formaldehyde and then coated with antigen at low pH. After the desired HA titer was attained, the cells were stored in liquid nitrogen. Antigen-coated cells stored at —20° gradually deteriorated during the course of 18 months.

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## A Comparative Study of the Leukemogenic Effects of Strontium-90 and X-Rays in Mice (33553)

T. Ito, K. Yokoro, A. Ito, and E. Nishihara (Introduced by Jacob Furth)

*Research Institute for Nuclear Medicine and Biology, Hiroshima University, Hiroshima, Japan*

Radiostrontium ($^{85}$Sr, $^{89}$Sr, and $^{90}$Sr) is selectively deposited in the skeleton and induces osteogenic sarcomas (1). Finkel (2) reported that it can also induce leukemia, although the incidence is low. While studying the induction of osteogenic sarcomas by $^{90}$Sr, we observed a high incidence of leukemia in a low leukemia strain of mice. In the present paper, the leukemogenic effect of $^{90}$Sr is compared with that of fractionated total-body X-irradiation.

*Materials and Methods. Animals.* Female mice of ICR/JCL strain, purchased from the Nihon Clea Co., Tokyo, Japan (CLEA), were employed. This strain stems from ICR/Ha, in which incidence of spontaneous leukemia is less than 1.0% (3). Mice were kept in metal cages, 10 animals in each, in an air-conditioned animal room and maintained on commercial pellets and water *ad libitum*. Thirty-three ICR/JCL female mice, of the line originally derived from CLEA and subsequently bred in this laboratory by brother-sister mating, have been maintained without any treatments as controls.

*$^{90}$Strontium.* $^{90}$Strontium chloride ($^{90}SrCl_2$), purchased from the Radiochemical Center, Amersham, Buckinghamshire, England, was diluted with physiological saline.

*X-irradiation*: X-irradiation was provided with a 180 kVp X-ray generator, the factors being 25 mA, HVL 1.18 mm Cu, with 0.5 mm Cu and 0.5 mm Al filters; it delivered at 50 rads/min at the TSD 65 cm.

Expt. I. The mice were divided into 4 groups at random. The first group was injected intraperitoneally with 1.0 $\mu$Ci of $^{90}$Sr/g of body weight at 5 weeks of age. The second group was thymectomized at 3 weeks of age and injected with 1.0 $\mu$Ci/g of $^{90}$Sr at 5 weeks of age as the first group. The third group was X-irradiated with 1000 R at 4 weeks of age over the right hind leg while other parts of the body were shielded with 10-mm thick lead plates, and was injected with 1.0 $\mu$Ci/g of $^{90}$Sr at 5 weeks of age. The fourth group was given only 1000 R over the right hind leg as the third group. Expt. I was started with 63 mice, 17 in the first, 14 in the second, 16 in the third, and 16 in the fourth group.

Expt. II. Expt. II was started 6 months after Expt. I. The mice were divided into 4 groups at random. The first two groups were treated in the same way as the first or the second group in Expt. I. The third group was given total-body irradiation in four doses of 170 R X-rays at 5-day intervals beginning at 4 weeks of age, according to the schedule of Kaplan and Brown (4). The fourth group was previously thymectomized at 3 weeks of age and given total-body irradiation of the same doses of X-rays as the third group. Expt. II was started with 83 mice, 17 in the first, 20 in the second, 22 in the third, and 24 in the fourth group.

The mice were sacrificed when moribund or showing typical symptoms of leukemia or osteogenic sarcoma. All animals were autopsied. Sections for microscopic examination

TABLE I. Incidence of Leukemia in Female ICR/JCL Mice following Treatment with $^{90}$Sr or X-ray.[a]

| Treatments | Incidence of leukemia | | |
|---|---|---|---|
| | Expt. I | Expt. II | Total |
| $^{90}$Sr alone | 11/17 (64.7) | 10/17 (58.8) | 21/34 (61.8) |
| Thymex + $^{90}$Sr | 12/14 (85.7) | 11/20 (55.0) | 23/34 (67.6) |
| Total-body X-ray | — | 17/22 (77.3) | 17/22 (77.3) |
| Thymex + total-body X-ray | — | 5/24 (20.8) | 5/24 (20.8) |
| Local X-ray + $^{90}$Sr | 12/16 (75.0) | — | 12/16 (75.0) |
| Local X-ray alone | 6/16 (37.5) | — | 6/16 (37.5) |
| No treatment[b] | — | 0/33 (0) | 0/33 (0) |

[a] Data up to 630 days following the treatment with $^{90}$Sr or X-rays; percentage given in parentheses.

