STUDIES ON THE MECHANISM OF LEUKEMOGENESIS
BY IONIZING RADIATION

School of Aviation Medicine
Randolph Air Force Base, Texas

January 1959
STUDIES ON THE MECHANISM OF LEUKEMOGENESIS BY IONIZING RADIATION

A. C. UPTON, M.D.

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

Air University
SCHOOL OF AVIATION MEDICINE, USAF
RANDOLPH AFB, TEXAS
January 1959
STUDIES ON THE MECHANISM OF LEUKEMOGENESIS BY IONIZING RADIATION

The induction of leukemia by ionizing radiation is influenced by many variables, including radiation intensity, radiation dose, fraction of the body irradiated, genetic differences in susceptibility, age at irradiation, sex, and other physiologic factors. The influence of these variables on the induction of leukemia varies with the hematologic type of leukemia induced. Irradiation increases the susceptibility of adult mice to filterable leukemogenic agents that, administered after irradiation, enhance the development of leukemia.

The high carcinogenic potency of ionizing radiation is well known (14, 15, 18). Among the many types of neoplasms induced by radiation, leukemia is prominent in human beings (66) and in mice (14). Although mice are, in general, susceptible to leukemia induction, their responsivenes to a given amount of radiation varies, according to the influence of genetic, physiologic, and radiologic factors. The effects of these variables have been investigated in a series of experiments summarized in this report.

RADIOLOGIC FACTORS

Relation between leukemia incidence and radiation dose

Apart from the influence of physiologic factors, the dose, dose rate, and hematologic type of leukemia in question affect the incidence of induced leukemia.

In mice of most strains, lymphomas are readily induced by whole-body irradiation. These neoplasms develop predominantly in the thymus as lymphosarcomas and frequently become generalized, with involvement of the peripheral blood. The relation between the incidence of these neoplasms and the radiation dose is nonlinear, a break in the dose-response curve occurring between 100 and 400 r, depending on the strain of mice in question (fig. 1). Granulocytic leukemia is less often encountered in mice, those of the RF strain being a notable exception. A dose of only 150 r greatly increases the incidence of granulocytic leukemia in these animals (14, 2). Paradoxically, as sublethal radiation dose levels are approached, the induction of granulocytic leukemia declines. This is attributed to mortality of potentially leukemic mice early in life from radiation-induced diseases other than leukemia since, when the incidence is adjusted to correct for intercurrent mortality, there is no decline in the induction rate (fig. 3). The shape of the dose-response curve for the dose range below 150 r is not definitely known. Although available data suggest that it is not linear (fig. 4), this, of course, does not necessarily imply the existence of a threshold.

The incidence of other types of leukemia and of lymphomas arising outside the thymus is not significantly increased by radiation.

Influence of radiation intensity

As first shown by Kaplan and Brown (29), the induction of thymic lymphomas may be greater for a given dose of X-rays when the radiation is administered in appropriately timed fractions than when it is administered in a single, brief exposure (63). A similarly complex time-intensity relation is suggested by the work of Mole (54). From these studies, there seems to be an optimal dose rate for lymphoma induction, below which the effectiveness of the radiation is diminished. At greatly

Received for publication on 5 August 1958.
Incidence of lymphomas induced in mice by a single exposure to ionizing radiation.

Δ RF females (28).
O RF males (63).
Δ C57BL (28).
□ LAF, (17).

Cumulative incidence of granulocytic leukemia induced in RF male mice by a single exposure to x-rays at 10 weeks of age. Each treatment group contained 65 to 70 mice (A. C. Upton and F. F. Wolff, unpublished data).
FIGURE 3

Incidence of granulocytic leukemia induced in RF male mice by a single exposure to x-rays at 5 to 6 weeks of age (62). The adjusted incidence is the observed incidence corrected for intercurrent mortality not attributable to leukemia (45).

FIGURE 4

Incidence of granulocytic leukemia induced in RF male mice by a single exposure to radiation at 6 to 8 weeks of age (62). The combined data for x-rays and thermal neutrons are plotted against the dose of radiation expressed in rem on the basis of 20-day lethality. The observed points depart significantly from the fitted straight line, i.e., \( x^2 = 3.857; P < 0.01 \); however, because this significance test and the 95 percent confidence intervals shown neglect the possibility of extrabinomial variation, no\'t must be interpreted with reservation (A. W. Simball, personal communication).
reduced dose rates, however, the incidence of leukemia exceeds the control levels (fig. 5), as noted even in mice exposed throughout life to only 0.11 r per day (47). This is consistent with the elevated incidence of leukemia in radiologists (60).

