The Histopathologic Study of the Development of the Irradiation-induced Leukemia in C57BL Mice and of Its Inhibition by Testosterone*

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SUMMARY

It is probable that the coexistence of two factors is necessary for the development of leukemia in the C57BL mouse exposed to total-body irradiation. The first factor is destruction, followed by regeneration, of thymic lymphocytes. The second factor is the postirradiation environment which provides circumstances such that the regeneration of the thymic lymphocytes becomes uncontrolled, leading to the development of leukemia.

Testosterone, by inhibiting the regeneration of thymic lymphocytes after radiation injury, removes one of the two essential factors, thereby reducing the incidence of leukemia in irradiated C57BL mice.

Leukemia may be readily induced in various strains of mice either by ionizing radiation or chemical carcinogens (26), estrogens (16, 18), or by combinations of these agents (39, 56). Treatment of mice with testosterone decreases the incidence of spontaneous (52) and irradiation-induced leukemia (17, 36).

The present investigation was undertaken with two purposes in mind: (a) to investigate the histogenesis of leukemia induced in C57BL mice by irradiation and (b) to determine the mechanism by which testosterone inhibits or decreases the incidence of leukemia after irradiation.

MATERIALS AND METHODS

Five- to 6-week-old female C57BL/6 mice were obtained from the Cancer Research Genetics Laboratory, University of California, and from the Roscoe B. Jackson Memorial Laboratory.

No more than six animals were placed in identical stainless steel cages, 10 X 7 X 4.5 inches in size. They were maintained on Purina Laboratory Chow, given water ad libitum by water bottles, and kept in identical laboratory conditions. The cages were cleaned with boiling water once a week. The animals were checked at least once a day.

In total, 340 animals were distributed into four groups (Table 1). Group I was kept as a control, Group II was treated with testosterone, Group III received total-body x-radiation, and Group IV received total-body x-radiation and testosterone.

Testosterone was given as testosterone propionate (10 mg/cc in sesame oil), 0.05 cc. intramuscularly.

<table>
<thead>
<tr>
<th>GROUP</th>
<th>TOTAL NO.</th>
<th>DIED OR SACRIFICED WITHIN 12 WK. AFTER X-RAY</th>
<th>DIED OR SACRIFICED BETWEEN 12 AND 27 WK. AFTER X-RAY</th>
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<tbody>
<tr>
<td>I. Control</td>
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<td>29</td>
<td>27</td>
</tr>
<tr>
<td>II. Testosterone-treated</td>
<td>63</td>
<td>42</td>
<td>21</td>
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<tr>
<td>III. X-radiated</td>
<td>104</td>
<td>71</td>
<td>22</td>
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<tr>
<td>IV. X-radiated and testosterone-treated</td>
<td>107</td>
<td>79</td>
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particularly in a single injection, starting from the first day of irradiation and continuing twice weekly for 10 weeks, or until the experiment was terminated.

Kaplan's method of fractionating total-body irradiation into four equal doses given at 4-day intervals (30) was followed. In a preliminary experiment, a 362-r total dose (338 r \( \times 4 \)) resulted in a 90 per cent mortality. In subsequent experiments the total dose was reduced to 500 and 812 r, given in four equal doses. Physical factors of irradiation for the x-ray unit were: 220 kvP, 15.0 ma., 0.5 mm. Cu and 1.0 mm. Al, master cone only, perforated plastic plate above treatment box, ½ inch in thickness, 50 cm. to mid-thickness of mice. HVL-1.3 mm. Cu, 50 r/min in air at 50 cm. Six animals held in a 6.5 \( \times \) 6.5 \( \times \) 7.5-inch cardboard box were exposed to irradiation at a time.

Two animals from each of Groups III and IV were sacrificed 24 hours and 4 days after each dose of irradiation. Beginning 1 week after the last dose of irradiation, two to four animals from each of the four groups were sacrificed almost on a weekly basis, until obvious leukemia developed in Group III. These animals were selected at random. In addition, all moribund animals were sacrificed, and autopsies were also performed on all animals found dead except those which were too autolyzed.

