ceiving uracil and cholesterol reduces the degree of cellular hyperplasia(2) but has not been observed to induce excessive colloid storage even when the dietary protocol of the present experiment was duplicated (unpublished data). Repetition of the present experiment with isolation of the various dietary factors and serial sacrifice is indicated.

Summary. Four Cebus monkeys, 2 males and 2 females, were maintained on a casein-corn oil diet containing 0.5% cholesterol for 86 weeks. For the greater part of this time the diet contained uracil (0.5-0.75%) and during 2 short feeding trials of 3 and 5 weeks duration orotic acid was also included in the diet (0.5%-1%). Uracil produced a further increment in serum cholesterol elevation beyond that due to dietary cholesterol alone as well as an elevation of serum urea. In one female concomitant feeding of orotic acid at the 1% level lowered the serum cholesterol. At autopsy the 2 females demonstrated large colloid goiters while the thyroids of the 2 males showed a mixed picture of both colloid goiter and that of colloid-poor hyperplasia. In one of the males this latter process was extreme and could not be distinguished from early carcinoma. It is assumed that these thyroid changes resulted from ingestion of uracil (or uracil and cholesterol), though the possible role of orotic acid cannot be completely excluded. Speculations on the pathogenesis of the two types of thyroid change are discussed as well as comparisons of the above results with those seen in rats.

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Radiation-Induced Leukemia in Germfree Mice.* (29423)

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The leukemogenic effect of whole-body administered X-rays in mice has been documented abundantly(1-5). Optimal conditions for eliciting leukemic disease in such mice have been defined as being related to age and frequency of exposure(6). Certain strains of mice, which have a low incidence of spontaneous leukemia, carry an occult leukemogenic virus which can be activated by exposure to moderate dosages of X-rays(7,8). However, irradiation does not accelerate the appearance of clinical disease in the high-leukemic C58 and AK strains of mice. Fur-

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thermore, the interrelationship of murine leukemia virus strains is inadequately defined.

The dissemination patterns of oncogenic viruses can be either "vertical" (trans-germinal) or "horizontal" (post-natal)(9,10). Factors involved in disease-spread among conventional mice are difficult to control because the animals, their food and bedding may be contaminated with viruses, some of which may be oncogenic(10); and the remote possibility of contaminations from human attendants should not be overlooked. The channel whereby the radiogenic form of leukemia is perpetuated to progeny is unknown. Gross has recovered leukemia virus from AK mouse fetuses(11), and virus-like particles have been observed in the tissues of fetal AK mice(12). Unidentified virus-like particles have been observed in thymus cells of the Lobund strain of germfree Swiss-Webster mice(13).

The present report is an inquiry into (a) the occurrence of leukemogenic agents in germfree mice, and (b) the route by which the agent(s) of radiogenic leukemia may be perpetuated through successive generations of mice.

Methods. Swiss-Webster strain mice were caesarian-delivered into the germfree environment by techniques already described(14, 15). The newborn germfree mice were hand-fed by stomach tube on a sterile diet(16) from birth until they reached weaning age. Thereafter, they were perpetuated as germfree mice by natural means through 21 successive generations. Five additional strains of mice (C3Hf, CFW, ICR, Balb/C, and C57 Bl) were each caesarian-delivered into the germfree environment, and the young were foster-nursed on germfree Swiss-Webster mother mice. After they reached weaning age, each strain was propagated by natural means in the germfree environment. Periodically, germfree mice of each strain were removed from the germfree isolators and "conventionalized" to the microbial flora of the animal house. The latter were the control animals for the experimental procedures described below. Spontaneous leukemia was not detected in the mice.

The germfree mice were examined thoroughly for parasites, protozoa, bacteria, and *Mycoplasma,* and the results were negative (17,18). They were examined for viral flora by standard procedures, as follows: (a) serology for antibodies to polyoma, "K," mouse hepatitis and adenoviruses; (b) susceptibility to vaccinia, reo, lymphocytic choriomeningitis and mouse adenoviruses, which indicated that they were neither resistant nor tolerant to the infections; (c) examinations of tissue cultures prepared from tissue explants and from trypsin-dispersed organs of germfree fetuses and newborn mice; (d) inoculation of tissue cultures prepared in (c) with extracts of germfree tissues; and (e) microscopic examinations of organs for histological evidence of infection. In all of the examinations noted above, no evidence of a microbial flora was detected.

Germfree and conventional mice of Swiss-Webster, C3H, and C57 Bl strains were examined for occult leukemia virus by the procedure described by Kaplan and Brown(19). 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/min as measured in air with a Victoreen condenser r-meter. At one month of age each mouse was given whole-body exposure 4 times to 150 r X-rays, at intervals of one week. The mice were thereafter maintained germfree or conventional for 6 months, and observed frequently for evidence of illness. Each mouse which showed symptoms of illness was removed from the germfree isolator or from the animal house. The sick mouse was examined hematologically, killed by cervical dislocation and examined by autopsy. Specimens of thymus and spleen tissues were collected and processed for examination by electron microscopy, and for storage in the dry ice box. Lymphatic and visceral tissues were fixed in Bouin's fluid, and then processed by standard procedures for histological examinations.

