phenomenon could possibly be related to altered differential gene expression. At day 11 the abnormally developing embryo had a molecular complement of acid phosphatases unparalleled in comparable normal development. This biochemical situation is similar to structural paraplasia wherein an abnormally developing embryo forms a structure "entirely foreign"(9) to normal embryology. Such structural paraplasia was illustrated by the presence of horseshoe kidney in the offspring of vitamin A-deficient rats.

Summary. As a result of a teratogenic maternal folic acid deficiency, discrete changes are produced in the molecular composition of abnormally developing embryos. Furthermore, these changes continue in the embryo after the mother has been returned to a complete ration. The adverse effects on the development of new enzymatic forms appeared to be both qualitative and quantitative. Although there was a general trend for delayed and asynchronous appearance of specific molecules in the abnormally developing embryos, this was not invariably the case and some of the enzymes did appear on schedule and at or near control amounts in the experimental animals. Thus the retardation in the appearance of certain enzyme forms resulting from folic acid deficiency would appear to be selective.

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Preliminary Observations on Spontaneous and Radiation-Induced Leukemia in Germfree Mice.* (29743)

H. E. WALBURG, JR., A. C. UPTON, R. L. TYNDALL, W. W. HARRIS AND G. E. COSGROVE

Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee

The induction of leukemia by ionizing radiation in conventionally reared mice has been a subject of intensive study for more than 20 years(1). With the implication of viruses in the pathogenesis of these neoplasms(2) it has become increasingly important to study the influence of host microbial flora on induction of leukemia and to determine the mode of transmission of any etiologic agents. Preliminary observations on X-ray-induced and spontaneous leukemia in germfree mice, associated with the appearance of "virus-like" particles in the leukemic tissues, are reported at this time because of their significance to further consideration of the pathogenesis of these neoplasms.

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Materials and methods. The mice used in this study included: 1) germfree and conventional noninbred ICR mice at least 4 generations removed from germfree ancestors supplied by the Lobund Laboratory of the University of Notre Dame, and 2) mice of the RF strain. The latter comprised mice from our noninbred production stocks and from our inbred line, which was originally brought to Oak Ridge by Furth in 1949(3,4). The germfree RF mice (RFM/Uf) were developed by caesarian section of a full-term

pregnant inbred RF mouse and foster-nursing of her litter (F55) on a germfree ICR female by the technique of the Lobund Laboratory; *i.e.*, the abdominal cavity was opened, the body of the gravid uterus was clamped with small umbilical forceps, severed proximally, and the entire uterus removed aseptically; the uterus was passed through a strong hypochlorite solution into a sterile plastic film isolator(5), the feti were removed from the uterus, dried, and when breathing regularly were placed in a cage with an ICR female which had delivered a litter less than 24 hours previously and had been separated from her offspring immediately preceding the introduction of the foster litter. The germfree RF mice of this litter were sib-mated at 8 weeks of age and their descendants similarly brother-sister mated to produce the offspring used in this study. The germfree mice of both strains were consistently negative on periodic examination for bacteria, yeasts, fungi, mycoplasma, internal and external parasites, and for all the viruses for which they were tested, *i.e.*, polyoma, reovirus 3, Theiler's virus, "K" virus, Sendai virus, pneumonia-virus-mouse, mouse adenovirus and mouse hepatitis virus. The conventional RF mouse colony, on the other hand, has been shown to harbor polyoma virus, reovirus 3, Theiler's virus, mouse adenovirus, and pneumonia-virus-mouse. Mice were irradiated with a GE Maxitron X-ray machine with the following factors: 300 kvp, 20 ma, hvl. 0.98 mm Cu, and dose rate approximating 100 r/min. The colony was inspected twice daily, and dead or moribund mice were necropsied, with histopathological examination whenever practicable. Examination of tissues with the electron microscope for "virus-like" particles was carried out as follows: organs were removed from the carcass, frozen, subsequently thawed, and homogenized in Tyrode's solution with a glass tissue homogenizer; the homogenate was centrifuged at 1200 $\times$ *g* for 15 minutes at 5°C, and the pellet discarded; the supernate was then centrifuged at 20,000 $\times$ *g* for 1 hour at 10-15°C and the pellet discarded; the resulting supernate was pelleted for electron microscopic examination by centrifugation at 102,000 $\times$ *g* for 15 minutes at 10-15°C; the pellets were resuspended in 1 or 2 drops of 0.15 M sodium citrate (pH 6.8), an aliquot of which was placed directly on a grid covered with a carbon film and negative stained with 1% phosphotungstate at pH 7.0(6).