Prior to sacrifice, tail blood was obtained for the determination of hemoglobin and of red blood cell, white blood cell, platelet, differential, and reticulocyte counts. These were performed by standard methods, except that micropipettes were used for the red and white blood cell counts. A direct method was used for the platelet count (54). The size of the thymus, liver, and spleen was measured. The intrathoracic organs (thymus, heart, lungs, and mediastinal lymph nodes) were removed en bloc. Representative tissue was obtained from liver, spleen, kidney, adrenal gland, aortic or mesenteric lymph nodes, and Peyer's patches. The right femur was removed and cut open so that the marrow was exposed to the fixative. All the tissues were fixed in Zenker formol solution (9:1 dilution) for 1–2 hours. After fixation the femur was decalcified in 5 per cent HNO\(_3\) for 20 minutes to an hour, depending on the age of the animals. After this the tissues were processed by a modified Maximow's technic (sectioning at 6 \( \mu \) in nitrocellulose, and staining by hematoxylin, cosin-azure II) (2). When the morphologic changes were minimal the specimens were re-examined as unknowns to avoid bias.

**Nomenclature.**—The term leukemia as used here refers to the disease variously called lymphoid tumor, lymphocytic neoplasm, lymphoma, lymphosarcoma of the thymus, lymphoblastoma, lymphatic leukemia, lymphogenous leukemia, lymphoid leukemia, lymphadenoma, malignant lymphoma, round-cell sarcoma, lymphosarcoma of Kundrat, and thymoma (12). When a neoplastic proliferation of abnormal lymphocytes was found the animal was classified as having leukemia.

**RESULTS**

**Tissues**

**Group I (Control)**

Leukemia was not observed in the animals in Group I during the period of 7 months following the initiation of the experiments (see Table 1). Specimens from these animals were used as controls for the other three groups.

**Group II (Testosterone-treated)**

Following continuous testosterone treatment gradual involution of the thymus occurred. By the end of 2 weeks it showed a definite decrease in size. By 10 weeks the thymus became markedly atrophic and occasionally was not visible macroscopically. When testosterone treatment was discontinued at the end of 10 weeks the thymus regained its normal size within the following 3 or 4 weeks. The thymus underwent further atrophy and usually could not be seen macroscopically when the testosterone was continued beyond 10 weeks.

The number of small lymphocytes in the thymic cortex was decreased within 2 weeks after the beginning of testosterone administration. This became more marked at 5 weeks (Fig. 2 vs. Fig. 1). The large lymphocytes and the reticulum cells became more prominent because of a marked decrease in the number of small lymphocytes. By 18 weeks the thymus was almost depleted of all types of lymphocytes (Fig. 3). Nevertheless, at no stage was there an increase in karyorrhexis or decrease in mitoses compared with those in the controls.

Following continuous testosterone administration no changes were observed in the myeloid tissue of the bone marrow or of the spleen. The lymphatic tissue of the spleen and the lymph nodes appeared to undergo a slight involution which was difficult to differentiate from the normal variation seen in control animals.

These findings, the involution of the thymus and lack of effect upon other lymphopoietic organs, confirm the observations made by Kaplan et al. (38).

To study further the more immediate effect of testosterone, an additional 21 animals were treated with 5 mg. of testosterone propionate intramuscularly and sacrificed at 2, 4, 9, 12, 24, 36, and 48 hours after treatment. Histopathologic study did not demonstrate any change which might explain
had been done previously (3). Animals which received 812 r had slightly more damage to hematopoietic tissue with slower regeneration than did animals receiving 500 r. Animals subjected to these two doses will be described together.

Twenty-four hours after first dose of irradiation.—In the animals sacrificed 24 hours after the first dose of irradiation the thymus was slightly decreased in size. Microscopic examination revealed karyorrhexis and a decrease in the number of small lymphocytes more marked in the cortex than in the medulla. Mitoses were also decreased in number. However, the small lymphocytes immediately under the capsule were hardly decreased in number. The lymphocytes in the medulla were increased in number. Eosinophils were slightly increased in number in the cortex. Changes in other hematopoietic tissues were not as striking as in the thymus. The bone marrow showed a decrease in cellularity, more marked in the erythroblastic series than in the granulocytic and megakaryocytic series. There was a compensatory expansion of the blood sinuses. Only a small amount of cellular debris was observed in the bone marrow. In the lymph nodes there was a slight reduction in the number of lymphocytes and a moderate amount of karyorrhexis. The spleen showed reduced lymphocytic and myeloid elements. The reticulum cells of the red pulp were more prominent than usual because of a marked reduction in the number of erythroblasts. These hematopoietic tissues had a lesser amount of nuclear debris than did the thymus.

