Significance of Thymus and Marrow Injury in Urethan Leukemogenesis*

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SUMMARY

Urethan at a dose level of 1 mg/gm body weight caused histologic damage and reduction of thymus weight in mice of strain C57BL. Thymic injury was only slightly less severe in 30- to 60-day-old mice than in 1- to 20-day-old mice. Urethan also produced bone marrow injury in mice of this strain, expressed as a loss of the capacity to promote thymic regeneration in irradiated test mice, which was age-dependent, occurring in donors 30 days of age or less, but not in donors 24–3 months old. Urethan-treated marrow from young donors was also significantly less effective than normal marrow in inhibiting lymphoma development in irradiated isologous hosts. It is concluded that the age-dependent capacity of urethan to act as a complete leukemogen is paralleled and presumably explained by the age-limited character of the marrow injury which it produces. Urethan leukemogenesis thus closely resembles radiation leukemogenesis in mice of strain C57BL with respect to the important role of concomitant injury to two tissues, the thymus and the bone marrow.

Urethan is a multipotent carcinogen (9) which is highly leukemogenic in mice when administered shortly after birth (4, 5). It is apparently less active in adult mice (2, 10) but is capable of potentiating the leukemogenic effect of other agents (2, 8).

Injury to two tissues, thymus and bone marrow, has been shown to be an important feature of leukemogenesis by ionizing radiation (6). Thymic injury is manifested histologically as well as by decrease in thymic weight. Bone marrow injury is expressed functionally as a decrease in the capacity of bone marrow cells to promote rapid thymic regeneration. Is injury to these two tissues also involved in urethan leukemogenesis? Are there age-dependent differences in urethan-induced injury which might explain the diminished leukemogenic potency of this agent in adult mice? Experiments relating to these questions are the subject of this report.

MATERIALS AND METHODS

Male and female mice of strain C57BL/Ka bred in this laboratory were used. They were caged in groups of eight to ten and maintained on Purine Laboratory Chow and water.

The effect of urethan on the thymus.—At 1, 20, 30, or 60 days of age mice were given intraperitoneal injections of urethan in distilled water, 1 mg/gm body weight. Starting at 24 hours after the urethan injection groups of five male and five female urethan-injected and normal control mice were sacrificed daily; the thymus was carefully excised and weighed to the nearest milligram on a torsion balance. In a parallel experiment young adult mice were irradiated with four doses of 168 r each, given at 7-day intervals. Fourteen days after the last irradiation half of the animals were given injections of urethan at the same dose level. Thymus weights were determined serially thereafter. The excised thymuses were fixed in Bouin’s fluid, sectioned, and stained with hematoxylin and eosin for histologic examination.

The effect of urethan on bone marrow.—Experiment 1: Female mice were weaned at 30 ± 3 days of age and randomly assigned to recipient groups in the experiment. These mice were given 300 r total-body irradiation. Within 2 hours after irradiation, 2 × 10⁶ nucleated marrow cells from variously treated donor mice were injected intravenously into the irradiated hosts. The age of the marrow donors was 24–3 months. One group of marrow donors had been given injections of a single dose of 20 mg. urethan; they were sacrificed 1, 3, or 7 days later, and the femoral marrow was removed, pooled, and suspended in Hanks solution. A second group of marrow donors had been given six daily 10-mg. injections of urethan; they were sacrificed, and the marrow was harvested 24 hours after the last urethan injection. Normal marrow was simultaneously obtained from a control donor group. The

* This investigation was supported in part by research grant CA-03352 from the National Cancer Institute, National Institutes of Health, United States Public Health Service.
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Received for publication May 23, 1964.

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thymic weights of the recipient animals were determined 21 days postirradiation.

Experiment 2: The relationship between body weight of the irradiated hosts and thymic response to injected marrow was examined. Female mice aged 30 ± 3 days were divided into two groups weighing 9–10 gm. versus 12–14 gm. at the time of irradiation. The experimental procedures were otherwise identical to those described in Experiment 1.