Results. The irradiated germfree mice showed no evidence of disease during the 4 months following the first dose of X-rays. Thereafter, individual germfree and conventional mice developed symptoms of rough-

TABLE I. Development of Leukemia in Germfree and in Conventional Mice Following Whole-Body Administration of X-rays.*

Mouse strain	Number	Microbial status	Development of leukemia in days†	No. leukemia / No. irradiated
C3H	11	Germfree	166, 171, 227, 227, 231, 238, 239, 239, 239, 254	10/11
C3H	7	Germfree	141	1/7
C3H	32	Conventional	133, 134, 172, 177, 188, 191, 191, 191, 261, 264, 264, 264, 265, 268, 277, 304, 304, 303	19/32
C3H	13	Conventional	143, 143, 158, 192, 209, 259	6/13
C3H	19	Conventional	137, 140	2/19
C57 Bl	7	Germfree	93, 96, 118, 120, 145	5/7
C57 Bl	8	Conventional	145, 177, 227, 300, 300	5/8
C57 Bl	8	Conventional	126, 129	2/8
Swiss-Webster	27	Germfree	133, 133, 134, 137, 140, 144, 146, 157, 166	9/27
" "	21	Conventional	129, 129, 150	3/21

* One-month-old mice administered 150 r x-rays at weekly intervals.
† Calculated from day of first treatment.

ness, kyphosis, weakness, dyspnea, and abdominal breathing. Palpable lymph nodes and elevated white blood cell counts were detectable in many of them. At 6 months after onset of X-ray treatment, leukemia was observed among germfree and conventional mice respectively, in 5/7 and 4/16 of the C57 Bl mice; in 3/18 and 11/64 of the C3H mice; and in 9/27 and 4/21 of the Swiss-Webster mice. As the observation periods of individual groups of irradiated mice were extended, the incidence of leukemia was increased (Table I). The experiments with observation periods under 300 days are incomplete.

The thymus glands were enlarged at least 6-fold in all of the leukemic mice, and in many instances occupied over half of the chest cavity. The mediastinal glands were frequently enlarged, and the thoracic cavity usually contained some serosanguinous fluid. In most instances the spleen and lymph nodes were enlarged significantly. The liver and kidneys were swollen, and the latter were occasionally pale in appearance. Prominent Peyer's patches were observed. The cecum was enlarged in the germfree leukemic as in the control mice.

The histological structures of the thymus, spleen, and lymph nodes were obscured by extensive infiltrations of large lymphoblastic cells (Fig. 1). The liver, kidneys, lungs and intestines, and occasionally the tissues of the heart and adrenal glands were heavily infiltrated with leukemic cells, many of them in mitosis (Fig. 2). White cells in the blood were lymphoblasts and "smudge" cells. Lesions in germfree mice were indistinguishable from those noted in the conventional counterparts.

Examinations of cells in the enlarged thymus gland by electron microscopy have revealed, in the cytoplasm, virus-like particles about 100 mμ in diameter. Examinations of additional specimens are still in progress. Newborn mice of the homologous genetic strain have been inoculated with filtrates of organ extracts, but sufficient time to record results has not yet passed.

In untreated germfree mice, the reticuloendothelial elements of visceral organs were underdeveloped. The lymph nodes contained cortical masses of lymphocytes (primary follicles), and an occasional germinal zone. The thymus glands were small and contained a narrow cortical layer of lymphoid cells. White blood cell levels did not exceed 12,000/mm^3.

Discussion. Leukemia has been induced by X-irradiation in 3 strains of "germfree" mice. Morphological evidence of virus has been observed in the cells of leukemic germfree mice, which we assume is a leukemogenic agent. We are attempting to confirm this by biological assay procedures. While this will be the first instance of virus detection in germfree mice, demonstrations of additional viruses may depend on the development of techniques for "unmasking" them. The search continues also for development of pro-

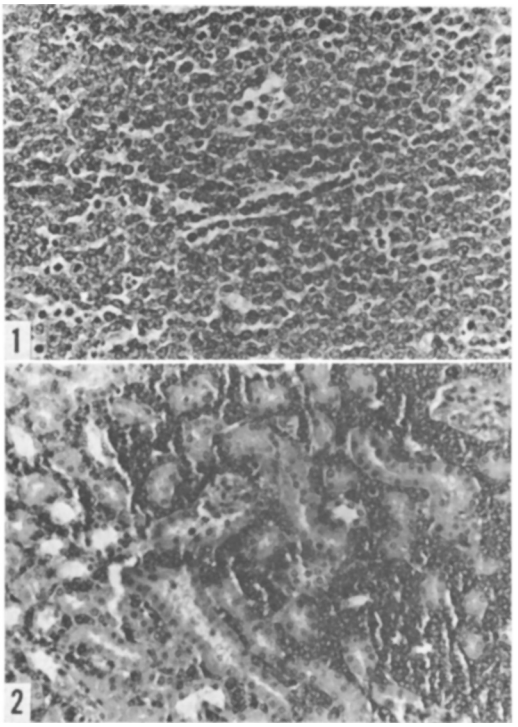

FIG. 1. Section of lymph node of germfree Swiss-Webster mouse in which leukemia was induced by x-rays. × 104.