[b] Data up to 360 days after birth.

were fixed in 10% neutral formalin, embedded in paraffin and stained with hematoxylin–eosin. Blood smears and imprints of hematopoietic tissues were stained with May–Grünwald–Giemsa and McJunkin's peroxidase stains. In a few cases, alkaline phosphatase activity of leukocytes in the blood smears and the tissue imprints was assayed by the method of napthol AS-MX phosphate (5).

*Leukemic cell transplantation.* Approximately 20% physiological saline suspension of leukemic cells was prepared, and 0.2–0.3 ml was injected intraperitoneally into young-adult mice of ICR/JCL strain to test the transplantability of the induced leukemias.

*Testing for virus.* A 20% homogenate in ice-cold physiological saline was made from blood, spleen, lymph nodes, or thymus of the leukemic mice induced by $^{90}$Sr or X-irradiation. It was centrifuged at 2000 rpm for 20 min and the supernatant fluid was recentrifuged twice each at 10,000 rpm for 10 min at 0° in a refrigerated centrifuge to remove the cell component. The final supernatant was injected intraperitoneally in newborn mice of ICR/JCL or ddN/JCL strain bred in this laboratory.

*Results. Incidence and type of induced leukemia.* Data on the incidence of leukemia developing up to 630 days following the treatments are presented in Table I. The purpose of Expt. I was to study the induction of osteogenic sarcomas by $^{90}$Sr in comparison with that by external irradiation. Unexpectedly leukemias occurred at a high rate in mice treated with $^{90}$Sr. $^{90}$Sr alone was fairly leukemogenic (61.8%). In contrast to X-irradiation, thymectomy of $^{90}$Sr-treated mice seemed to enhance the leukemogenicity (67.6%), although this increase was statistically not significant. Local X-irradiation alone was weakly leukemogenic (37.5%). Fractionated total-body X-irradiation was most highly leukemogenic (77.3%). This was lowered by thymectomy to 20.8% ($p<0.01$). In control mice, no leukemia occurred during 360 days from birth.

The latent periods of leukemia in various groups are shown in Fig. 1. In $^{90}$Sr-treated mice, the peak of leukemia development was between 90 and 210 days following injection, and was not affected by thymectomy or local X-irradiation. In mice treated with total-body X-irradiation, the peak was similar to that in $^{90}$Sr-treated mice. In thymectomized total-body X-irradiated mice and in locally X-irradiated, leukemias developed with a longer latent period.

The leukemias induced by $^{90}$Sr, including those occurring in thymectomized or locally X-irradiated mice, did not appear to be of thymic origin. There was a systemic enlargement of lymph nodes with moderate to marked hepatosplenomegaly. The thymuses were normal or atrophic. The leukocyte count of the leukemic mice varied from 1650 to 107,500/mm$^3$ (av: 75,400/mm$^3$), and in the