Partial-body versus whole-body irradiation:

Induction of either granulocytic leukemia or lymphoma is greatly inhibited when a small fraction of the body is shielded from radiation (table I). It is noteworthy that, although such shielding essentially abolishes induction of lymphomas, it does not completely prevent induction of granulocytic leukemias. The effects of partial irradiation of different regions of the body and the possible importance of the small fraction of the radiation dose penetrating tissue beneath the shield are being investigated.

![Graph showing the incidence of leukemia in RF female mice exposed 25 hours daily throughout life to 70° gamma rays or Po-Be neutrons. Each treatment group contained 100 to 200 animals.](image)

**FIGURE 5**

Incidence of leukemia in RF female mice exposed 25 hours daily throughout life to 70° gamma rays or Po-Be neutrons. Each treatment group contained 100 to 200 animals. (A. C. Upton, J. A. Sproul, Jr., and M. L. Randolph, unpublished data).

**TABLE I**

Effects of partial-body shielding on leukemia induction by x-rays (63)

<table>
<thead>
<tr>
<th>X-ray dose*</th>
<th>Number of mice</th>
<th>Tissue exposed</th>
<th>Mean age at death (mo.)</th>
<th>Leukemia incidence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(r, (gm.·cm.)†</td>
<td></td>
<td></td>
<td></td>
<td>Myeloid</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>314</td>
<td>None</td>
<td>19.1</td>
</tr>
<tr>
<td>150</td>
<td>3,000</td>
<td>104</td>
<td>Whole body</td>
<td>15.6</td>
</tr>
<tr>
<td>300</td>
<td>6,000</td>
<td>104</td>
<td>Whole body</td>
<td>12.5</td>
</tr>
<tr>
<td>450</td>
<td>9,000</td>
<td>105</td>
<td>Whole body</td>
<td>10.3</td>
</tr>
<tr>
<td>450</td>
<td>6,000</td>
<td>86</td>
<td>Upper 2/3 body§</td>
<td>16.0</td>
</tr>
</tbody>
</table>

* 250 kVp, 50-100 r per minute, hvl 0.44 mm of Cu.
† 80-100 kvp.
‡ RF males, 8-9 weeks old at irradiation.
§ Pelvis and lower extremities shielded with lead.
|| Incidence adjusted (cf. 63) to correct for intercurrent mortality from causes other than leukemia.
jection of nonirradiated, viable, isologous bone
marrow (32) or spleen (7) cells after irradiation
has also been noted. On the basis of exist-
ing evidence, it seems that these procedures
have in common the effect of providing normal
hemopoietic cells that rapidly colonize and re-
populate the irradiated marrow and lymphoid
tissue of the recipient (8). Hence, it is logical that
they promote recovery of the weight of the
irradiated thymus (4). The mechanism
whereby partial shielding or injection of mar-
row inhibits leukemogenesis is unknown, but
since lymphomas are induced in nonirradiated
thymic tissue when it is implanted into ir-
radiated recipients (31, 34, 43, 44), it is
evident that systemic, radiation-induced dis-
turbances can themselves cause neoplastic
change in the thymus. Kaplan (28) has there-
fore postulated that the leukemogenic influence
is an exaggerated stimulation of growth elicited
by repair mechanisms and that only in the
presence of nonirradiated marrow cells can
thymic regene-
ration take place promptly enough to prevent
this excessive stimulation. In view of the possible
trole of viral
agents in leukemogenesis (12), however, it is
conceivable that the anti-oncogenic action of
intact marrow or spleen cells results from re-
covery-promoting effects on the irradiated im-
mune system of the host (3, 48 49).

PHYSIOLOGIC ACTORS

Strain differences

Susceptibility to the spontaneous develop-
ment and induction of leukemia varies greatly
from one strain to another. Within the same
strain, susceptibility to the leukemogenic ac-
ion of any one inducing agent is not necessarily
correlated with susceptibility to others (38).
Although strain differences have heretofore
been ascribed to genetic variables, it has also
been suggested that the high incidence of
lymphoma in certain strains may be caused by
leukemogenic viruses that are transmitted
from one generation to another in the germ
cells (21). Even susceptibility to the leuke-
mogenic action of such filterable agents, how-
ever, varies from one strain of mice to another
(13, 22, 64), presumably because of genetic
differences.

