Induction of Leukemia in Rats with Mouse Leukemia (Passage A) Virus.* (26512)

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A mouse leukemia virus (passage A) originally isolated from spontaneous Ak leukemia and then passed by serial cell-free passage through newborn C3H mice(1) was employed in this study. When inoculated into less than 10-day-old C3H/Bi mice, this virus, now in its 27th passage, consistently induces lymphatic leukemia in over 95% of the inoculated animals after an average latency of less than 3 months.

An attempt was made to determine whether this mouse leukemia virus could also induce leukemia in rats.

Materials and methods. Animals. From a nucleus of randomly bred Sprague-Dawley rats received in June 1960 through the courtesy of Dr. J. B. Moloney 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.

Leukemic extracts of 20% concentration were prepared in the usual manner(1) from thymic and mesenteric lymphoid tumors, as well as spleens and livers pooled from several C3H/Bi mice with passage-A-virus-induced leukemia.

Following centrifugation, at 0°C, at 3,000 rpm for 15 minutes, then at 9,500 rpm for 5 minutes, the final supernate was passed through Selas, porosity 02, filter candles, and immediately used for inoculation of newborn, less than 1 day old, Sprague-Dawley rats. All inoculations were intraperitoneal (0.5 ml each). The animals were weaned when about 3 weeks old, separated by sexes, and kept for observation.

Experimental. In the first experiment, a newborn litter consisting of 6 females and 5 males was inoculated with the leukemic filtrate. Eight of the 11 rats (73%) developed leukemia at 3 to 4 months of age; the remaining 3 rats are still in good health at 5 months of age. Among the leukemic rats, 3 had very large thymic lymphosarcomas filling out almost the entire chest cavity; 3 other rats had very large thymic lymphosarcomas, but also very large spleens, large livers, and moderately large mesenteric lymphosarcomas; the remaining 2 rats had a generalized stem-cell leukemia (Fig. 1). No enlargement of inguinal or axillary lymph nodes was noticed in any of these animals. Only 2 rats had a high peripheral (tail) white blood count; the highest count was 403,000 WBC per cu mm with 95% stem cells (Fig. 2). In other rats, the peripheral blood changes were moderate, with slight, if any, elevation of the white cell counts and only occasional presence of blast cells; all leukemic rats, however, had anemia.

In a second experiment, a litter consisting of 7 newborn rats was inoculated with the leukemic filtrate; 4 rats (57%), thus far, have developed leukemia at 4 to 4½ months of age. One had a generalized stem-cell leukemia (188,000 WBC per cu mm), and one a generalized lymphatic leukemia; 2 had very large thymic lymphosarcomas with no other pathology. The remaining 3 rats now 5 months old, are still in good health.

In a third experiment, now in progress, 3 newborn litters, consisting of a total of 21 rats, were inoculated with the leukemic filtrate; 12 rats have thus far (57%) developed leukemia at 2½ to 3 months of age; the remaining 9 are still in good health, but are only 3 months old now. Among the leukemias observed, 4 were thymic lymphosarcomas, 7, were generalized lymphatic leukemias and one was a stem-cell leukemia.

Discussion. Experiments reported here suggest that newborn rats of the Sprague-Dawley strain are susceptible to the leukemogenic action of the mouse-leukemia-derived

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FIG. 1. Rat of Sprague-Dawley strain inoculated (.5 ml i.p.) when less than 1 day old with mouse leukemia (passage A) filtrate. As a result, generalized leukemia developed after a latency of 4 mo. Note large mediastinal and mesenteric tumors, very large spleen, and large liver. Blood examination showed stem-cell leukemia (see Fig. 2). Bone marrow was also leukemic.

passage A virus. The incidence of leukemia thus far induced in rats varied from 57 to 73%; however, among the inoculated animals, those that remained free from leukemia are still young, and some may develop leukemia later. It is our impression that final incidence will exceed 80%, and will thus be only slightly lower than that observed following inoculation of the same virus into suckling mice of susceptible strains. Rats of the Sprague-Dawley strain are not homogeneous, and susceptibility may vary from one litter to another. The incidence of spontaneous leukemia in rats of this stock is very low, and probably does not exceed 2% in old animals, but is difficult to assess since no exact data are available. We have not yet seen a spontaneous leukemia among our untreated animals of this strain.