FIG. 2. Section of kidney of germfree Swiss-Webster Mouse in which leukemia was induced by x-rays. × 104.

cedures whereby the occult leukemogenic agent(s) might be excluded from our germfree mice.

Areas of immunocytological activity have been detected occasionally in the cortical follicles of lymph nodes in germfree mice. This has been attributed to antigenic stimuli in the diet; and experiments to prove this with antigen-free, soluble diets are in progress(20). It may be conceivable that such reaction or germinal zones in the lymph nodes are in response to an occasional maturation of leukemia virus, or to some other, as yet undetected, agent. If reactions in the lymph nodes are diet-related, then the leukemogenic agent will be truly "occult" in nature; if not, then the lymph node response may provide a very sensitive indicator of infection.

The Swiss-Webster mouse strain was the first to be adapted to germfree status in the present series at Lobund Laboratory. They had no contact with predecessors except *in utero,* and they were reared and maintained on a heat sterilized diet. The leukemogenic factor has thus been perpetuated "vertically" either through germinal cells, or through the placenta, from mother to offspring. At no time following caesarian-delivery into the germfree isolator were they exposed to known "contamination." Since the germfree C3H and C57 Bl mouse strains were each derived from infected parents we can assume that they were not contaminated through contact with germfree Swiss-Webster foster-mother mice. Germfree CFW, ICR, and Balb/C mouse strains have now been subjected to X-irradiation, and we await the developments with interest.

Summary. Lymphatic leukemia was elicited by whole-body X-irradiation in germfree mice of Swiss-Webster, C3H, and C57 Bl strains. The disease was not observed in untreated germfree control animals. The leukemic lesions resembled those induced by X-irradiation in conventional counterpart animals. Cytoplasmic viral inclusions were noted by electron microscopy in cells from the enlarged thymus glands. In analyzing the technical details whereby the animals were entered into and maintained under germfree conditions, one can conclude that the occult leukemogenic agent was perpetuated in successive generations of mice by "vertical" passage, through the ovum or through the placenta.

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Effects of Hypertransfusion and Erythropoietin on Labeling of Rat Megakaryocytes by Tritiated Thymidine.* (29424)

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Ninety to one hundred percent of all rat megakaryocytes became labeled during the 48- to 96-hour period after a single intravenous injection of tritiated thymidine (H^3TdR)(1). From this, Feinendegen *et al* (1) concluded that "recognizable megacaryocytic elements originate from unrecognized precursors which continuously synthesize DNA for a period of at least 1 to 3 days prior to maturation into recognizable megacaryocytic precursors." Their conclusion was based on the assumption that availability time of H^3TdR is short.

Initially it was considered that H^3 was available for desoxyribonucleic acid (DNA) labeling for only a few minutes after intravenous injection of H^3TdR because of the rapid plasma clearance of H^3TdR(2,3). Recent studies, however, point to the fact that the H^3 may be available much longer(4-9) either from reutilization or continued availability of H^3, possibly as thymidine itself (9). Thus, an alternative explanation for the megakaryocyte labeling curve might be delayed incorporation of H^3 either directly into megakaryocytes or their precursors.

Reutilization of DNA or its breakdown products has been considered to be minimal because of dilution(3) and rapid degradation by the liver(10). Thus, it has not been thought to influence importantly studies of marrow cell kinetics with H^3TdR. However, extruded nuclei of erythroid cells provide a substantial source of DNA available for local reutilization(11). To evaluate this possibility we studied H^3TdR labeling of rat megakaryocytes after suppression of erythropoiesis by hypertransfusion or stimulation by erythropoietin.

Materials and methods. Female Sprague-Dawley rats, weighing 148-197 g and having free access to Purina rat chow and water, were injected intravenously with H^3TdR,‡ 1 μc/g body weight at time 0. They were serially sacrificed with ether at 30 min, 12 hr, 24 hr and daily thereafter. Bone marrow smears prepared from a split femur were fixed in absolute methyl alcohol. After dipping in Kodak NTB-3 emulsion, the slides were stored for 30 days at —20°C. After developing, they were stained with Giemsa or Wright's and Giemsa stain. For each rat 250 megakaryocytes were classified into 3 groups using the criteria of Feinendegen *et al*(1). These cells were also scored for nuclear grain count, and correction was made for background. Platelets were counted by the method of Brecher and Cronkite(12) from cardiac blood drawn into versene just prior to sacrifice.

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‡ Schwarz Bioresearch, Inc., 1.9 c/mmole, diluted with sterile saline to 500 μc/ml.