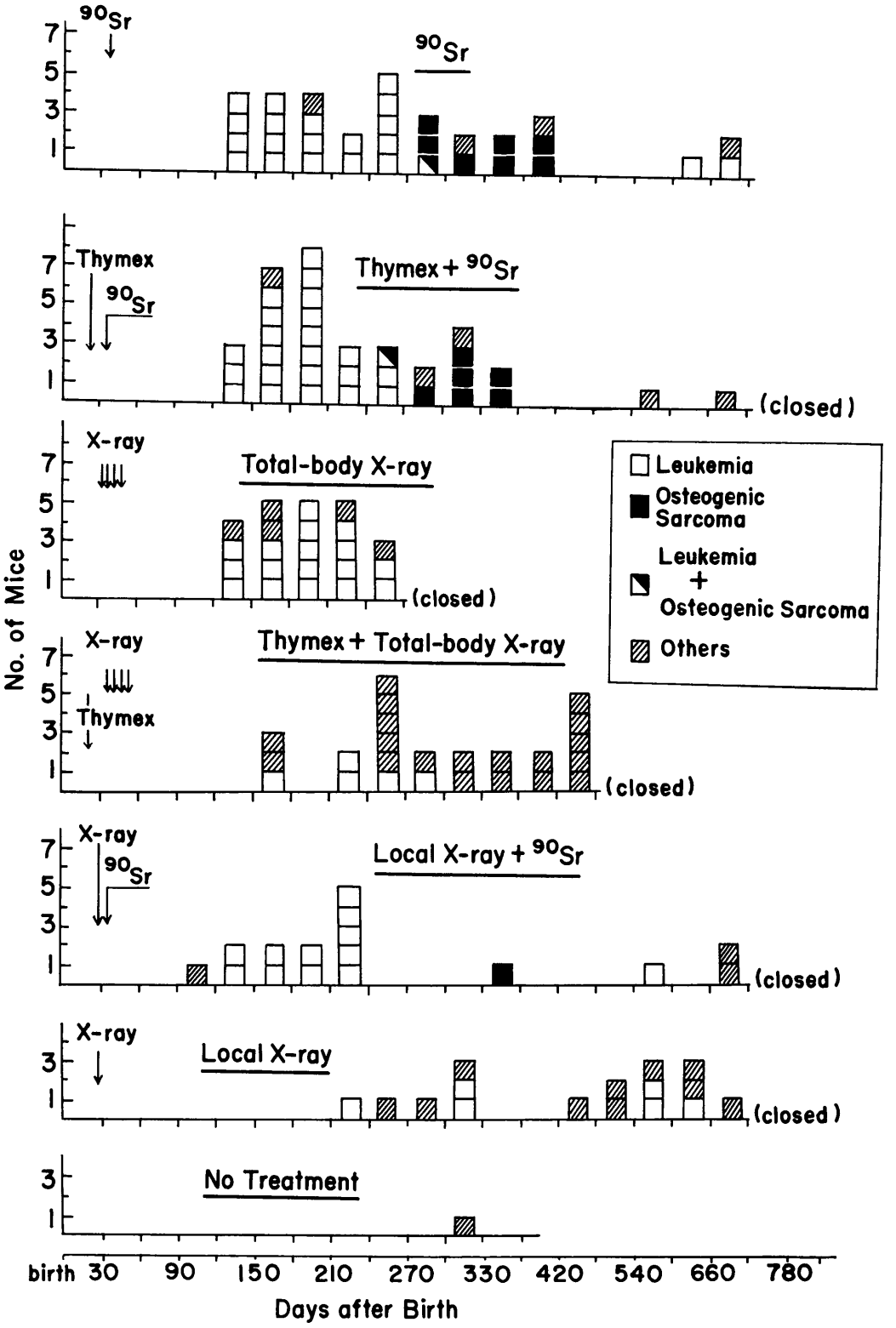


FIG. 1. Histogram of the latent periods of leukemia and osteogenic sarcoma induced by $^{90}$Sr and X-rays.

majority of cases, numerous leukemic cells were observed in the peripheral blood. Most of these leukemias were cytologically of lymphoblastic, reticulum cell, or stem cell type. The incidence of these three types was almost equal, although that of reticulum cell type was slightly higher in thymectomized mice. One of $^{90}$Sr-induced leukemias was erythremic, showing a picture similar to that in Friend or Rauscher leukemias (6).

Of 17 cases with leukemia induced by total-body X-irradiation in nonthymectomized mice, 16 were of thymic origin. One mouse had greatly enlarged lymph nodes but no thymic tumor. The 16 cases with thymic tumors were cytologically lymphoblastic. The exceptional case with no thymic tumor was of stem cell type. The leukemias induced by total-body X-irradiation in thymectomized mice showed a systemic enlargement of lymph nodes with moderate hepatosplenomegaly and were lymphoblastic or of stem cell type. The leukemias induced by local X-irradiation were macroscopically of nonthymic origin and cytologically of lymphoblastic or reticulum cell type. No myeloid leukemia developed in any group.

*Transplantability and transmissibility of induced leukemia.* The leukemias, induced either by $^{90}$Sr or X-rays, were almost invariably transplantable into young-adult ICR/JCL mice of both sexes, and the recipients died within about 4 weeks following the inoculation. An invasion by leukemic cells in the peripheral blood, greatly enlarged lymph nodes, especially in the abdominal cavity, and hepatosplenomegaly were observed. But thymus was not involved. Cytological studies revealed that the type of leukemia was the same as that of the respective cell donor.