Four days after first dose of irradiation.—The thymuses of the animals sacrificed 4 days after the first dose of irradiation and immediately prior to the second dose were approximately two-thirds of normal size. The cortex was repopulated by large lymphocytes, and mitoses were increased in number. The normal architecture, with cortex more cellular than medulla, had also been regained. Regeneration in the other hematopoietic tissues appeared to be somewhat less advanced than in the thymus. In the spleen and marrow regeneration of erythroblasts was more advanced than that of granulocytes and megakaryocytes.

From second dose to 24 hours after fourth dose.—Following each dose of irradiation, the hematopoietic tissues underwent damage and regeneration. Because the recovery after each dose of irradiation was far from complete at the time the following dose was given, the hematopoietic tissues became increasingly atrophic. Twenty-four hours after the last dose of irradiation the thymus was reduced to about one-third to one-fourth of the normal size. Its capsule became much thickened. The cortex could not be differentiated from the medulla (Fig. 4A). The number of lymphocytes in the cortex was decreased (Fig. 4B).

The marrow was more bloody than normal owing to an expansion of the sinuses. A few myelocytes, erythroblasts, and megakaryocytes remained. The spleen was reduced to about a third of normal size. The red pulp was almost completely depleted of myeloid and lymphatic cells. The white pulp was reduced in size, containing only a few lymphocytes. The lymph node was also very atrophic. Reticular and plasma cells were the dominant cell types.

Regenerative phase (24 hours to 6 weeks after fourth dose of irradiation).—Thymus: One week after irradiation the thymuses had increased in size to approximately half the size of the controls. The thymic cortex had been densely repopulated with lymphocytes (Fig. 5A). These cells were somewhat larger, and the nuclear chromatin was more delicate than that of the small lymphocytes which were the dominant cells in control animals (Fig. 5B vs. Fig. 1B). However, the cortical lymphocytes seen 1 week after irradiation appeared to be smaller than the lymphocytes seen 4 days after each dose of irradiation. Mitotic figures were still frequent.

Two weeks after irradiation the thymus was two-thirds of normal in size. The number of small lymphocytes in the thymic cortex had increased (Fig. 6). The majority of these lymphocytes, however, were still somewhat larger than those in the normal thymus (Fig. 6B vs. 1B). Three weeks after irradiation the thymus was decreased slightly in size owing to a moderate reduction in the number of lymphocytes in the cortex (Fig. 7). A similar temporary involution had been reported by Kaplan and Brown (33). Mitotic figures were seen in approximately normal number. There was no increase in karyorrhexis.

After this there was a gradual increase in size of the thymus and in number of thymic lymphocytes. The thymus regained normal size and structure in 5–6 weeks after irradiation (Fig. 8).

Spleen: One week after irradiation the spleen had almost regained normal size owing to an extremely active erythroblastic regeneration in the red pulp. These erythroblasts occurred in large clumps, occupying more than half of the red pulp. The white pulp had increased in size because of an accumulation of medium-sized lymphocytes. How-
ever, this regeneration was much less active than that of the erythroid series. Regeneration of the granulocytes and megakaryocytes was not yet observed.

Two weeks after irradiation the spleen became even larger than the control. Grossly visible nodules composed of dense aggregations of erythroblasts were seen in the red pulp. The regeneration of the white pulp was less than that of the red pulp. For the first time megakaryocytes and granulocyte precursors were found among erythroblasts.

During the following weeks there was a decrease in numbers of erythroblasts and myelocytes in the red pulp; the white pulp increased in size due to a reaccumulation of lymphocytes. By 5–6 weeks after irradiation the spleen again appeared normal.

