of leukemia; 2 males are still apparently well at 12 mo of age.

TABLE V. Forms of Leukemia Developing in Rats Inoculated with Passage A Virus, then Thymectomized.

Exp group	No. leuk. rats exam.	No. dev. lymph. leuk.*	Lymph. leuk. inc. (%)	No. dev. myeloid leuk.	Myeloid leuk. inc. (%)	No. dev. stem-cell leuk.‡	Stem-cell leuk. inc. (%)
Thymectomized rats	48	21	43.7	7†	14.6	20	41.7
Litter-mate controls	52	32	62	3	6	17	32

* In the thymectomized group 1 rat developed reticulum-cell sarcoma and 3 rats Hodgkin's-like lesions in the spleen. In control group 4 rats had Hodgkin's-like lesions in spleen and lymph nodes.

† One rat in this group developed erythro-myeloid leukemia.

‡ Four rats in thymectomized group, and 2 in control group, had a shift toward the myeloid form. In addition, in the thymectomized group, 1 rat had a Hodgkin's-like lesion.

all instances, bone marrow and microscopic sections of spleens, livers and lymphoid tumors were also examined.

The incidence of myeloid leukemia was higher in thymectomized rats (14.6%) than in control rats (6%). Moreover one of the thymectomized rats developed erythro-myeloid leukemia, never before observed in our experiments on rats.

It appears, therefore, that removal of thymus in rats had an influence on susceptibility to the leukemogenic action of the passage A virus which could be compared essentially to that previously observed in mice(6). However, the influence of thymectomy was apparently less pronounced in rats; there was only a relatively slight delay in development of leukemia in rats of this group as compared with non-thymectomized controls. The increase in incidence of myeloid leukemia in thymectomized rats was appreciable, but less striking than in mice. When C3H(f) mice injected with passage A virus were thymectomized, development of leukemia was either inhibited, or considerably delayed(5,6); furthermore, a substantial number developed myelogenous leukemia, a few developed chloroleukemia, and occasionally the erythroblastic form(6).

Discussion. When the mouse leukemia virus was first isolated from spontaneous leukemia(1), it appeared that this agent had a relatively narrow host range. Later a more potent virus strain was isolated from spontaneous Ak mouse leukemia and passed serially through newborn C3H mice(2). The pathogenic potency of this strain, designated "passage A," gradually increased; when inoculated in recent experiments into newborn mice of several strains, such as C3H, C57 Brown/cd, BALB/c, Swiss (N.I.H.) and I, this virus induced a high incidence of leukemia after a relatively short latency in mice of all strains tested(7).

Passage A virus is also pathogenic for rats (3). The results reported here indicate that the leukemogenic potency of passage A virus for rats increased after only one or 2 serial passages in this species; incidence of induced leukemia increased to almost 100%, and average latency decreased to less than 3 months. The virus retained its pathogenic potency for the mouse, even though it was carried in rats. Thus after several rat-to-rat passages it induced a high incidence of leukemia when inoculated into newborn mice.

The fact that a leukemic mouse virus can also induce leukemia in rats was first reported by Graffi and his co-workers(8); they observed that a leukemic mouse virus, isolated from a transplanted mouse tumor, was not only leukemogenic for mice(9), but also induced leukemia following inoculation into newborn rats(8,10). Most of the rats used for inoculation were of the Wistar strain. Total incidence of induced leukemia was only 10%; however, in a few individual experiments up to 50% of the inoculated rats developed leukemia. After passage through rats, the virus retained its pathogenic potency for mice. Moloney, confirming and extending Graffi's observations, reported that a leukemic virus isolated from a transplanted mouse sarcoma was leukemogenic not only

for mice(11) but also for newborn rats of either the Sprague-Dawley or Osborne-Mendel strains(12).

It is evident from results reported here that leukemic passage A virus can induce a variety of forms of leukemia and lymphomas in rats. Among the forms most frequently observed were lymphatic leukemia, stem-cell leukemia and myeloid leukemia; lymphosarcomas were observed very frequently in the leukemic rats; reticulum-cell sarcomas were less frequent, but occurred also. In a few leukemic rats Hodgkin's-like lesions were observed in lymph nodes and spleens. There was a marked and rapidly progressing anemia in most of the leukemic animals. The development of extensive myxomatous swellings in the subcutaneous tissues of some of the leukemic rats and its possible relation to the inoculated leukemic virus require further study.

Thymectomy of virus-injected rats delayed somewhat the development of leukemia, and increased the incidence of myeloid form; one rat in the thymectomized group developed erythro-myeloid leukemia. The influence of thymectomy on the susceptibility of rats to the leukemogenic action of passage A virus was therefore essentially similar to, but apparently less striking than, that previously observed in mice(5,6).