In order to study the possible role of a virus in radiation leukemogenesis, extracts (presumably cell-free) of several leukemias induced either by $^{90}$Sr or total-body X-irradiation was injected into newborn ICR/JCL and ddN/JCL mice. Of 12 cases of leukemia originally induced by $^{90}$Sr or X-rays, 5 cases (3 cases by $^{90}$Sr and the other 2 cases by X-rays) were transmitted by the extracts, with a latency of 14–60 weeks, in both strains. The leukemogenic potency of the extracts varied from 7 to 57%. One of the leukemias induced by $^{90}$Sr has been transmitted in three successive animal passages up to date. The latencies in the successive passages varied from 10 to 54 weeks. Most of the leukemias induced by the extracts involved lymph nodes and the spleen. In a few cases, thymic tumors were also observed.

*Alkaline phosphatase activity of leukemic cell.* The alkaline phosphatase test was carried out in 15 mice with leukemia. In 4 cases with thymic tumors induced by total-body X-irradiation, all leukemic cells were alkaline phosphatase negative. In 13 cases with no thymic tumors (3 induced by $^{90}$Sr, 1 by total-body X-irradiation, 9 by the extracts from the leukemias produced by $^{90}$Sr or total-body X-irradiation), 40–100% of leukemic cells were positive. These alkaline phosphatase positive cases were lymphoblastic or of stem cell type.

*Other modes of death.* No acute death was observed in mice of any experimental group. Severe anemia and leukopenia caused death in two mice (one treated with $^{90}$Sr alone, another treated with local X-irradiation and $^{90}$Sr), 78 and 147 days after the treatments, respectively. Of 84 mice treated with $^{90}$Sr, 16 developed osteogenic sarcomas after a latency of 210–360 days (Fig. 1). Two of these mice had both leukemia and osteogenic sarcoma. No additive effects of local X-irradiation on the induction of osteogenic sarcoma by $^{90}$Sr were observed. A mouse treated with local X-irradiation and $^{90}$Sr died of a localized lymphoma developed in the retroperitoneal region 515 days after the treatments, being histologically lymphosarcoma. Adenocarcinoma of the lung was observed in a thymectomized total-body X-irradiated mouse 400 days after the treatments. Intercurrent deaths observed in some other mice were mainly due to pneumonia.

*Discussion.* Radiostrontium is selectively deposited in the skeleton as in calcium, damages the bone tissue, and induces bone tumors as a late effect (1). Carcinomas also occur in the neighborhood of heavy deposition as in nasal and oral cavities, and exter-

nal auditory meatus (7).

Although much is known about the damage caused by radiostrontium in bone marrow, little is known about its leukemogenicity. The reported incidence of leukemia in mice given radiostrontium is rather low (less than 20%) while that of bone tumors can approach 100% (1, 2, 8). In our experiments, more than 60% of female ICR/JCL mice given $^{90}$Sr developed leukemia after rather short latent periods. Van Putten and De Vries (9) found 44% lymphosarcomas and 19% reticulum cell sarcomas in $^{90}$Sr-treated female (CBA/Rij × C57B1/Rij)$F_1$ mice. However, it is not clear whether the induction of these diseases was by $^{90}$Sr, because the latent periods were very long (more than 500 days) and the incidences in their controls were similarly high (lymphosarcomas 35%, reticulum cell sarcomas 29%). The assumed $^{90}$Sr merely shortened the latent periods.

Host factors (species, strain, sex, age, etc.), no doubt, play an important role in the induction of leukemia as well as factors associated with the isotope and its administration (mass number, dose, method of administration, etc.). It has been reported the ICR/Ha and ICR/JCL mice develop spontaneous leukemias only exceptionally (3, 10). No leukemia developed in our 33 control mice up to 360 days of age and in 20 non-treated male mice of this strain maintained for other purposes up to 400 days. In our present experiments most leukemias of various cell types developed within 210 days following the administration of $^{90}$Sr. Thus, $^{90}$Sr exhibited a high leukemogenicity during "leukemia free age" in this strain of mice.