Bone marrow: The bone marrow at the completion of irradiation was very atrophic as described above. However, in contrast to the splenic red pulp, some myeloid elements were demonstrable in the marrow 24 hours after the last dose of irradiation. One week after irradiation there was a remarkable regeneration of all myeloid cells. The blood sinuses were decreased in size. The cellularity of the bone marrow was approximately 50–60 per cent that of normal. Approximately 80 per cent of the marrow elements at this stage were erythroblasts, as compared with 20–25 per cent in control animals. The successive changes consisted of increasing cellularity with further decrease in size of blood sinuses. Granulocytes and precursors were increased so that in 2–3 weeks their number was greater than that of the erythroblasts. The bone marrow attained normal appearance in 5–6 weeks.

Lymph node: Analysis of lymph nodes was difficult because of their small size and variation in histologic appearance. A gradual accumulation of lymphocytes was observed, and lymphoid follicles were reformed in the cortex of the lymph node so that a normal appearance was attained within 5–6 weeks after irradiation.

In summary, after 500 r or 812 r of fractionated total-body irradiation, all the hematopoietic tissues, after an initial phase of necrosis and atrophy, had returned to normal in 5–6 weeks.

Over-regeneration of the thymus and the development of leukemia.—Six weeks after the last dose of irradiation, the thymus began to increase in size due to cortical hypertrophy. In those animals in which the size of the thymus still remained normal the cortex had expanded at the expense of the medulla, due to a proliferation of large lymphocytes (Fig. 9). These cells were twice as large as normal small lymphocytes (Fig. 9B vs. Fig. 1B).

The cytoplasm was scanty, and the nuclei were more delicate than those of normal lymphocytes. Mitotic figures were increased in number. This proliferative change usually involved the entire cortex of the thymus. In accordance with the observations of Carnes, there was no evidence to indicate that the proliferative changes originate anywhere except in the thymic cortex (6). However, in some cases it was localized to a small area or to one lobe of the gland. During this period there was no alteration in the lymph node, spleen, liver, bone marrow, and peripheral blood.

It was not possible to decide exactly when the leukemia began, because the change from normal was so gradual. The first unequivocal histologic evidence of leukemia was found in the thymus of an animal sacrificed 19 weeks after irradiation, similar to what had been observed by Kaplan on the basis of serial biopsies of the thymus in similar experimental material (27). The peripheral blood of this animal was still completely normal, and there was no enlargement of any organs at autopsy. On microscopic examination a striking abnormality was found in the thymus. The medulla was greatly reduced in size, with corresponding hypertrophy of the cortex (Fig. 10A). The latter consisted of dense sheets of stem cells (Fig. 10B). These cells were large in size, with scanty cytoplasm and pale-staining nuclei containing one or two small nucleoli. Other organs were normal in microscopic appearance.

In the following 15-week period after this first observation of the malignant change, 22 out of 35 animals in this group were found to have leukemia. In eight of these 22 animals the leukemic process was confined to the thymus without involvement of any other organ, and in two of these it was noted only in a part of the thymus.

Those animals which had widespread leukemia could be recognized in most cases by their inactivity, weight loss, and lusterless tails. Most of them had respiratory distress owing to an enlarged thymus, frequently associated with a straw-colored or serosanguinous pleural effusion. Liver, spleen, and lymph nodes were always markedly enlarged. Microscopic examination revealed extensive infiltration of these organs with medium or large lymphocytes (Fig. 11, A–F). In addition, the bone marrow and renal cortex were similarly involved. Leukemic tissue was not found in the renal medulla.

Group IV (Irradiation plus Testosterone)

Thymus: Twenty-four hours after the first dose of irradiation, the hematopoietic tissues were similar to those in animals which received irradiation...
tion alone. In contrast, at 4 days after the first dose of irradiation, the thymus had fewer lymphocytes than did corresponding animals in Group III.

At 24 hours after the last dose of irradiation fewer lymphocytes were found in the thymus of Group IV than of Group III animals. Other hematopoietic organs showed a degree of atrophy similar to that seen in animals in Group III.

One week after irradiation the thymus was about one-third to one-half the size of the thymus of corresponding animals in Group III. Microscopic examination revealed an increase in number of large lymphocytes in the thymic cortex of animals in Group IV. However, the number of lymphocytes and of mitotic figures was much less than that seen at a corresponding time in the thymuses of Group III (Fig. 12 vs. Fig. 5).