Experiment 3: The relationship between the age of the bone marrow donors and thymic regeneration in irradiated hosts was examined. The donors were of two ages: 30 days or 75–90 days. Female mice 30 ± 3 days old were given two whole-body irradiations of 200 r at weekly intervals. Within 1–2 hours after the second irradiation these hosts were treated with either urethan-treated marrow or normal marrow from marrow donors of each age group; 2 × 10⁷ nucleated cells of marrow were injected intravenously. The urethan-treated marrow donors were given injections of urethan in six daily doses of 0.5 mg/gm body weight, and the marrow was pooled in Hanks solution 24 hours after the last urethan injection. The thymic weights of the recipient animals were determined 21 days postirradiation.

Experiment 4: The effect of injection of urethan-treated marrow on lymphoma development in irradiated host mice was compared with that of normal and irradiated marrow. Female mice 30 ± 3 days old were irradiated

<table>
<thead>
<tr>
<th>TABLE 1</th>
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<tbody>
<tr>
<td>FIELD OF URETHAN TREATMENT OF ADULT MARROW DONORS ON THYMIC REGENERATION IN IRRADIATED AND MARROW-RECIPIENT MICE</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>X-ray dose to host mice (r)</th>
<th>Urethan dose to marrow donors</th>
<th>Interval to marrow harvest (days)</th>
<th>Mean final body weight (gm.)</th>
<th>Mean thymic weight ± standard error (mg.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>1</td>
<td>16.5</td>
<td>68 ± 1.86</td>
<td></td>
</tr>
<tr>
<td>300</td>
<td>Control</td>
<td>15.0</td>
<td>30.8 ± 2.15</td>
<td></td>
</tr>
<tr>
<td>300</td>
<td>20 mg. X 1</td>
<td>14.7</td>
<td>52.7 ± 1.45</td>
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</tr>
<tr>
<td>300</td>
<td>20 mg. X 1</td>
<td>15.3</td>
<td>49.8 ± 1.6</td>
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</tr>
<tr>
<td>300</td>
<td>10 mg. daily X 6</td>
<td>14.9</td>
<td>58.9 ± 2.83</td>
<td></td>
</tr>
<tr>
<td>300</td>
<td>10 mg. daily X 6</td>
<td>15.6</td>
<td>60.7 ± 2.43</td>
<td></td>
</tr>
</tbody>
</table>

* Strain C57BL/Ka female mice 33 ± 3 days old at the time of irradiation; sacrificed for thymic weight determination 21 days later.
† Strain C57BL/Ka female mice 75–90 days old.
‡ 2 × 10⁷ nucleated marrow cells in Hanks solution were injected intravenously into host animals within 2 hours after irradiation.

<table>
<thead>
<tr>
<th>TABLE 2</th>
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<tbody>
<tr>
<td>THE EFFECT OF HOST BODY WEIGHT ON THE THYMIC RESPONSE TO URETHAN-TREATED BONE MARROW*</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>X-ray dose to host mice</th>
<th>Urethan dose to marrow donors</th>
<th>Interval to marrow harvest (days)</th>
<th>Mean final body weight before x-ray = 12–14 gm.</th>
<th>Mean thymic weight ± standard error (mg.)</th>
<th>Mean final body weight before x-ray = 9–10 gm.</th>
<th>Mean thymic weight ± standard error (mg.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>1</td>
<td>17.1</td>
<td>72.1 ± 1.9</td>
<td>14.6</td>
<td>61.2 ± 3.2</td>
<td></td>
</tr>
<tr>
<td>300</td>
<td>None</td>
<td>—</td>
<td>16.1</td>
<td>72.1 ± 3.2</td>
<td>14.1</td>
<td>36.4 ± 2.62</td>
</tr>
<tr>
<td>300</td>
<td>20 mg. X 1</td>
<td>1</td>
<td>15.6</td>
<td>69 ± 3.52</td>
<td>15.0</td>
<td>59 ± 2.58</td>
</tr>
<tr>
<td>300</td>
<td>20 mg. X 1</td>
<td>1</td>
<td>15.7</td>
<td>55.6 ± 1.5</td>
<td>15.4</td>
<td>61 ± 1.42</td>
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<tr>
<td>300</td>
<td>10 mg. daily X 6</td>
<td>1</td>
<td>16.5</td>
<td>52.8 ± 1.47</td>
<td>13.5</td>
<td>28 ± 2.21</td>
</tr>
</tbody>
</table>