The spontaneous incidence of myeloid leuk-
emia is so low in most strains of mice and other
laboratory animals that only sporadic cases
have been reported in the literature (10).
Granulocytic leukemias have been induced in
mice, however, by administration of indol (6,
11), benzol (45), and ionizing radiation (16).
The unusually high susceptibility of RF mice
to induction of granulocytic leukemia by radia-
tion is transmitted in part to F, hybrid prog-
geny in the one strain combination (BALB/c
female x RF male) tested so far (table II).

Influence of age

Although in man acute leukemia is relatively
common in childhood, leukemia is rare in im-
mature animals of other species. In mice it is
a disease of adult life, increasing in frequency
with age (5). Age also affects susceptibility
to induction of leukemia by x-rays, its influence
varying with the hematologic type of leukemia
in question (table III). The decreases in sus-
ceptibility to lymphoma induction with age,
observed previously with x-rays (25), chemical
agents (55), and ACTH (58), occurs more
rapidly than might be expected on the basis
of thymic involution alone (27). This suggests
the influence of other age changes, such as
endocrine alterations.

The resistance of newborn mice to induction
of myeloid leukemia by irradiation, despite
maximal susceptibility to lymphoma induction,
is unexplained. Preliminary experiments sug-
gest that this resistance persists during the
first several weeks of life, decreasing only
gradually as sexual maturity is approached (A.

In man, as in the mouse, acute lymphatic
leukemia is probably induced more commonly
in irradiated children than in irradiated adults
(56).
TABLE II

Relative susceptibility of parental and F1 hybrid strain mice to induction of granulocytic leukemia by x-rays*

<table>
<thead>
<tr>
<th>Strain</th>
<th>Sex</th>
<th>Number of mice</th>
<th>X-ray dose (r)</th>
<th>Leukemia incidence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RF</td>
<td>M</td>
<td>101</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>RF</td>
<td>F</td>
<td>97</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>BALB/c</td>
<td>M</td>
<td>74</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>BALB/c</td>
<td>F</td>
<td>70</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>BALB/c x RF</td>
<td>M</td>
<td>94</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>BALB/c x RF</td>
<td>F</td>
<td>101</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>RF</td>
<td>M</td>
<td>104</td>
<td>300†</td>
<td>38</td>
</tr>
<tr>
<td>RF</td>
<td>F</td>
<td>101</td>
<td>300†</td>
<td>12</td>
</tr>
<tr>
<td>BALB/c</td>
<td>M</td>
<td>82</td>
<td>300§</td>
<td>9</td>
</tr>
<tr>
<td>BALB/c</td>
<td>F</td>
<td>79</td>
<td>300§</td>
<td>4</td>
</tr>
<tr>
<td>BALB/c x RF</td>
<td>M</td>
<td>99</td>
<td>300§</td>
<td>25</td>
</tr>
<tr>
<td>BALB/c x RF</td>
<td>F</td>
<td>91</td>
<td>300§</td>
<td>16</td>
</tr>
</tbody>
</table>

† Whole-body exposed to 250-kv x-radiation at 5-6 weeks of age.
‡ Whole-body exposed to 250-kv x-radiation at 13-17 weeks of age.
§ Whole-body exposed to 250-kv x-radiation at 12-24 weeks of age.

TABLE III

Influence of age at irradiation on susceptibility to induction of leukemia (68)

<table>
<thead>
<tr>
<th>Age at irradiation* (days)</th>
<th>Number of mice†</th>
<th>Mean survival (mo.)</th>
<th>Leukemia incidence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>69</td>
<td>12.4 (±3)</td>
<td>6 (±3)</td>
</tr>
<tr>
<td>35-42</td>
<td>104</td>
<td>12.5 (±7)</td>
<td>48 (±7)</td>
</tr>
<tr>
<td>65-75</td>
<td>65</td>
<td>14.7 (±9)</td>
<td>59 (±9)</td>
</tr>
<tr>
<td>175-185</td>
<td>107</td>
<td>15.8 (±9)</td>
<td>51 (±9)</td>
</tr>
<tr>
<td>Control</td>
<td>60</td>
<td>19.6 (±3)</td>
<td>8 (±3)</td>
</tr>
</tbody>
</table>