Many investigators have reported that removal of the thymus eliminates both the occurrence of spontaneous leukemias (11) and their induction by X-rays (12), chemicals (13), and virus (14). We confirmed this in X-irradiated ICR/JCL mice. However, thymectomy did not reduce the incidence of $^{90}$Sr-induced leukemia but seemed to promote it. There is a difference in the leukemogenic mechanism between external irradiation with X-rays and internal irradiation with $^{90}$Sr. The presence of the thymus appears to be a prerequisite for lymphoma induction by total-body X-irradiation. In induction of leukemia by $^{90}$Sr, some extrathymic sites seem to play a role. Since the irradiation from the decay of $^{90}$Sr-induced leukemia might be expected to be of bone marrow origin. Most leukemias induced by $^{90}$Sr were of lymphoblastic, reticulum cell, or stem cell type. No myeloid leukemia was found in our studies. It has been suggested that thymic lymphoma is not always the result of a direct effect of X-rays upon the thymus (15, 17). Joneja and Stich (17) suggested on the basis of chromosomal changes in hematopoietic tissues that X-irradiation produces progenitors of leukemic cells in bone marrow or spleen and these cells migrate into the injured thymus where they proliferate. We suppose that similar progenitor cells can be induced in bone marrow by a direct effect of $^{90}$Sr, and they migrate into various lymphoid and other tissues, but not into the thymus. This difference might be attributed to the fact that thymus does not seem to sustain any injuries due to $^{90}$Sr as it does following total-body X-irradiation. This contention may be further supported by the fact that the type of $^{90}$Sr-induced leukemia was not restricted to the lymphoid cell type regardless of the presence or absence of the thymus. The thymic environment is said to be necessary for the lymphoid differentiation of undifferentiated stem cell.

In the present study, the mice were given a single intraperitoneal administration of 1.0 $\mu$Ci of $^{90}$Sr/g of body weight. It is possible that there is a more efficient dose to induce leukemia. Nilsson (8) noted that the rate of leukemia development does not always increase with the increase of the accumulated doses of $^{90}$Sr. The higher dose of radiostrontium might kill the transformed cells or suppress the proliferation of these cells.

There are several reports implicating a virus in leukemogenesis by X-rays in mice (18, 19) and by $^{32}$P (20). In our present studies, 5 cases of leukemia (3 induced by $^{90}$Sr and 2 by total-body X-irradiation) were transmitted by leukemic cell extracts. Electron-microscopic and immunologic studies of the

virus are currently being carried out in this laboratory.

It is reported that murine thymic lymphoma cells have a strong alkaline phosphatase activity, whereas normal lymphocytes and thymocytes have none (cf. 21). In the present study, alkaline phosphatase activity was also observed in the leukemic cells of nonthymic origin which were induced by $^{90}$Sr, total-body X-irradiation, or by leukemic extracts. Further studies are needed to clarify this problem.

The latency of the osteogenic sarcomas induced in the $^{90}$Sr-treated is much longer than that of leukemia (1). This explains the lower incidence of osteogenic sarcomas in our mice.

*Summary.* The leukemogenicity of $^{90}$Sr was studied in comparison with that of fractionated total-body X-irradiation in the low leukemia ICR/JCL strain of mice. Fractionated total-body X-irradiation of 680 R yielded leukemia in 77.3% of mice, of which all but one were of thymic origin. A single intraperitoneal dose of 1.0 $\mu$Ci/g of body weight also had a high leukemogenic effect (61.8%). The $^{90}$Sr-induced leukemias were cytologically of lymphoblastic, reticulum cell, or stem cell type, not of thymic origin. No myeloid leukemia occurred in these mice. Thymectomy inhibited the leukemogenic effect of total-body X-irradiation as reported by others, while the induction of leukemia by $^{90}$Sr was not inhibited by thymectomy. It is postulated that X-irradiation produces progenitors of leukemic cells in bone marrow or spleen, and these cells migrate into the injured thymus where they proliferate, while the similar progenitor cells can be produced in bone marrow by direct effects of $^{90}$Sr. As grafted lymphomas they migrate into lymphoid and other tissues but not preferentially into the thymus. Three cases of leukemia induced by $^{90}$Sr and 2 cases by X-rays were transmitted by presumably cell-free extracts. It is suggested, therefore, that a viral factor may take part in the leukemia induction by $^{90}$Sr as it does in induction of leukemia by X-rays.

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