* Twenty-one days after irradiation.
† Strain C57BL/Ka female mice 33 ± 3 days old at the time of irradiation; sacrificed for thymic weight determination 21 days later.
‡ 2 × 10⁷ nucleated marrow cells in Hanks solution were injected intravenously into host animals within 2 hours after irradiation.
TABLE 3
THE EFFECT OF THE AGE OF URETHAN-TREATED MARROW DONORS ON THYMIC REGENERATION IN IRRADIATED RECIPIENT MICE

<table>
<thead>
<tr>
<th>Urethan dose to marrow donors</th>
<th>Donor age (months)</th>
<th>Mean mouse weight (gm.)</th>
<th>Mean thymic weight ± standard error (mg.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>2.5-3</td>
<td>16.3</td>
<td>65.1 ± 1.86</td>
</tr>
<tr>
<td>None</td>
<td>1</td>
<td>16.3</td>
<td>56.4 ± 1.97</td>
</tr>
<tr>
<td>12 mg. daily × 6</td>
<td>1</td>
<td>16.1</td>
<td>23.0 ± 2.33</td>
</tr>
</tbody>
</table>

with four doses of 168 r each at 7-day intervals. After the last irradiation, the mice were divided into four groups and treated as follows:

Group A.—No further treatment.
Group B.—Normal marrow (1.5 × 10⁸ nucleated cells pooled in Hanks solution) was injected intravenously 2 hours after the last irradiation of the host.
Group C.—The mice serving as marrow donors received 300 r total-body irradiation. One day later their marrow was pooled in Hanks solution, and 1.5 × 10⁸ nucleated cells were injected intravenously into irradiated host mice.
Group D.—The marrow donors were treated with four daily injections of urethan, 0.5 mg/gm body weight each. Their marrow was harvested 24 hours after the last urethan injection, and the irradiated recipients were given injections intravenously of 1.5 × 10⁸ nucleated cells.

The mice in all groups were sacrificed and autopsied when lymphomas became grossly detectable. Animals found dead were also routinely autopsied. The latent period for tumor development was taken as the interval from the date of the first irradiation to the date of autopsy.

RESULTS

Injury to the thymus by urethan.—Urethan treatment produced thymic injury, which was manifested both histologically and as a decrease of thymus weight (Chart 1). The decrease in thymus weight began 24 hours after a single urethan injection in all age groups tested and reached its maximum 4 days after urethan injection. Five days after urethan injection thymic weight began to increase again, and on the 8th day there was complete recovery. Histologic examination revealed a pattern similar to that following irradiation (6), with initial pyknosis, fragmentation, and depletion of cortical lymphoid elements (Figs. 1, 2), followed by regeneration.

In mice given injections of urethan shortly after birth or at 20 days of age, thymic weight decreased to about half the normal control weight. In 30- and 60-day-old mice urethan caused thymic weight to decrease by about 30 per cent below normal control levels.

In the irradiated mice, urethan caused only a slight and transient additional decrease in thymus weight, which was demonstrable about 4 days after urethan injection and thereafter returned to the radiation-involut ed baseline.

The effect of urethan on bone marrow.—In the first experiment (Table 1) marrow from urethan-treated donor mice (about 75-90 days old) was as effective as normal marrow in promoting thymic regeneration in irradiated recipients.