In the following weeks the thymus rapidly decreased in size to such an extent that frequently it was not visible macroscopically. The thymus consistently showed a marked reduction in the number of lymphocytes (Figs. 13 and 14). It was composed primarily of cords of reticular cells with only a few lymphocytes. The thymus remained atrophic as long as the testosterone treatment was continued. The thymus fully regenerated within 3–4 weeks after cessation of 10 weeks of testosterone treatment. During the following 1–2 weeks except in two animals, there was no evidence of the hyperproliferation seen in the thymus of Group III animals. However, in two animals of Group IV at sacrifice the thymic cortex contained large lymphocytes, although it was normal in size. Two other animals in Group IV died of leukemia, proved by post-mortem examination (Table 1) at 5 months after irradiation.

Myeloid Tissue: Regeneration of the myeloid tissue of the bone marrow and red pulp of the spleen in Group IV was not significantly different from that in the animals which received x-radiation alone (Group III). A moderate retardation of the lymphatic tissue of the spleen and lymph nodes was observed. However, this was rather difficult to interpret because of variation of the amount of lymphatic tissue even in the controls.

**Peripheral Blood**

- **Group I (Control)**
  
  Peripheral blood counts of 50 control animals are summarized in Table 2A.

- **Group II (Testosterone-treated)**
  
  Peripheral blood counts were done in 40 animals from the testosterone-treated group. The hemoglobin and red count were not significantly different from those of the controls. The white blood cells fluctuated around the lower limit of normal. Blood counts were obtained from an additional 30 animals prior to and after treatment with testosterone in sesame oil or sesame oil alone. There was a drop in white count in both groups similar to that described for Group II animals. Therefore, the decreased white count in the testosterone-treated animals was apparently not due to a specific action of testosterone. The platelet count was not altered by treatment with testosterone.

**Group III and Group IV**

There was little difference in the blood counts of the animals which received 500 r alone and those which received 500 r and testosterone during the first 6 weeks after treatment (Charts 1–4). However, the hemoglobin returned to normal faster in the animals which received 812 r and testosterone than in the animals which received 812 r alone. The blood counts of animals with leukemia are presented in Tables 2B and 2C. The counts of animals of both Group III and Group IV not developing leukemia were within normal range.

The peripheral blood was normal in all but one animal when leukemia was confined to the thymus (Table 2B). In this animal (No. 6) there was a mild anemia.

Some animals (Table 2C) with widespread leukemia had a mild anemia and thrombopenia and...
a moderate lymphocytosis and reticulocytosis. Numerous smudge cells were usually present in blood smears. Most of the white blood cells were small to large atypical lymphocytes. A few reticular cells were also seen in the blood smear of one animal.

**MISCELLANEOUS OBSERVATIONS**

Loss of hair was observed following prolonged testosterone treatment (Groups II and IV). These animals also showed a markedly atrophic X zone of the adrenal cortex similar to that in the previous observation (9, 10, 49, 55). After testosterone was discontinued, this zone regenerated very rapidly.

The differences in reaction to irradiation of Group III and Group IV animals were:

1. Repopulation by lymphocytes of the thymus of Group III and not of Group IV in the first 6 weeks after irradiation.
2. Uncontrolled proliferation of medium and large lymphocytes beginning at 6 weeks after irradiation in the thymus of Group III, in contrast to failure of regeneration of lymphocytes in Group IV as long as treatment with testosterone was continued.
3. Development of leukemia in Group III and lack of development of leukemia in all but two animals in Group IV.

**DISCUSSION**

**INCIDENCE OF LEUKEMIA IN C57BL MICE**

Strain C57BL mice has a 5-8 per cent incidence of spontaneous leukemia occurring primarily after 1 year of age (18, 30). In the present study no animal lived longer than 9 months so, as anticipated, leukemia did not develop in control animals (Group I). Kaplan and Brown (30) reported an incidence of lymphoid tumors in up to 98 per cent of C57BL female mice exposed to 951 r fractionated total-body x-radiation begun at the age of 33 ± 3 days. In this study a large number of irradiated animals were sacrificed before they had lived long enough to develop leukemia. Consequently, the incidence of leukemia is lower than that reported by Kaplan and Brown (30).

