The Effects of Cortisone on the Development of Spontaneous Leukemia in Mice and on Its Induction by Irradiation

By A. C. Upton and J. Furth

The influence of pituitary, adrenal, thyroid, and gonadal hormones on lymphoid tissues is now recognized and the antilymphoid action of the adrenal cortex well known. Cortisone has proven to be effective in the treatment of lymphomas of laboratory animals and of man. It was found earlier that thymectomy or prolonged stress, which causes involution of the thymus, prevents the development of lymphoma in high-leukemia mice; therefore, it seemed desirable to investigate the effects of transient cortisone-induced lymphoid atrophy upon spontaneous lymphoma formation, as well as upon the induction of leukemia by ionizing radiation. The experiments here described confirm and extend the observations of Woolley and Peters and of Kaplan, et al., published while the present studies were in progress.

Materials and Methods

The mice used for the investigation of spontaneous lymphoma were of the high-leukemia strain AKR. Those employed in the study of radiation-induced leukemia were of the strain RF. The mice were x-irradiated by a G. E. Maxitron-250, with the following factors: 250 kvp., 30 ma., 3 mm. Al filtration (beryllium window), 93.7 cm. TSD, 57 r per minute. In each of the experiments the animals were caged and observed throughout life under standard laboratory conditions. Purina laboratory chow and drinking water were available ad libitum, unless otherwise stated, supplemented once weekly by chopped carrots, lettuce, and cabbage. All mice were autopsied at death, and histologic sections were prepared, as needed, to establish the major pathologic diagnoses.

Experiment 1

Cortisone (Merek) was injected subcutaneously, 1.0 mg. (0.04 ml.) daily for three successive days, into one hundred one AKR male mice 2 to 4 months of age. Sixty-three litter mates received simultaneously injections of cortisone vehicle (1.5 per cent benzyl alcohol in physiologic saline) in comparable amounts (0.04 ml.). All mice were observed until natural death or killed in extremis.

Experiment 2

To produce a more prolonged thymic and lymphoid atrophy, three series of injections of cortisone were administered to forty-nine male and fifty-two female AKR mice. The dosage and route of administration were as above, given at 9, 16, and 23 weeks of age. Simultaneously, litter mates received injections of cortisone vehicle, as above, (forty-five males and forty-one females) or were subjected to acute starvation, consisting of withdrawal of food but not of water (thirty-four males and sixty-six females).
TABLE 1.—Effect of Cortisone on Induction of Leukemia in RF Mice by Irradiation

<table>
<thead>
<tr>
<th>Type of treatment</th>
<th>Sex</th>
<th>No. of mice</th>
<th>Per cent incidence of leukemia</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>myeloid</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>thymic</td>
</tr>
<tr>
<td>Cortisone</td>
<td>♂</td>
<td>62</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>♀</td>
<td>73</td>
<td>4</td>
</tr>
<tr>
<td>Cortisone vehicle</td>
<td>♂</td>
<td>71</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>♀</td>
<td>79</td>
<td>0</td>
</tr>
<tr>
<td>X-irradiation</td>
<td>♂</td>
<td>65</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>♀</td>
<td>60</td>
<td>18</td>
</tr>
<tr>
<td>Cortisone before x-irradiation</td>
<td>♂</td>
<td>60</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>♀</td>
<td>70</td>
<td>12</td>
</tr>
<tr>
<td>Cortisone after x-irradiation</td>
<td>♂</td>
<td>69</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>♀</td>
<td>62</td>
<td>21</td>
</tr>
</tbody>
</table>

Experiment 3

Male and female mice of the RF strain, 8 to 12 weeks of age, were injected subcutaneously with cortisone, 1.0 mg. daily on three successive days, beginning one week before or two to three weeks after 350 r whole body x-irradiation (table 1). Cortisone vehicle was injected simultaneously into litter mates in comparable amounts.

RESULTS AND DISCUSSION

Effects of Cortisone on the Development of Spontaneous Lymphoma

Experiments 1 and 2. In AKR mice three successive daily injections of cortisone brought about a severe thymic and lymphoid atrophy, lasting several weeks; regeneration, however, was complete within sixty days. This transient lymphoid atrophy was followed by a slight reduction in the incidence of lymphoma (fig. 1). Three sets of cortisone injections resulted in a more sustained, although not permanent, lymphoid involution, which was correlated with a greater inhibition of lymphoma development. This was manifested by a delayed onset and reduced total incidence of lymphoma (figs. 2 and 3). It is noteworthy that at 10 months of age the cumulative incidence of lymphoma in cortisone-treated male mice was only 10 per cent, as compared with 55 per cent in the controls, while at 20 months the figures were 67 per cent and 84 per cent, respectively, indicating the relative magnitude of the delay in onset of lymphoma. After acute starvation lymphoma formation appeared to be delayed rather than prevented. There was no lasting weight reduction in the acutely starved animals; therefore, the effects on leukemia are attributed to nonspecific stress, mediated through the pituitary-adrenal mechanism, and not to underfeeding, which also inhibits the development of lymphoma in mice of this strain. The higher incidence of spontaneous lymphoma in the female conforms to earlier observations and may be correlated with the leukemogenic effects of estrogen. Correspondingly, the antileukemic action of cortisone was less effective in females. Quantit-
EFFECTS OF CORTISONE ON DEVELOPMENT OF SPONTANEOUS LEUKEMIA

Fig. 1.—The effects of three successive daily injections of 1.0 mg. of cortisone at 2 to 4 months of age on the development of spontaneous lymphoma in AKR male mice.

tative differences in antileukemic potency of the various experimental measures studied can be attributed to variations in the intensity and duration of corticoid stimulation.