* 300 r of whole-body, 250-kv x-radiation, 80-100 r per minute
† Male mice of the RF strain.
‡ Incidence adjusted to correct for mortality not attributable to leukemia

Gonadal factors

Although in many, but not all, strains of mice estrogens enhance and androgens inhibit lymphoid tumor formation, the basis for these effects is yet unknown (36). Kaplan et al. (33) pointed out the close correspondence between the action of various hormones on thymus weight and their influence on lymphoma formation. Agents that promote thymic growth tend to augment leukemogenesis, and vice versa, with the exception of estrogen. The effect of castration on lymphoid induction in irradiated RF mice (table IV) suggests that estrogen exerts a co-leukemogenic action on the thymus in this strain.

Castration does not abolish the relatively high susceptibility to granulocytic leukemia in males, and ovariectomy, although raising susceptibility in females, does not increase it to the male level. A sex difference in the incidence of the disease therefore persists even after gonadectomy (table IV). This difference is being explored further in males and females gonadectomized at birth and in castrates treated with androgens and with estrogens. Likewise, the influence of the estrus cycle on granulocyte formation (2) is being correlated with susceptibility to myeloid leukemogenesis.

Effects of thymectomy

Removal of the thymus prevents not only the spontaneous development of mediastinal lymphomas (41, 52) but also the induction of such neoplasms by chemicals (42) and radiation (26) (table V). In the absence of the thymus, other lymphoid tissues undergo neoplasia in response to irradiation (table V), an effect comparable to that noted by Kirsch-
TABLE IV
Effects of gonadectomy on leukemia induction by x-rays in R\textsuperscript{m} mice (62)

<table>
<thead>
<tr>
<th>X-ray dose (r)</th>
<th>Sex</th>
<th>Number of mice</th>
<th>Mean age at death (mo.)</th>
<th>Leukemia incidence* (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>M</td>
<td>314</td>
<td>19.1</td>
<td>6 (±2) 9 (±3)</td>
</tr>
<tr>
<td>0</td>
<td>M†</td>
<td>103</td>
<td>18.5</td>
<td>6 (±2) 16 (±3)</td>
</tr>
<tr>
<td>300</td>
<td>M</td>
<td>104</td>
<td>12.5</td>
<td>48 (±7) 20 (±5)</td>
</tr>
<tr>
<td>300</td>
<td>M†</td>
<td>117</td>
<td>13.5</td>
<td>43 (±7) 24 (±6)</td>
</tr>
<tr>
<td>0</td>
<td>F</td>
<td>97</td>
<td>20.0</td>
<td>4 (±2) 6 (±2)</td>
</tr>
<tr>
<td>0</td>
<td>F†</td>
<td>118</td>
<td>19.5</td>
<td>3 (±2) 9 (±3)</td>
</tr>
<tr>
<td>300</td>
<td>F</td>
<td>101</td>
<td>12.0</td>
<td>14 (±4) 53 (±9)</td>
</tr>
<tr>
<td>300</td>
<td>F†</td>
<td>102</td>
<td>12.7</td>
<td>27 (±7) 34 (±7)</td>
</tr>
</tbody>
</table>

* Incidence adjusted (cf. 35) to correct for mortality not attributable to leukemia.
† Gonadectomised 1 week before irradiation; i.e., at 4-6 weeks of age.

TABLE V
Effects of thymectomy on leukemia induction by x-rays in RF mice (63)

<table>
<thead>
<tr>
<th>Mice dying with leukemia</th>
<th>Mean age at all causes (mo.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Myeloid</td>
</tr>
<tr>
<td>X-ray dose (r)</td>
<td>Number of mice* Percent</td>
</tr>
<tr>
<td>0</td>
<td>314 3 16.3 5 15.5 29 20.6 19.1</td>
</tr>
<tr>
<td>450</td>
<td>105 23 10.8 21 8.5 9 16.8 10.3</td>
</tr>
<tr>
<td>450†</td>
<td>120† 29 10.0 1 6.0 30 13.1 11.0</td>
</tr>
</tbody>
</table>

* Males, irradiated at 5-6 weeks of age.
† Thymectomised 1 week before irradiation.

baum and L'ebert (37) in thymectomized mice treated with methylcholanthrene. Hence, although in intact mice the thymus is apparently the lymphoid tissue of maximal sensitivity, neoplasia may be induced in other lymphoid organs by appropriate stimulation, depending on strain variations in susceptibility (63). The reactivity of the thymus may be related to its high lymphopoietic activity (1). This in turn may result from growth stimulation by local humoral factors such as the lymphocytosis principle (53), which is produced by the thymus and elevated in animals with lymphoid leukemia. Hence not only may cells of the thymus become neoplastic themselves, but the thymus may exert a leukemogenic action on lymphoid cells formed in other organs (cf. 61). The thymus apparently does not, however, affect the development of granulocytic leukemia (table V).