**PATHOGENESIS OF LEUKEMIA**

These studies and those of Kaplan, Nagareda, and Brown (38) suggest that the development of leukemia in C57BL mice exposed to irradiation depends on the coexistence of two factors: (a) a thymus in which the lymphocytes undergo degeneration, atrophy, and regeneration, and (b) factor(s) other than the thymus.

The thymus.—The special role of the thymus in the development of spontaneous and induced leukemia in mice has long been recognized. Enlargement of the thymus was always found in spontaneous leukemia in Ak strain mice (14, 51). Thy-mectomy reduces the incidence of spontaneous leukemia (14, 42, 51) and of leukemias induced by methylcholanthrene (43), irradiation (35, 35), and by leukomogenic virus (20, 46). Law (41), working with the high-leukemia RIL and the low-leukemia C3Hb mice, observed a marked increase in the incidence of leukemia in (C3Hb × RIL)F1 bearing grafts of RIL thymic tissue as compared with control (C3Hb × RIL)F1 mice. Our observations and prior observations (25, 26, 32) that the first evidence of leukemia was found in the thymus and
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CHART 3.—White blood cell counts in C57BL mice given total-body x-radiation with and without testosterone treatment (0.5 mg. testosterone propionate in sesame oil twice a week).

that the thymus was involved in every animal which had leukemia further support the concept that the thymus plays a special role in the development of leukemia in these mice.

The thymus, similar to other hematopoietic tissues, undergoes a degeneration, atrophy, and regeneration following total-body irradiation. However, unlike other tissues, the thymus later becomes the site of an uncontrolled lymphocytic proliferation leading to the development of leukemia. The importance of this cycle of degeneration, atrophy, and regeneration in the thymus is supported by prior experiments. Arnesen (1) noted that the thymus atrophied prior to the development of spontaneous leukemia in AKR/O mice. Shielding the thymus in total-body irradiated mice, which prevents degeneration of the thymic lymphocytes, also prevents the development of leukemia (24).

Leukemia develops when nonirradiated thymic grafts are implanted subcutaneously into thymectomized, irradiated mice, and, furthermore, the leukemia arises from the transplanted thymus (37). These observations apparently contradict

CHART 4.—Platelet counts in C57BL mice given total-body x-radiation with and without testosterone treatment (0.5 mg. testosterone propionate in sesame oil twice a week).
our hypothesis that the thymus must undergo degeneration, atrophy, and regeneration (repopulation by lymphocytes) in order for leukemia to develop. However, thymic lymphocytes are almost entirely destroyed and then regenerate when the thymus is transplanted (5, 6, 7, 53). Thus, the sequence of events after irradiation (degeneration, atrophy, and regeneration) is duplicated regardless of whether the newly formed lymphocytes are derived from the host (44, 45), from the nonirradiated thymic graft (37), or from both sources.

The thymus is unique in being the only lymphopoietic organ in which endodermal tissue is intimately mixed with mesenchymal tissue (50). The thymus reacts differently to irradiation from other lymphatic tissues as shown by the following observations: (a) thymic lymphocytes are more sensitive to irradiation than other lymphocytes; (b) regeneration after irradiation is more rapid; and (c) at about 3 weeks after irradiation, as already reported by Kaplan and Brown (33), the regeneration of the thymus is interrupted by a transitory partial involution. It is not known whether these differences account for the special role played by the thymus in the development of leukemia.

Factor(s) other than the thymus.—Irradiation of the thymus alone does not cause leukemia (94), even though the thymus goes through successive phases of degeneration, atrophy, and regeneration. Transplantation of a normal thymus into nonirradiated mice, which also induces the same sequence of events, does not result in leukemia. However, transplantation of a nonirradiated thymus into a thymectomized irradiated mouse causes leukemia (36), and an abnormal regeneration of the thymic lymphocytes occurs (5). Therefore, there must be some factor(s) other than the thymus needed to produce leukemia in the total-body-irradiated mouse.