It appears highly significant that even transient, cortisone-induced lymphoid atrophy inhibited the subsequent development of lymphoma. Since this effect was enhanced by repeating the injections of cortisone and thus preventing lymphoid regeneration, it would appear that a drastic reduction in the incidence of lymphoma, such as results from thymectomy, might be obtained by maintaining cortisone-induced suppression of lymphoid growth throughout life. This assumption is supported by the experiments of Woolley and Peters.

The lymphoid tumors developing after cortisone treatment were indistinguishable from those occurring in the vehicle-injected mice; both were characteristically thymic in origin. Thus, it is evident that transient involution of the thymic cortex did not uniformly abolish the leukemogenic influence of the thymus. The mechanism whereby lymphoma formation is inhibited by cortisone is not clear, but it is probable that leukemic and potentially-leukemic lymphocytes and lymphoblasts are eliminated by the cortisone-induced atrophy, while their stem cells in the stroma are not destroyed.

Effects of Cortisone on the Induction of Leukemia by Irradiation

Irradiation significantly increased the incidence of lymphomatosis in the female, principally by hastening the onset and augmenting the number of thymic
lymphomas, as noted earlier. The cumulative incidence of leukemias of various types is plotted against age in figure 4. In the female, cortisone alone or in conjunction with irradiation had no significant effect on the development of lymphoid tumors. Kaplan, et al. reduced the incidence of radiation-induced lymphomas in C57 black mice of both sexes by cortisone treatment postirradiation; however, their cortisone treatments were much more prolonged than those used in this experiment.

In the male, as in the female, irradiation caused an increased incidence of thymic lymphoma (p < 0.01); however, the combined frequencies of all types of lymphoma were not significantly altered (fig. 4), because nonthymic lymphoid tumors were reduced in number. The formation of nonthymic lymphomas could have been masked or prevented by the induction of myeloid leukemia by irradiation. Cortisone given before irradiation acted synergistically, increasing the incidence of thymic and other lymphomas over and above that caused by irradiation alone. This leukemogenic action indicates that the lymphoid tissues were more susceptible to the induction of lymphoma by irradiation when in an early regenerative state following cortisone-induced atrophy than when in their normal condition. Likewise, Kaplan and Brown observed that the sensitivity of the C57 black mouse to lymphoma induction by irradiation is greatest for a given total dose if the irradiation is fractionated at intervals of about four to eight days. They postulated that the heightened susceptibility depended upon the
increased mitotic activity, notably that of the reticulum, associated with regeneration. This is supported by the limited data of Deansley and of our own, assuming that the susceptibility of the cell to leukemogenic forces parallels its radiosensitivity, being maximal at the time of mitosis. However, a precise correlation between mitotic activity and sensitivity to leukemogenesis is yet to be made.

Cortisone administered after irradiation appeared to inhibit lymphoma induction in the male, although the effect was of questionable significance (p = 0.09). This finding conforms to the observations of Kaplan, et al. However, that lymphoma induction should be inhibited by both cortisone treatment postirradiation, which delays lymphoid recovery, and bone marrow infusion postirradiation, which accelerates lymphoid recovery. Apparently, different mechanisms are involved in these processes, and their understanding will require further study.

Cortisone, as irradiation, induces a drastic lymphoid and thymic atrophy, but unlike the latter it is not leukemogenic. This may be due to absence of mutagenic action or to failure of cortisone to injure the myelopoietic system. It is suggested by recent studies that partial shielding of the bone marrow during irradiation, as injection of bone marrow postirradiation, which hasten recovery of the bone
FIG. 4.—The effects of three successive daily injections of 1.0 mg. of cortisone at 8 to 12 weeks of age, beginning one week before or two to three weeks after 350 r whole body x-irradiation, on the induction of leukemia by irradiation in RF male and female mice. (From J. Furth and A. C. Upton: in: Ciba Foundation Symposium on Leukemia Research, London, J. A. Churchill, 1954.)
Fig. 5.—Promyelocytes and myeloblasts in smear of peripheral blood of mouse with myeloid leukemia. (Wright-Giemsa, X 750.)

Fig. 6.—Spleenic imprint of mouse with myeloid leukemia, composed almost entirely of primitive myeloid cells. (Wright-Giemsa, X 900.)

Fig. 7.—Myeloid leukemic infiltration of bone marrow, with extensive necrosis. (Hematoxylin and eosin, X 50.)