Passage A virus can also induce a variety of forms of leukemia in its host of origin, *i.e.*, in mice. During systematic experiments carried out recently in our laboratory it has become clear that passage A virus is not only able to induce lymphatic leukemia in mice, but that it can also induce stem-cell leukemia or myelogenous leukemia(7), and that under certain experimental conditions the same virus can induce in mice a variety of less frequently occurring forms, such as chloroleukemia, monocytic leukemia and occasionally even the erythroblastic form(6). In certain strains of mice, such as C3H, removal of thymus following inoculation of passage A virus was necessary for induction of the myeloid, instead of the lymphatic, form of leukemia. In other strains of mice, however, such as BALB/c, development of myeloid leukemia was also observed in non-thymectomized animals(7).

It is now apparent that the leukemic virus employed in our studies is of a considerable and versatile leukemogenic potential; it can induce a spectrum of different forms of leukemia, and lymphomas in mice and rats. The form of induced disease depends to a considerable extent on the individual response of the infected host. The leukemic "passage virus," employed in our experiments, is comparable perhaps to the "fixed virus" of rabies described by Pasteur, as contrasted with the "wild virus" encountered in animals developing disease spontaneously. The virus isolated directly from spontaneous mouse leukemia is only occasionally potent; more often such a "wild virus," as in the case of "street virus" in rabies, may be of relatively low potency. Exceptions, however, exist, and in some instances a potent leukemic virus can be isolated directly from naturally occurring disease, *i.e.*, from "spontaneous" leukemia. Kirsten and his co-workers observed recently(13) that a mouse leukemia virus isolated directly from spontaneous Ak mouse leukemia was sufficiently pathogenic to induce leukemia following inoculation into newborn Wistar rats, without prior serial mouse-to-mouse passage.

Summary. 1. Passage A leukemic virus, initially isolated from spontaneous Ak mouse leukemia and passed serially through newborn mice, is pathogenic not only for mice, but also for Sprague-Dawley or Osborne-Mendel rats. 2. The virus could be recovered from rats and passed serially in rats. After one or 2 passages in this species, the incidence of leukemia induced in rats following inoculation of this virus increased to almost 100%, and the latency decreased to less than 3 months. 3. In rats the virus induced most frequently lymphatic or stem-cell leukemia, in about 7% also the myeloid form. Lymphosarcomas, and less frequently reticulum-cell sarcomas or Hodgkin's-like lesions, were found in lymph nodes and spleens in some of the leukemic animals. 4. After passage through rats, the virus induced an incidence of leukemia varying from 87 to 97% when inoculated into newborn mice. 5. Thymectomy of rats that had been inoculated with the virus, slightly delayed the development of leukemia; incidence of myeloid leukemia was higher in the thymectomized rats; moreover, one rat in this group devel-

oped erythro-myeloid leukemia.

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Neutralization of Specific Reagins in Monkeys Passively Sensitized by Cross-Reactive Allergy Sera. (28217)

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It is well recognized clinically that individuals with pollenosis due to grass often exhibit skin reactions when tested with pollens of related grasses. Patients with insect allergy usually are hypersensitive to several related species within a genus and, often to different genera within an order(1,2). Recently it was demonstrated(3,4) that sera from some individuals hypersensitive to the dust from castor bean pomace will passively sensitize the skin of monkeys to castor pollen and also to the pollen and seeds of other species and genera of the spurge (Euphorbiaceae) family.

In the past it was extremely difficult to study the mechanisms of human allergic cross-sensitization and allergic cross-reactions because it was thought to be necessary to utilize direct skin tests upon allergy patients or the Prausnitz-Küstner tests upon passively sensitized human volunteers(5). The problem of testing for cross-reacting allergies in passively sensitized human beings is also complicated by the phenomenon of "hyporesponsiveness" described by Bowman and Walzer(6) and confirmed by others(7). These workers have shown that, in human beings, wheals at passively sensitized sites produce an impairment in the subsequent responsiveness of such sites to restimulation. The hyporesponsiveness at a previously reacted site usually involves a tissue factor and an antibody factor in variable degree. The tissue factor represents a completely reversible change which diminishes rapidly and disappears within 4 weeks. The immunological change supposedly represents injury by the wheal to all of the reagins at a passively sensitized site; it is nonspecific and is not reversible.

Because of the domestic and world-wide importance of the production and processing of castor beans and of the widespread occurrence of severe allergy to castor beans and castor bean pomace, the U. S. Department of Agriculture encouraged additional research studies utilizing passively sensitized monkeys in an effort to elucidate the nature of allergic cross-reactions to castor bean pollen and pomace and to other plants of the spurge family. This project required our having access to a large population of castor-allergic individuals, and a population sufficiently large does not exist in the United States.

Panzani has been treating patients allergic to the dust from castor oil factories in the city and environs of Marseille, France. His first case was described in 1951, and the num-

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