What, then, is the nature of the factor(s) other than the degeneration and regeneration of the thymic lymphocytes? Shielding the spleen (47, 48), or leg (28, 31), as well as intravenous injection of bone marrow of splenic cells (8, 34, 48), prevents leukemia in irradiated mice. These procedures do not alter the initial degenerative effect of total-body irradiation upon the thymic lymphocytes. However, regeneration of thymic lymphocytes is more rapid when total-body irradiation is modified by leg shielding (29) or injection of bone marrow

### TABLE 2

**BLOOD COUNTS**

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<tr>
<th>ANIMAL NO.</th>
<th>Hb. gM, %</th>
<th>RBC (10^6 / \text{cc} ) MM</th>
<th>WBC (10^6 / \text{cc} ) MM</th>
<th>PLATELETS (10^6 / \text{cc} ) MM</th>
<th>RETICS. %</th>
<th>DIFFERENTIAL</th>
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<tr>
<td>1</td>
<td>14.2</td>
<td>9.60</td>
<td>13.20</td>
<td>944.0</td>
<td>4.6</td>
<td>% Lymphocytes</td>
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<td>2</td>
<td>13.9</td>
<td>9.22</td>
<td>7.70</td>
<td>889.0</td>
<td>3.6</td>
<td>% Neutrophils</td>
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<td>3</td>
<td>14.8</td>
<td>8.72</td>
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<td>14.3</td>
<td>10.74</td>
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<td>8</td>
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<td>9.80</td>
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<td>14.2</td>
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<td>40 M, 53 L, (45 Sm)</td>
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<td>10</td>
<td>14.3</td>
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<td>13.45</td>
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<td>15.2</td>
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<td>17.4</td>
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<td>13.4</td>
<td>23.05</td>
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<td>Atypical lymphocytes and reticular cells</td>
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<td>13.7</td>
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<td>19.35</td>
<td>79.0</td>
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<tr>
<td>16</td>
<td>14.5</td>
<td>47.30</td>
<td>95.7</td>
<td>15.0</td>
<td>46 S, 39 L</td>
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</table>

* Mean and standard variation based on 50 controls.

Hb = hemoglobin; RBC = red blood cells; WBC = white blood cells; Retics. = reticulocytes; S = small lymphocytes; M = medium lymphocytes (lymphoblasts); L = large lymphocytes (stem cells); Sm = smudge cells (ruptured lymphocytes) per 100 WBC.
This would suggest that, for leukemia to develop in C57BL/6 mice, regeneration of thymic lymphocytes must take place in a specific environment such as that provided by total-body irradiation. Injection of hematopoietic cells or protection of these cells probably modifies the post-irradiation environment through cellular re-population (13, 19) rather than by humoral factor (21).

Chart 5 illustrates the interrelationship of the two factors needed for the development of leukemia in C57BL mice exposed to total-body irradiation.

Mechanism of the Protective Effect of Testosterone against Leukemia in Mice

A relation between sex hormones and leukemia in mice has been long recognized. The incidence in females is higher than in males in certain strains (51, 52); estrogens (15, 40) increase and testosterone decreases (17, 32) the incidence, and ovariectomy but not orchiectomy (51) reduces the incidence of spontaneous leukemia. On the contrary, Murphy (52), working with spontaneous leukemia in Rockefeller Institute strain of mice, reported that orchiectomy resulted in an increase in the incidence of leukemia, whereas ovariectomy had no significant effect. However, these findings are not applicable to all strains of mice, because Kaplan (25) did not find that gonadectomy had a significant influence on the incidence of irradiation-induced leukemia in C57BL mice.

Daily injection of 500 μg of testosterone into rats causes more reduction in the weight of the thymus than of the lymph node and of the spleen (53). These results agree with our observation and that of Kaplan et al. (38) that injection of testosterone causes a greater reduction in the number of lymphocytes in the thymus than of the spleen or lymph nodes. This may be due to direct destruction as found with glucocorticoids (11, 58) or to an inhibition of production of the lymphocytes or a combination of these factors. An inability to demonstrate destruction of lymphocytes after injection

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**Chart 5**—Mechanism of the development of leukemia in mice exposed to irradiation.
of testosterone does not exclude this possibility, because under the influence of low-grade but prolonged irradiation one may completely deplete a hematopoietic organ without any demonstrable increase in karyorrhexis or decrease in mitotic activity (22).