Influence of splenectomy

Because the spleen is invariably enlarged and infiltrated with leukemic cells in granulocytic leukemia of the RF mouse (3), as in myelogenous leukemia of man, the effects of splenectomy on the induction of this disease were investigated. Removal of the spleen either one week before or as late as one month after irradiation markedly inhibits the development of granulocytic leukemia without affecting the induction of lymphomas (table VI). The mechanism of these effects remains to be determined. It is conceivable, however, that the spleen, by virtue of its myelopoietic activity in the mouse, may constitute a major source of leukemic cells in this species. On the other hand, the possibility that the spleen may elaborate a diffusible leukemogenic substance...
TABLE VI
Influence of splenectomy on susceptibility of RF male mice to leukemia induction by x-rays

<table>
<thead>
<tr>
<th>X-ray dose (r)</th>
<th>Operation*</th>
<th>Number of mice</th>
<th>Leukemia incidence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Myeloid</td>
</tr>
<tr>
<td>0</td>
<td>None</td>
<td>381</td>
<td>4</td>
</tr>
<tr>
<td>300†</td>
<td>None</td>
<td>104</td>
<td>38</td>
</tr>
<tr>
<td>300‡</td>
<td>Splenectomy before</td>
<td>52</td>
<td>13</td>
</tr>
<tr>
<td>65</td>
<td>None</td>
<td></td>
<td>54</td>
</tr>
<tr>
<td>65</td>
<td>Splenectomy before</td>
<td></td>
<td>22</td>
</tr>
<tr>
<td>66</td>
<td>Splenectomy after</td>
<td></td>
<td>29</td>
</tr>
<tr>
<td>58</td>
<td>Sham before</td>
<td></td>
<td>33</td>
</tr>
<tr>
<td>53</td>
<td>Sham after</td>
<td></td>
<td>54</td>
</tr>
</tbody>
</table>

* Splenectomy or sham-splenectomy (laparotomy) 1 week before or 1 month after irradiation.
† Whole body exposed to 250-kvp x-radiation at 5-6 weeks of age.
‡ Whole body exposed to 250-kvp x-radiation at 10 weeks of age.

or may somehow be a favored site for growth of neoplastic myeloid cells must not be overlooked. In short, the importance of the spleen in the development of granulocytic leukemia should probably be compared with that of the thymus in the development of lymphomas.

The effects of sham-splenectomy before irradiation on the incidence of both myeloid leukemia and lymphoma resemble the action of cortisone (60) and are for this reason possibly attributable to surgical stress. Why a similar effect on lymphoma induction was not observed with splenectomy itself cannot be explained; however, splenectomy does not affect the development of lymphomas in AKR mice (52) or in irradiated C57BL mice (26).

Other physiologic factors

In addition to the influences already mentioned, the activity of the adrenal cortex affects the induction of lymphoid tumors in mice, hypercorticism inhibiting and hypocorticism enhancing lymphoma formation (cf. 61). Other hormones have also been reported to influence the growth of leukemic cells, but the significance of their effects is equivocal (33, 36, 61).

EFFECTS OF EXTRANEOUS AGENTS

Turpentine

Because of its leukocytosis-promoting activity, turpentine was administered in conjunction with radiation to determine whether stimulation of granulopoiesis would enhance the induction of myeloid leukemia. Preliminary studies disclosed that myeloid hyperplasia in the marrow and spleen were maximal 7 to 9 days after intramuscular injection of turpentine. Hence, to irradiate the animal at the peak of heightened granulopoietic activity, irradiation was carried out one week after injection. In addition, animals were irradiated immediately before injection of turpentine so that the marrow would be stimulated in the irradiated state.

