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Leukemia in Germfree Rats.* (30837)

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Leukemia in mice is a virus disease which is manifested either spontaneously or subsequent to whole-body exposure of the host to X-rays(1). The agent(s) of mouse leukemia induces leukemic disease in rats provided that they are inoculated shortly after birth(2,3, 4); however, the viruses are not considered indigenous to rat populations. Spontaneous leukemia has occurred infrequently in rats (5,6); and induction of leukemia in rats by a variety of chemical and physical agents has been of relatively insignificant incidence(7, 8). When spontaneous leukemia did appear in rats, the etiological agent(s) was not clearly identifiable(9).

Rats and mice have been maintained and propagated under defined germfree conditions for several years(10,11); and they have become at least comparable in quality to the conventional stock from which they were derived. One anatomical anomaly observed in germfree animals has been the development of very large fluid-filled, thin-walled ceca, which, in some instances, become so large as to occupy half of the abdominal cavity and thereby interfere with normal function. Also, the reticuloendothelial system is underdeveloped, but functionally intact. Virus-like particles have been observed in individual thymic tissues derived from the 6 strains of germfree mice; and leukemia has been induced in large numbers of mice among all of the strains by whole-body X-irradiation(12, 13). It is assumed that at least some of the observed particles are leukemia virus. In addition, virus-like particles have been observed in thymic tissues of preleukemic and leukemic germfree AKR mice(14). Virus associated with leukemia is the only agent thus far detected in germfree mice.

Spontaneous leukemia has not been observed during the past 4 years among the 4000 germfree rats which have been propagated at Lobund Laboratory. While a microbial agent has not yet been detected in germfree rats, the search continues. Germfree rats develop neoplastic diseases(15). Spontaneous breast tumors have been observed in aged germfree Wistar strain rats. Breast tumors have been induced in germfree Sprague-Dawley rats which had been fed 7, 12 dimethylbenzanthracene (DMBA) when 50 days of age. Fibrosarcomas were induced in 3 strains of germfree rats by a single subcutaneous injection of sterilized 3-methylcholanthrene in olive oil. In all instances, the lesions which developed were indistinguishable from those which were induced by the same procedures in conventional counterpart rats.

It was of significant interest to determine (a) if the tissues of germfree rats contain virus-like particles, (b) if leukemia could be induced in germfree rats by X-irradiation, and (c) if leukemia could be induced in them by a strain of mouse leukemia virus.

Methods. Animals. The germfree rats used in this study were of the Wistar, Fischer, and Sprague-Dawley strains. The Wistar strain was in the 20th germfree generation, the Fischer in 6th, and Sprague-Dawley in 3rd generation by random brother-sister mating. They were propagated in steel isolator units,

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and maintained in flexible plastic isolators during the periods of experimental observations(10,11). They were fed steam sterilized diets L-462 or 5010C and water *ad libitum*, and maintained on steam sterilized sawdust bedding. The animals were examined at regular intervals for bacterial flora(16), and for symptoms and signs of disease. Spontaneous leukemia was not observed in any of the untreated control germfree rats which were maintained under the same germfree status as the experimental rats.

Electron microscopy. Weanling germfree rats of the 3 strains were killed by cervical dislocation. Thymic, spleen, and lymph node tissues were excised quickly, fixed in osmic acid(17) and processed as epon-embedded thin sections for examination in the electron microscope(18).

Irradiation. Groups of germfree rats, approximately 30 days old, were given whole-body exposure to 4 doses of X-rays, each of 200 r, at intervals of 7 days. The source of radiation was a 260 KVP Picker therapy X-ray machine. It was operated at 245 KVP and 15 MA with a filtration of 1.0 mm Al and 0.25 mm Cu at a dose rate of 43-45 r/ mice, as measured in air with a Victoreen condenser meter. The rats were observed for as long as possible under germfree conditions and thereafter under conventional conditions for a total period of 12 months. Animals which appeared abnormal were autopsied and examined for gross and histological evidence of disease.