Testosterone had a relatively insignificant effect upon the degeneration of thymic lymphocytes induced by total irradiation. In contrast, testosterone (a) inhibited the regeneration of lymphocytes in the thymus which occurred during the 6 weeks after irradiation and (b) inhibited the malignant proliferation of lymphocytes which began 6 weeks after irradiation. Testosterone did not significantly modify the effect of irradiation on other hematopoietic tissues.

We, therefore, suggest the following mechanism to explain the inhibition of the development of leukemia in C57BL mice exposed to irradiation and injected with testosterone. We have already indicated the probability that two factors (regeneration of the thymus and the post-irradiation environment) must be present in order for leukemia to develop. Testosterone, by preventing the regeneration of the thymus, removes one of the two essential factors (see Chart 5F). Furthermore, when testosterone was discontinued after 10 weeks the thymus regenerated and leukemia did not develop in animals followed for an additional 5 months. On the other hand, the development of leukemia is not inhibited if treatment with testosterone is not begun until 3 weeks after irradiation (38). Therefore, not only must the two factors be present, but they must coexist.

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Fig. 1.—Normal thymus. (A) Normal architecture; there are more lymphocytes in the cortex than in the medulla, X100. (B) Cortex of Fig. 1A composed of dense sheet of small lymphocytes, X1300.

Fig. 2.—Thymus after 5 weeks of testosterone treatment. (A) Decrease in width and in concentration of small lymphocytes in cortex, X100. (B) Cortex of Fig. 2A; fewer small lymphocytes and more prominent reticulum cells than in Fig. 1B, X1300.

Fig. 3.—Thymus after 18 weeks of testosterone treatment. (A) Marked reduction in size and no differentiation of cortex from medulla, X100. (B) Same thymus as in Figure 3A; even in the most cellular area there is a great decrease in concentration of small lymphocytes and increased prominence of reticulum cells, X1300.
Fig. 4.—Thymus 24 hours after the last dose of total-body irradiation. 
(A) Decrease in the number of cortical lymphocytes, X100. 
(B) Cortex of Fig. 4A, X1300.

Fig. 5.—Thymus 1 week after irradiation. 
(A) The cortex has been repopulated with large lymphocytes, X100. 
(B) Note comparatively large lymphocytes in the cortex versus normal small lymphocytes of Fig. 1B, X1300.

Fig. 6.—Thymus 2 weeks after irradiation. 
(A) Expansion of cortex due to increase in number of lymphocytes, X100. 
(B) Lymphocytes are smaller than those seen at 1 week (Fig. 5B) after irradiation, X1300.
Fig. 7.—Thymus 3 weeks after irradiation. (A) Partial involution due to a decrease in number of cortical lymphocytes, X100. (B) Higher power of Fig. 7A; decrease in number of lymphocytes, X1300.

Fig. 8.—Thymus, 6 weeks after irradiation. (A) Normal-appearing, compare with Fig. 1A, X100. (B) Higher power of Fig. 8A, normal appearing lymphocytes, X1300.

Fig. 9.—Thymus 8 weeks after irradiation. (A) Cortex has expanded at the expense of the medulla, X100. (B) Proliferation of large lymphocytes in the cortex, X1300.
Fig. 10.—Thymus 12 weeks after irradiation with definite evidence of leukemia. (A) Reduction in size of medulla due to cortical hypertrophy, ×100. (B) Extensive proliferation of stem cells in cortex, ×1200.

Fig. 11.—Leukemic infiltration in advanced leukemia. (A) Thymus, ×1200. (B) Bone marrow, ×300. (C) Liver, ×300. (D) Spleen, ×300. (E) Lymph node, ×100. (F) Renal cortex, ×100.
Fig. 12.—Thymus 1 week after irradiation and testosterone. (A) Lesser cellularity of cortex than in Group III at same time (Fig. 5A), X100. (B) Lesser number of lymphocytes than in Fig. 5B, X100.

Fig. 13.—Thymus 2 weeks after irradiation and testosterone. Decrease in size due to a reduction in the number of lymphocytes, X100.

Fig. 14.—Thymus 3 weeks after irradiation and testosterone. (A) Further atrophic change, X100. (B) Lymphocytes are markedly decreased in number with increased prominence of reticulum cells, X100.


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The Histopathologic Study of the Development of the Irradiation-induced Leukemia in C57BL Mice and of Its Inhibition by Testosterone

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