The results of this experiment (table VII) suggest that injection of turpentine before irradiation does not affect the induction of granulocytic leukemia, but the induction of lymphoma is enhanced, possibly through stress. Turpentine given after irradiation, however, augments the induction of granulocytic leukemia without affecting lymphoma formation. Although this enhancement is not of high statistical significance, the increased incidence of granulocytic leukemia greatly exceeded that noted in any previous experiment.

The relation between the enhancing action of turpentine in myeloid leukemogenesis and the role of "promoting" agents in chemical carcinogenesis warrants further investigation. It is conceivable that the action of turpentine combined with the effects of homeostatic repair mechanisms to overstimulate proliferation of
TABLE VII
Action of turpentine on leukemia induction by x-rays in F344 male mice

<table>
<thead>
<tr>
<th>X-ray dose and material injected</th>
<th>Number of mice</th>
<th>Mean age at death (mo.)</th>
<th>Leukemia incidence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 r - saline</td>
<td>67</td>
<td>19.7</td>
<td>Thymic: 9 (±4) Myeloid: 2 (±1)</td>
</tr>
<tr>
<td>0 r - turpentine</td>
<td>59</td>
<td>18.7</td>
<td>Thymic: 6 (±4) Myeloid: 6 (±4)</td>
</tr>
<tr>
<td>300 r - saline</td>
<td>65</td>
<td>14.2</td>
<td>Thymic: 59 (±10) Myeloid: 8 (±7)</td>
</tr>
<tr>
<td>300 r - turpentine</td>
<td>68</td>
<td>12.8</td>
<td>Thymic: 73 (±10) Myeloid: 5 (±3)</td>
</tr>
<tr>
<td>Turpentine - 300 r</td>
<td>69</td>
<td>14.1</td>
<td>Thymic: 57 (±12) Myeloid: 16 (±5)</td>
</tr>
</tbody>
</table>

† Whole body exposed to 250-kvp x-radiation at 10 weeks of age. Turpentine, 0.1 ml, or an equal volume of saline injected intramuscularly 1 week before or 1 hour after irradiation.
‡ Incidence adjusted (cf. 35) to correct for intercurrent mortality not attributable to leukemia.

myelopoietic cells. Neoplasms of these cells might therefore arise through the same mechanism as that postulated to induce lymphomas in the thymus (28).

Effects of filtrates
The pioneer work of Gross (19) and the confirmatory observations that only lymphomas but an increasing variety of other neoplasms may be induced by filterable agents (12) raise the possibility that radiocarcinogenesis may involve the action of viruses or related factors.

To determine whether irradiation increases susceptibility to the leukemogenic action of cell-free filtrates of leukemic tissue, adult mice of the RF strain were injected with filtrates prepared from AKR mice bearing transplanted lymphomas. Because of the observations of Schwartz et al. (57), filtrates of brain tissue were used. In each of two experiments (Table VIII), the injection of filtrates from leukemic AKR mice enhanced the induction of thymic lymphomas, whereas the injection of filtrates from normal mice did not do so. Furthermore, nonirradiated adults were resistant to this effect. Irradiation in adult life therefore seems to enhance the susceptibility of mice that are otherwise susceptible (13) only in infancy. The decreased incidence of granulocytic leukemia

TABLE VIII
Enhancement of lymphoma formation in irradiated RF mice by inoculation with filtrates of lymphomatous tissue

<table>
<thead>
<tr>
<th>X-ray dose (r)</th>
<th>Filtrate injected†</th>
<th>Number of mice</th>
<th>Leukemia incidence§ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>None</td>
<td>67</td>
<td>Thymic: 4 Myeloid: 4 Nonthymic: 4 Total: 4</td>
</tr>
<tr>
<td>0</td>
<td>Normal brain</td>
<td>45</td>
<td>Thymic: 2 Myeloid: 2 Nonthymic: 2 Total: 2</td>
</tr>
<tr>
<td>0</td>
<td>Lymphomatous brain</td>
<td>54</td>
<td>Thymic: 2 Myeloid: 2 Nonthymic: 10 Total: 10</td>
</tr>
<tr>
<td>450</td>
<td>Tyrode's solution</td>
<td>84</td>
<td>Thymic: 27 Myeloid: 5 Nonthymic: 9 Total: 31</td>
</tr>
<tr>
<td>450</td>
<td>Normal brain</td>
<td>48</td>
<td>Thymic: 32 Myeloid: 4 Nonthymic: 10 Total: 10</td>
</tr>
<tr>
<td>450</td>
<td>Lymphomatous brain and lymphoid tissue</td>
<td>84</td>
<td>Thymic: 20 Myeloid: 11 Nonthymic: 21 Total: 32</td>
</tr>
<tr>
<td>450</td>
<td>Lymphomatous brain</td>
<td>83</td>
<td>Thymic: 12 Myeloid: 22 Nonthymic: 19 Total: 41</td>
</tr>
</tbody>
</table>