Virus inoculation. Rat adapted strain A leukemia virus, kindly supplied by Dr. Ludwik Gross, was inoculated intraperitoneally (0.1 ml) into 45 newborn Sprague-Dawley rats. One rat was killed every 7 days during each of the next 5 weeks in order to detect early stages of disease. The rest of them were maintained and observed under germfree conditions for the following 6 months. Sick animals were removed to the laboratory for examination by autopsy, by conventional histologic, and by electron microscopic techniques.

Results. Electron microscopy. Representative thymic tissue specimens from 10 individual germfree rats of each of the 3 strains did not contain clear-cut evidence of virus-like particles. Some budding structures were observed in the membranes (Fig. 1) and in the walls of intracytoplasmic vacuoles from 2 Wistar rats, which might be interpreted as "A" particles.

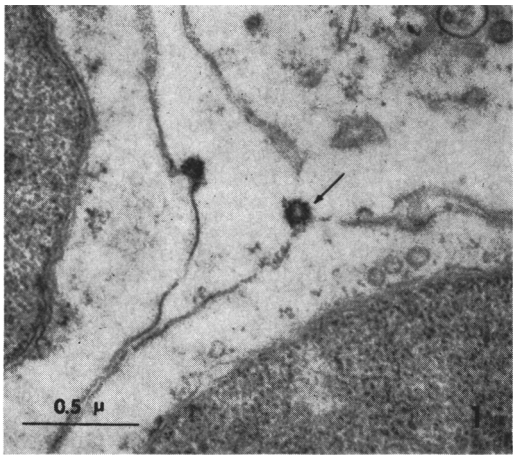

FIG. 1. "A" type particles in thymus cells of germfree Wistar rat. × 30,000.

Irradiation. All of 43 Sprague-Dawley, 49 Wistar, and 12 Fischer germfree rats survived the cumulative effects of 800 r X-rays. Bacterial contaminants appeared in them after 3 to 6 months of observations; so they were thereafter maintained as conventional animals. At 6 months after irradiation, 1 Wistar and 1 Sprague-Dawley rat developed enlarged thymus glands which were about 15 times normal size, hard and fibrous. The thymus tissue was completely replaced by hyalinized fibrous tissues. The lymph nodes of the 2 rats were underdeveloped: they were small and the cortical lymphoid follicles lacked "reaction" centers. This in no way resembled the leukemic thymoma induced in mice by X-rays, nor the thymoma induced by Gross virus, as described below. Instead, the tissues resembled the tissues of mice which had been thymectomized at birth.

Between 9 and 12 months after irradiation, 6 of the 49 irradiated Wistar rats developed adenofibromas of the breast. In the same interval, 7 of the 43 irradiated Sprague-Dawley rats developed breast tumors: 6 were adenofibromas and 1 was adenocarcinoma. One of 12 Fischer rats developed adenocarcinoma of the breast at 11 months. The breast tumors could not be differentiated from those tu-

mors which developed spontaneously in germfree Wistar rats, nor from those which were induced by DMBA in germfree Sprague-Dawley rats(15). Control germfree rats of the same age, sex, and strain remained free of disease during the course of the experiment.

Virus inoculation. Five rats were examined periodically after inoculation of strain A leukemia virus in order to detect early histological changes in their tissues. None was observed. Five baby rats were destroyed by the mothers shortly after inoculation. Individuals among the 35 remaining rats started to get sick at 11 weeks after inoculation. In one experimental group, 7 of 8 rats developed leukemia within a range of 79 to 116 days (average 97 days). In a second group 23 of 24 rats developed leukemia within a range of 62 to 202 days (average 88 days). Three rats could not be examined because of cannibalization. Symptoms of leukemia usually developed suddenly. The sick rats lost weight and manifested severe dyspnea; they died shortly after first detection of symptoms. In 24 (74%) of the sick rats the only lesions consisted of a turgid thymoma, which frequently occupied half of the chest cavity, and the lymph nodes and spleen were within normal limits of size. In 2 of the sick rats, the thymus glands were small, but their lymph nodes and spleens were enlarged. Four of the sick rats showed enlargement of the thymus, the lymph nodes, and spleen, as well as abnormally elevated numbers of lymphoid cells in the circulation. The normal histological structures of the thymus glands were completely replaced in leukemic rats by large lymphoblastic cell types, many of them in mitosis. When other organs (lymph nodes, spleen, liver and kidney) were enlarged, infiltrations with the same type of lymphoblastic cells were responsible for it. In all of the germfree rats, the cecum was thin-walled, enlarged, and filled with watery fecal material, similar to that observed in control germfree rats.