Fig. 8.—Diffuse myeloid leukemic infiltrations of liver. (Hematoxylin and eosin, X 150.)

Fig. 9.—Myeloblastic transformation of bone marrow. (Hematoxylin and eosin, X 150.)

Fig. 10.—Myeloid leukemic infiltration of adrenal. (Hematoxylin and eosin, X 150.)
marrow and lymphoid apparatus, inhibit lymphoma induction. Thus, in leukemogenesis, indirect or systemic factors appear to be important as well as local effects on lymphoid tissues.

The incidence of spontaneous lymphoid tumors in the nonirradiated male (14 to 21 per cent) and female (22 to 26 per cent) RF mice used in these studies is considerably higher than that observed earlier. This is attributed to genetic changes in the strain, confirmed by other recent experiments, probably due to accidental interbreeding of the AKR and RF lines during the process of moving the colony from laboratories at Cornell University to Oak Ridge.

*Radiation-Induced Myeloid Leukemia*

The occurrence of myeloid leukemia in both sexes of RF mice was drastically increased by a single dose of 350 r. Most currently studied radiation-induced leukemias are lymphoid and thymic. The observations made in Japan among human survivors of atomic bomb radiation indicating that a single exposure to ionizing radiation may produce a high incidence of myeloid leukemia lend significance to the similar finding in RF mice.

The disease in mice, as observed earlier, attained a peak incidence at 11 to 14 months of age (fig. 4). Its frequency was not affected by cortisone in the male (fig. 4). In the female, however, administration of cortisone prior to irradiation appeared to inhibit the induction of myeloid leukemia. Although the statistical significance of this effect is doubtful and its mechanism unexplained, it deserves verification and further study.

Since radiation-induced myeloid leukemia has not been previously described in detail, some of its salient features will now be summarized. Mice dying of myeloid leukemia were cachectic and pale. The spleen was characteristically greatly enlarged, smooth, and grayish-pink. The lymph nodes were but slightly enlarged, at times grayish-green (chloroleukemia), and the thymus was atrophic. Diffusely scattered subpleural and pulmonary petechiae, representing small hemorrhagic infarcts, were commonly encountered. Microscopically, leukemic infiltrations were consistently present in the bone marrow (figs. 7 and 9) and, to a lesser extent, in the spleen (fig. 6), liver (fig. 8), lymph nodes, and other organs (fig. 10). While the radiation-induced myeloid leukemias did not differ morphologically from the few spontaneously occurring cases available for study, necrosis of the bone marrow (fig. 7) was a common finding in the irradiated mice with myeloid leukemia (present in fifty-five of sixty-five cases) while lacking in the nonirradiated animals. This lesion may represent a late complication of irradiation; it is possible, however, that the pathogenesis of myeloid leukemia is related to this marrow injury. The degree of maturation of leukemic myeloid cells was variable, most cases being predominantly myelocytic or promyelocytic in type. Leukemic cells were present in the peripheral blood (fig. 5), which was typically anemic; leukostasis and leukemic thrombi occurred commonly in sections of blood vessels, notably those of the lung.

Neoplasms of reticulum cell type, including monocytic leukemia, occur late in life in mice of this strain, and they were encountered in all treatment groups (table 1). Their incidence was not significantly affected by cortisone.
EFFECTS OF CORTISONE ON DEVELOPMENT OF SPONTANEOUS LEUKEMIA

SUMMARY

The development of spontaneous lymphoma in a high leukemia strain of mice was inhibited by administration of cortisone, although the hormone-induced atrophy of the thymus and other lymphoid tissues was only transient.

Inhibition of lymphoma formation resulted from only three successive daily injections of cortisone but was greater if the cortisone administration was prolonged for three months.

A high incidence of myeloid leukemia was induced in a low-leukemia strain of mice by a single exposure to 350 r of x-radiation.

Cortisone was without effect on the induction of myeloid leukemia.

Cortisone administered preirradiation increased, and postirradiation decreased, the incidence of radiation-induced lymphomas in male mice.

SUMMARIO IN INTERLINGUA

Le disveloppamento de leucemia spontanee in un stirpe de muses de alte susceptibilitate a leucemia esseva inhibite per le administration de cortisona, ben que le atrophia inducite per le hormon in le thymo e le altere texitos lymphoide esseva solo transiente.

Le inhibition del formation de lymphomas resultava de injectiones diurne de cortisona in solo tres dies successive, sed illo esseva plus marcate con un administration de cortisona prolongate pro tres menses.

Un alte frequentia de leucemia myeloide esseva inducite in muses de un stirpe de basse susceptibilitate a leucemia per un singule exposition a 350 r de radios X.

Cortisona esseva sin influentia super le induction de leucemia myeloide.

Le frequentia de lymphomas inducite per radiation in muses mascule esseva altiate per le administration de cortisona ante le radiation; illo esseva reduceite per le administration de cortisona post le radiation.

REFERENCES

A. C. UPTON AND J. FURTH

The Effects of Cortisone on the Development of Spontaneous Leukemia in Mice and on Its Induction by Irradiation

A. C. UPTON and J. FURTH