Results. ICR mice surviving 600-900 R of acute X-irradiation have been observed for up to 10 months after exposure. The cumulative incidence of leukemia in the irradiated germfree mice is as high as, or higher than, in the irradiated conventional mice (Fig. 1). It is higher in both irradiated groups than in the unirradiated groups, which do not differ significantly in incidence of spontaneous leukemia. Germfree RF mice exposed to 300 R of X-rays at 4-6 weeks of age, likewise, show a higher cumulative incidence than their conventional counterparts (Fig. 2). All leukemias examined histopathologically have been lymphocytic, and about half of them have involved the thymus, either exclusively or with generalized lymphomatosis. In the one germfree leukemic RF mouse examined with the electron microscope thus far, ultracentrifugates of homogenized liver and thymus tissue revealed tailed particles morphologically similar to those observed consistently in conventional leukemic mice of the same strain and similar to the Moloney (7) and Rauscher(8) viruses. Such particles have not been detected in tissues from nonleukemic mice. Both germfree and conventional mice dying with leukemia have manifested glomerulosclerosis on histologic examination of the kidney, the lesions resembling those described earlier in conventional mice (9).

Discussion. The detection of spontaneous and radiation-induced leukemia in germfree mice has important implications concerning the relationship of the microbial flora to the pathogenesis of these neoplasms. Those elements of the "conventional" flora which have been shown, by the testing procedures employed, to be absent from our germfree mice may be inferred to be unnecessary for the development of either spontaneous or radiation-induced leukemia. Furthermore, these neoplasms can arise in the absence of mi-