The lymph nodes of normal control germfree rats were usually small, and contained cortical aggregates of small lymphoid cells. "Germinal" or "reaction" zones have been observed infrequently in them. The lymph nodes from rats with only lymphatic thymomas were enlarged but they were usually free of abnormal cells. They contained increased numbers of "reaction" zones in the cortical follicles and some plasma-type cells in the medullary cords of the lymph nodes.

Virus-like particles, 80 to 100 mμ in diameter, were observed in and around the thymoma cells of the rats which had been inoculated with Gross virus (Fig. 2). They were indistinguishable from similarly-located particles in (a) the thymus glands of disease-free germfree mice, (b) in tissues from germfree mice with radiation-induced leukemia, and (c) in germfree mice which had been inoculated with Gross passage A leukemia virus.

Discussion. Leukemia was not induced in germfree rats by the same irradiation procedures which were shown to be effective in mice. This could mean either that the rats were virus-free, or that the X-ray dose did not provide the stimulus by which the disease could be initiated. A mechanism for leukemogenesis does exist in rats, as exemplified in their response to Gross virus. There was no decisive structural evidence of virus-like particles in thymic tissues from colony specimens of germfree rats, only "budding" extrusions from the cell membrane which have been interpreted as type "A" particles. The procedures used in electron microscopy at this Laboratory are accurate, since by the same procedures virus particles have been detected with ease in the thymic tissues of mice(13).

Germfree Sprague-Dawley rats were as susceptible to Gross passage A leukemia virus as conventional rats. The disease induced in most of the germfree rats seems to be limited to the thymus glands, thereby differing somewhat from the disease induced in conventional rats(4) and in germfree mice. Germinal or "reaction" zones were observed in the lymph nodes of rats bearing only thymomas. Since such changes are seldom found in normal germfree rats, the changes may reflect either an immunogenic response of the host, or initial stages in development of the leukemic lesion in the lymph node. The lymph node lesion in mice appears to develop