† Tissue homogenates from three AKR donors pooled in Tyrode's solution at 3°C, and then centrifuged for 50 minutes at 1,200 x g. Centrifugate filtered through Seda No. 0.64 filter with Escherichia coli under a negative pressure of 8 mm of Hg. 0.1 ml of filtrate (equal A660 colony-forming) inoculated intravenously into each recipient within 1 hour after irradiation.
‡ Mice 10 weeks old at irradiation.
§ Analysis at 15 months after inoculation.
Incidence of leukemia in RF male mice exposed to 550 r of x-rays at 10 weeks of age followed by intravenous inoculation of cell-free tissue filtrates. The donor materials are as follows: O pooled normal AKR brain (table VIII), □ pooled lymphomatous AKR brain (table VIII), △ pooled brain from irradiated RF mice developing granulocytic leukemia (29 mice in treatment group), ○ Tyrode's solution (9 mice in treatment group) (A. C. Upton and F. F. Wolff, unpublished data).

In animals of this group (fig. 6), is ascribed to heavy intercurrent mortality of these mice from lymphomas.

To ascertain whether filterable leukemogenic agents are also present in radiation-induced leukemia, we are examining the tissues of irradiated mice developing granulocytic leukemias and lymphomas for such agent. As yet we have no conclusive data, but preliminary results of our initial experiments strongly suggest that leukemogenic filtrates may be obtained from irradiated mice with granulocytic leukemia (fig. 6).

It is noteworthy that the one filtrate obtained from mice with granulocytic leukemia that has been tested thus far enhanced the induction of granulocytic leukemia only and did not significantly affect the incidence of lymphomas. Specificity was also noted with the filtrates obtained from lymphomatous AKR mice (table VIII), and preliminary results suggest specificity for filtrates obtained from irradiated RF mice developing thymic lymphomas. The failure of these filtrates to induce neoplasms different from those of the donor type contrasts with the observations of Gross (20), Stewart et al. (59), Latarjet and De Jaco (39) and others. It does not, however, indicate any real conflict, because the oncogenic effects of the filterable agents reported thus far have varied widely, depending on such factors as the strain of the donor and recipient,
methods of preparation of the filtrate, number of transplant generations of the donor neoplasms prior to filtration, serial passages of the agent through brain tissue, and cultivation of the agent in vitro. Whether the specificity of the leukemogenic effects of the two types of filtrates we have observed to date indicates the existence of two distinctly different types of agents remains to be determined.

Whether the agents are either free deoxyribonucleic acid (24) or nucleoprotein (40), or of both types, it is not clear where they come from, how radiation affects their production, what role they play in neoplasia, or how consistently they are present in radiation-induced tumors. Until further information is available, any one or a combination of three conceivable mechanisms may be postulated: (1) The agents are oncogenic viruses of low infectivity, which invade the animal from its environment after depression of its immunologic defenses by irradiation; this would presumably occur only with relatively large doses of radiation. (2) The agents exist in the host prior to irradiation as temperate or latent proviruses and are activated to a tumorigenic state by radiation; if this process is comparable to the induction of lysogenicity in bacteria, it might occur in response to minute doses of radiation (51). (3) The agents are fortuitously synthesized by radiation, through disturbance of normal nucleic acid formation (23). Elucidation of this question will require a better understanding of viruses and of the fundamental effects of radiation on the cell.

**SUMMARY**

The induction of leukemia by ionizing radiation is influenced by many variables, including radiation intensity, radiation dose, fraction of the body irradiated, genetic differences in susceptibility, age at irradiation, sex, and other physiologic factors.

The influence of these variables on the induction of leukemia varies with the hematologic type of leukemia induced.

Irradiation increases the susceptibility of adult mice to filterable leukemogenic agents that, administered after irradiation, enhance the development of leukemia.

The author is grateful to Dr. A. W. Kimball of the Mathematics Panel for statistical analysis of data.

**REFERENCES**