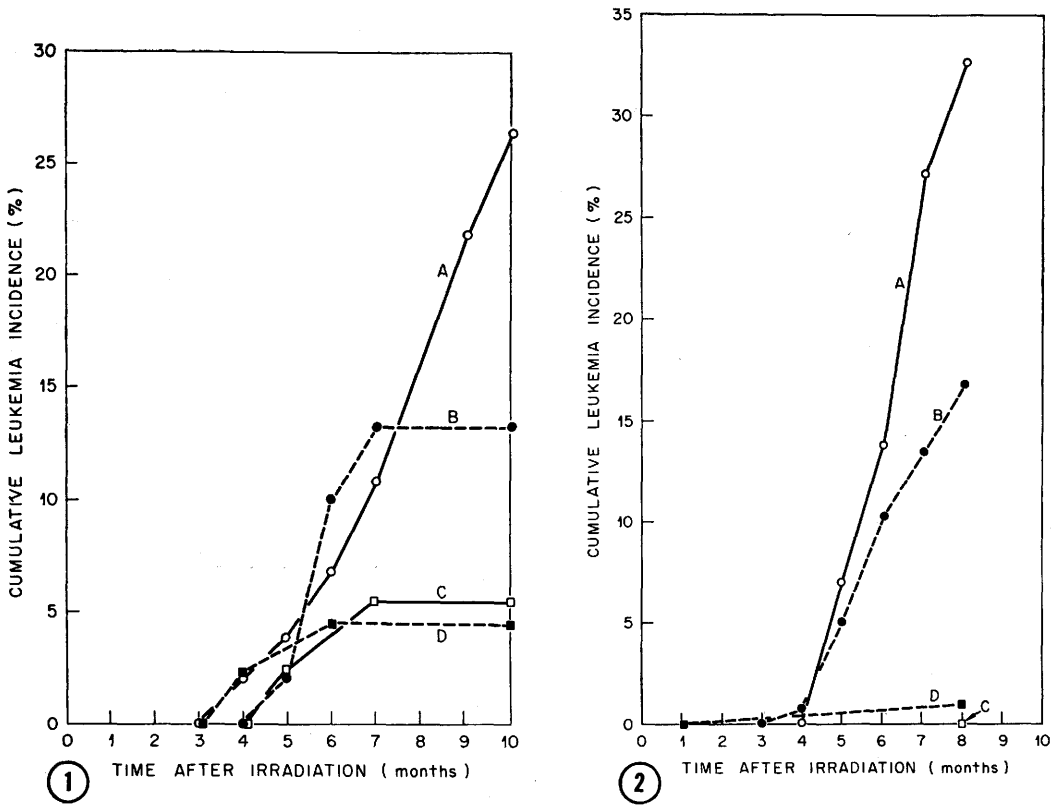

FIG. 1. Cumulative incidence of leukemia in germfree and conventional ICR mice in relation to time after irradiation. A. O——O, Irradiated germfree mice (31 males and 22 females). B. ●-----●, Irradiated conventional mice (36 males and 15 females). C. □——□, Nonirradiated germfree mice (25 males and 20 females). D. ■-----■, Nonirradiated conventional mice (24 males and 23 females).

FIG. 2. Cumulative incidence of leukemia in germfree (RFM/Uf) and conventional RF mice in relation to time after irradiation. (Data for conventional mice from Upton *et al*(15), and A. C. Upton and J. W. Conklin, unpublished data.) A. O——O, Irradiated germfree mice (12 males and 16 females). B. ●-----●, Irradiated conventional mice (104 males and 94 females). C. □——□. Nonirradiated germfree mice (11 males and 15 females). D. ■-----■, Nonirradiated conventional mice (267 males and 1081 females).

crobial contamination from the environment. At the same time, our data are consistent with the hypothesis of vertical transmission of a leukemia virus; *i.e.*, certain radiation-induced leukemias of conventional RF mice involve the action of a leukemogenic virus (10) which may be conceived to have been transmitted to the germfree mice *via* the fertilized ovum, the placenta, or the milk(2,11). The interpretation of a viral etiology is further supported by the occurrence of characteristic "leukemia-virus-like" particles in the tissue of a leukemic germfree animal, although the exact nature of the particles and the transmissibility of the leukemias by cell-free materials remain to be established.

Since the germfree mouse has been found to be more sensitive to the effects of inoculated Friend leukemia virus(12), our data are consistent with the view that the germfree mouse is, likewise, more susceptible to the leukemogenic effects of an endogenous leukemia virus "activated" by irradiation. The explanation for this may lie in the relatively poor development of the immunological defense system of the germfree mouse, particularly of the lymphatic system(13), although the absence of an "interfering" virus, or other explanations, must also be considered. Ethylene oxide sterilization, for ex-

ample, has been implicated in the production of carcinogens in mouse bedding material (14), and ethylene oxide was used to sterilize our isolators and contained cages. Bedding, food, and water were all sterilized with high pressure steam, however, and the isolators themselves were thoroughly purged of ethylene oxide and dried before use. Hence, it is questionable whether there was any contact of the mice with ethylene oxide residues. Furthermore, the cumulative incidence of spontaneous leukemia was as high in conventional as in the germfree mice, and supplies for the conventional RF mice had no contact whatever with ethylene oxide.

Glomerulosclerosis in conventional RF and CF-1 mice rendered leukemic by injection of radiation-induced myeloid leukemia virus or Rauscher virus has been noted earlier(9). The occurrence of a similar glomerulosclerosis in our leukemic germfree mice indicates that the pathogenesis of this lesion is not dependent on the presence of the conventional microbial flora. Its relation to leukemia is under study.

Summary. Germfree ICR and RF mice X-irradiated in a single exposure to 300-900 R have been observed for leukemia for periods up to 10 months after irradiation, along with nonirradiated controls. Preliminary data indicate that the cumulative incidence of leukemia in the irradiated germfree mice is as high as, or higher than, in the irradiated conventional mice. The incidence is higher in both irradiated groups than in the unirradiated germfree and conventional controls, which do not differ in spontaneous leukemia incidence. All leukemias examined thus far histopathologically have been lymphosarcomas. Tissues of a germfree RF mouse with X-ray-induced leukemia contained tailed "virus-like" particles similar to those noted in conventional mice and associated with transmission of the disease. Both germfree and conventional mice with leukemia showed renal glomerulosclerosis.

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