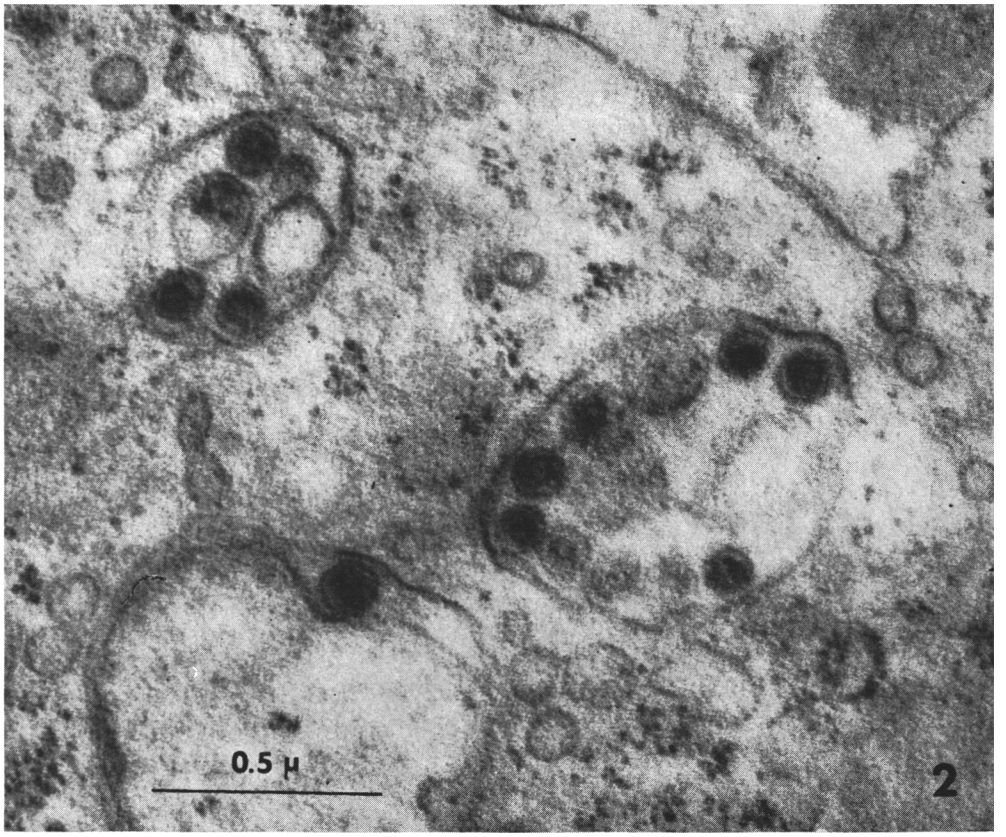

FIG. 2. Gross passage "A" leukemia virus in thymoma cell of germfree Sprague-Dawley rat. × 60,000.

through an irreversible enlargement of the germinal zones(19). It is unlikely that the virus inoculum contained other viruses in addition to leukemia virus, since the lymph nodes remained unchanged prior to the appearance of thymoma in inoculated rats.

Summary. Germfree rats of 3 strains (Wistar, Fischer and Sprague-Dawley) were examined for leukemogenic potential. (a) Particles of indefinite nature were observed only in 2 of 10 Wistar rats. (b) Leukemia could not be induced in the rats by whole-body administered X-rays, and (c) germfree Sprague-Dawley rats were as susceptible to Gross passage A leukemia virus as conventional rats.

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Studies of Fasted, Water-deprived Intact and Adrenalectomized Dogs Injected with Glucocorticoids.* (30838)

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These experiments are concerned with (a) the increase in plasma volume of fasted, water-deprived, intact and adrenalectomized dogs induced by large i.v. doses of Δ-1-dehydrohydrocortisone (prednisolone, Schering); (b) establishment of the minimum effective amount of steroid required to raise the plasma volume under the conditions imposed by these experiments; (c) the ineffectiveness of solubilized desoxycorticosterone for potentiating the plasma volume-raising action of prednisolone and (d) alterations in blood and plasma constituents of food and water-deprived animals without adrenals while receiving massive doses of glucocorticoid.

Materials and methods. Seven intact and 17 long term, vigorous, adrenalectomized dogs were used. The animals were thoroughly table trained and all injections were by vein. The chemical methods used for the analyses were those employed in earlier studies from this laboratory(1). Plasma volume was determined according to recommendations of Gregersen and Rawson(2) and Chien and Gregersen(3), using a standardized, weighed sample of the dye T1824 which was read at 620 mμ on a Beckman D B Spectrophotometer. The morning after drawing control samples, food was withheld and 24 hours later water was also withdrawn. Prednisolone was then given i.v. in divided doses of 25 mg each at 10 a.m., 6 and 11 p.m. At 8 a.m. the following day an additional 25 mg was injected followed by a second 25 mg dose 1.5 hours later, making a total of 125 mg administered within the final 24 hours of the fast. The interval between the last injection and sampling was 1.5 hours in all experiments.

Results. Table I A records the data obtained from 7 intact animals employed as controls for the dogs without adrenals. Each received 125 mg prednisolone (free alcohol) solubilized by the method described previously(4). The plasma volume increased by 27.4% within 1.5 hours accompanied by hemodilution and a rise in blood sugar.

Table I B. After a 48-hour fast and withdrawal of water during the final 24 hours of food deprivation, each adrenalectomized dog of this series was given 175 mg of the glucocorticoid in 5 divided doses of 35 mg each at 8 a.m., 2, 6, and 11 p.m. and at 8 a.m. the following day. The reactions of the starved animals to the prednisolone were (1) large increase in plasma volume which rose 35.7% for an average kg increase of 15 ml or a total of 287.1 ml per dog; (2) hemodilution associated with the volume rise; (3) marked elevation of the blood sugar. Changes in plasma Na, Cl and K were not significant, hence the data on these electrolytes are omitted from Table I.

Table I C. The prednisolone was reduced to 125 mg in this series. However, despite the smaller dosage the plasma volume rose 28.3% above control readings. Hemodilution was present and blood sugar increased.

Table I D. Attempts were undertaken to determine the approximate minimum dose of prednisolone necessary to induce elevation of the plasma volume of fasted, water-deprived

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