![Fig. 1. Thymus of infant C57BL mouse 24 hours after injection of urethan, 0.5 mg/gm. Extensive pyknosis and karyorrhexis of lymphoid elements of the cortex are evident. Hematoxylin-eosin, × 400.](image1)

![Fig. 2. Between 24 and 48 hours after urethan, most of the cellular debris is cleared away, revealing the lymphoid depletion of the cortex. × 400.](image2)
test animals. There was no apparent difference in activity attributable to the two different urethan dosage schedules employed. Marrow harvested 24 hours after a single 20-mg. dose of urethan was slightly less effective than marrow harvested 2 or 6 days later, suggesting that injury sustained by the marrow under these conditions was transient and almost fully repaired within 24 hours.

In the second experiment the possible relationship between thymic regeneration and the body weight of the irradiated, marrow-injected test mice was evaluated. The results are presented in Table 2. Normal marrow and marrow taken from donors after a single injection of urethan had similar effects on thymus weight in animals of both body weight groups. However, marrow from donors that had received daily injections of urethan, though active in the high body weight group, failed to promote thymic regeneration in the animals of low body weight, which were probably physiologically immature.

In these experiments the marrow donors had been randomly available adult C57BL mice, approximately 2½–3 months old. When the results of Experiment 2 indicated that body weight is a significant variable, our third experiment was set up to test the effect of the age of the bone marrow donors on thymic regeneration. The results are presented in Table 3. Normal marrow, whether taken from 1-month-old or from 2½- to 3-month-old donors, was equally effective in promoting repair of the irradiated thymus. Urethan-treated marrow was as effective as normal bone marrow in promoting thymic regeneration when collected from 2½- to 3-month-old donors, but was ineffective when obtained from 30-day-old donors. These results thus confirm and extend the results of Berman and Kaplan (3), who used 30-day-old mice both as donors and recipients. In their experiments, urethan-treated marrow was found to be ineffective in promoting thymic regeneration. It is concluded that urethan causes functional bone marrow injury, expressed as a loss of the capacity to promote thymic regeneration in young mice (up to 30 days of age); the marrow of older mice seems to be relatively refractory to this kind of injury in the dose range studied, perhaps in part by virtue of its more rapid repair.

Experiment 4 was set up to study lymphoma development in irradiated mice given injections of bone marrow from variously treated donors. The results are presented in Chart 2 and Table 4. Control mice (Group A), which were irradiated but received no marrow, yielded a 91 per cent lymphoma incidence, whereas only 40 per cent of mice injected with normal marrow after irradiation (Group B) developed lymphomas. Irradiation of the donor animals (Group C) abolished the protective effect of injected marrow on lymphoma development; indeed, both the high incidence (100 per cent) and short latent period in this group suggest that the irradiated marrow may even have had a slight enhancing effect on lymphoma development. Urethan-treated marrow from 30-day-old donors (Group D) was significantly less effective than normal marrow in preventing lymphoma development; 73 per cent of this group developed lymphomas. Thus, in young mice urethan mimics the injurious effect of ionizing radiation (6) on the bone marrow with respect to two apparently related functional end-points: (a) capacity to promote thymic regeneration and (b) capacity to prevent lymphoma development in irradiated host mice.

DISCUSSION

Berenblum, Boiato, Fiore-Donati, and Trainin (2) found that lymphoma development in irradiated hosts was inhibited equally well by either normal or urethan-treated bone marrow; however, their marrow donors were 7–8 weeks old at the time of the first urethan injection and 10–11 weeks old when killed. Our data suggest that at this age urethan may have little or no injurious effect on the marrow. The present results indicate that urethan has the capacity to injure both the thymus and the bone marrow in young mice up to about 1 month of age. If concomitant injury to these two tissues is essential for lymphoma induction, it would follow that urethan should act as a complete leukemogen in this age group, and it does (45). In older mice, urethan is almost as active with respect to thymic injury but is ineffective with respect to bone marrow injury. The absence of bone marrow injury may explain the diminished capacity of urethan to act as a complete leukemogenic agent in older mice, whereas its positive action as a co-leukemogenic agent in such mice (1, 8) may be attributable to its persistent thymolytic activity.

REFERENCES

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*Cancer Res* 1964;24:1926-1931.

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