Whereas it is relatively easy to recognize leukemia in the live mouse, the difficulties are more pronounced in the rat. Labored breathing may be indicative of a thymic lymphosarcoma, but is diagnostic only in more advanced phases of the disease. Enlarged spleen is another sign of certain forms of generalized leukemia in the rat, but is not always present; furthermore, it may not be easy to palpate the abdomen of adult rats and detect a moderate spleen enlargement. Blood counts are reliable as a diagnostic aid, but only in cases in which leukemia involves the peripheral blood; quite frequently, however, the disease may be aleukemic. Thus the investigator must frequently base his diagnostic considerations in live rats not only on peripheral blood examination, but also on the general appearance and behavior of the animals, their relative strength, weight, and manner of breathing. Curiously, the inguinal and axillary lymph nodes were not prominently enlarged in most leukemic rats thus far observed in our studies, thus adding to diagnostic difficulties; in mice, the often, although not always, prominently enlarged peripheral lymph nodes in either spontaneous, or filtrate-induced, leukemia facilitate diagnosis in the live

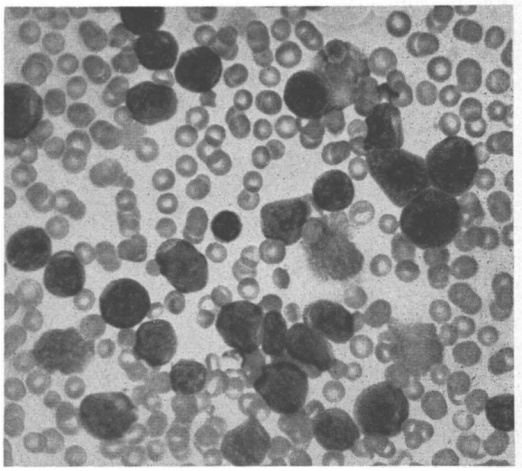

FIG. 2. Blood smear from tail of a rat in which leukemia developed following inoculation of mouse leukemia (passage A) virus (see Fig. 1). WBC 403,000/mm³. 95% stem cells. Magnification 520 × (oil immersion). Wright and Giemsa stain.

animal. No diagnostic difficulties were encountered, however, once the leukemic rats died or were sacrificed, and their internal organs were inspected. The large thymic tumors, frequently enlarged spleens and livers, and mesenteric tumors, could hardly be missed. The blood morphology was diagnostic only in some animals; in others, the disease was aleukemic, with only insignificant changes in blood morphology. Similarly, microscopic sections of internal organs, such as spleen, liver, or kidneys, showed infiltration with leukemic cells only in animals with generalized leukemia. The femoral bone marrow of the leukemic rats was examined in several instances, and was usually found to be leukemic; however, in at least 2 of those leukemic animals in which the disease was limited to very large thymic lymphosarcomas, the bone marrow was found to be non-leukemic. This was consistent with our previous observations on virus-induced mouse leukemia (Gross, unpublished), in which occasionally mice with typical, but early, leukemia had non-leukemic bone marrow. It is possible that the leukemic process may initially develop in the thymus, or in another organ such as spleen, then spread rapidly to other organs of the host, including also the bone marrow. Accordingly, the bone marrow may represent a secondary, and not necessarily a primary, site of the disease.

It is of considerable interest that a mouse leukemia virus could induce leukemia in rats, even though the same virus has been observed to display a specific affinity for particular inbred strains of mice(2). The host specificity of the Ak-leukemia-derived mouse virus was more pronounced in our early studies(3) in which extracts of relatively low potency were employed. Even the more recently employed potent passage virus, however, is still more leukemogenic for certain inbred strains of mice, such as C3H/Bi, or C57 Brown/cd, than for others, such as C3H/An, BALB/c, or A(2). The curious phenomenon was thus observed that a leukemic virus may display specificity for certain strains of mice, or even for certain sublines of inbred strains(3), yet the same virus may be able to cross a species barrier and induce a similar disease in animals of 2 different species.

Other oncogenic mouse viruses also have been observed to induce leukemia in rats. Graffi and Gimmy(4) observed development of leukemia following inoculation of newborn rats of either Sprague-Dawley, or Wistar strains with a leukemogenic virus isolated from transplanted mouse tumors; the incidence of leukemia, however, induced with this mouse virus in rats was low and averaged only 10%(5). Moloney, employing for inoculation a leukemogenic mouse virus isolated from transplanted mouse sarcoma 37, was able to induce an incidence of leukemia exceeding 70% in rats of the Sprague-Dawley strain(6).

It thus appears that a mouse leukemia virus may induce leukemia also in rats. The tissue-culture-grown parotid tumor (polyoma) virus(7), which is essentially an oncogenic virus originally isolated from leukemic mouse tissues(8), also induces tumors not only in the host of the origin, *i.e.*, in mice, but also in hamsters(9), and rats(10). The oncogenic viruses may be in general much less species-specific than might have been anticipated.

Addendum. As this manuscript goes to press, additional data became available suggesting that the leukemic virus could be readily recovered from rats in which leukemia had been induced with passage A filtrate, and could then be passed from rat to rat. From leukemic organs of one of the rats in which leukemia developed following inoculation of passage A (mouse leukemia) filtrate, a filtered (Selas 02) extract was prepared in the usual manner, and inoculated into 9 newborn Sprague-Dawley rats. After a short latency of only 2 months, 8 out of the 9 inoculated rats (89%) developed leukemia; two rats had very large thymic lymphosarcomas, and the remaining 6 had a generalized lymphatic leukemia.

Summary. 1. A mouse leukemia virus, originally isolated from spontaneous Ak leukemia, then passed serially through newborn mice (passage A), and consistently leukemogenic for suckling mice of susceptible strains, was found to be leukemogenic also for newborn rats. 2. Following intraperitoneal inoculation of passage A filtrates into newborn Sprague-Dawley rats, leukemia developed in

from 57% to 73% of the inoculated animals, after a latency varying from 2½ to 4½ months. 3. Either localized thymic lymphosarcomas, or generalized lymphatic leukemia, involving also spleen, liver, and mesenteric lymph nodes, developed in the inoculated animals. A few rats developed acute stem-cell leukemia.

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Serum Free Cholesterol and Atheroma in Young Cockerels. (26513)

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Partial conversion of free to combined cholesterol upon incubation of normal human serum has been reported(1). Thirty-one to 50% decrease in free cholesterol occurred when serum from 20 normal patients was incubated(2). Incubation of serum from most patients with active coronary atherosclerosis resulted in less than normal conversion(3). These observations suggested the value of studying the relationship between serum free cholesterol not combined upon incubation and development of atheroma in normal and atherosclerotic young cockerels.

Material and methods. Approximately 4 wk old White Leghorn cockerels were used. Normal birds were fed a commercial chick growing mash *ad lib.** Atherosclerosis was induced by feeding the basal diet containing .5% cholesterol deposited from ethyl ether solution. Atherosclerotic condition was evaluated as % area of the aorta, brachiocephalic, and iliac arteries involved in plaque formation. Pooled blood serum from each group was incubated 30 hr at 37-38°C. Other methods employed have been described(4).

Results and discussion. Extent of artery involvement and serum cholesterol data before and after incubation were determined in 3 series of groups of birds, representing normal, mild spontaneous, and severe cholesterol-induced atherosclerosis respectively (Tables I, II, II). Total cholesterol values did not change appreciably upon incubation. Last column in each table shows the % original free cholesterol remaining after serum incubation.

Of particular interest is the incubation increase of % uncombined free cholesterol from normal (17 to 45) through mild spontaneous (45 to 56) and severe cholesterol-induced atherosclerosis (57 to 87%). The range, 17 to 45%, for normal birds suggests an increasing tendency toward atherosclerotic development although plaques were not grossly observable (Table I). Corresponding results, 45 to 56%, associated with the first appearance of plaques as mild spontaneous atherosclerosis in birds on normal diet support this suggestion (Table II).

Plaques were always found when more original free cholesterol remained than was combined upon incubation. They were not found when less than 45% remained. Approximately 57% marked the beginning of severe atherosclerosis. This narrow range for mild spontaneous artery involvement represents a

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