**The Role of Periodic and Interrupted Treatment of Newborn and Infant Mice with Urethan on Leukemogenesis**

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**Summary**

The significance of the number of urethan injections and the length of interruption of treatment on the leukemogenesis in newborn mice were studied. Five-tenths mg of urethan was injected i.p./gm of body weight to C57BL x C3H F1 mice for a total of 3, 5, or 6 times. The 1st injection was given when the mice were less than 24 hr of age, while others followed at 3-day intervals. This periodicity was interrupted in 2 groups for 9 or 21 days following the 3rd treatment.

The group that received all 6 injections of urethan at 3-day intervals developed leukemia in significantly higher proportion of mice than the groups in which similar treatment was interrupted for 9 or 21 days, or which received 3 or 5 injections continuously.

The results demonstrated that continuous and periodic treatment with urethan starting at newborn age was significantly more efficient in inducing leukemogenesis than if such a treatment was interrupted for varied periods of time. It was postulated that the presence or absence of the immature cells in the thymus during the urethan treatment may be causally related to these results.

**Introduction**

The history of urethan leukemogenesis in mice and the significance of newborn age in genesis of this neoplasia has been presented recently (9). It has been found that the response of the mice to repeated and periodic treatment of urethan decreased significantly within the 1st week of life (9). Our original studies did not reveal this phenomenon (unpublished data). The mice in that series of experiments were treated for 3 consecutive days with urethan starting at 1, 4, or 7 days of age. The additional 3 treatments were given after 30 days and only threshold leukemogenic response (2-3%) has been observed in all groups.

An integrated series of experiments were instituted to test the role of dose, periodicity, and continuity of urethan treatment on the leukemogenesis of newborn and infant mice. The 1st part of these studies showed direct relationship between the total dose of urethan delivered and the incidence of leukemia (9). The present paper communicates the 2nd part of these investigations, substantiating the importance of the sufficient continuity of urethan treatment of newborn and infant mice for the effective leukemogenesis.

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**Materials and Methods**

**MICE.** The experimental animals were of F1 generation of C57BL and C3H inbred mice, raised in our laboratory (9). Conceived females were allocated at random to experimental groups. Their offspring were weaned at about 30 days of age, at which time they were numbered, recorded, and housed in plastic cages in sets of 10. Sanicel was used as bedding. The mice were kept in a temperature-controlled laboratory at 78°F, and were fed Rockland diet and given water ad libitum.

Throughout the experiment, mice were weighed and inspected periodically. Following death, animals were autopsied and specimens were taken from all thymuses, lymph nodes, spleen, liver, kidneys, and lungs regardless of gross pathology. The tissues were fixed in 10% formalin, processed and stained with eosin.

**URETHAN.** White, crystalline urethan (ethyl carbamate), reagent-grade was utilized. Solution of 10% concentration was always made in redistilled water shortly before use.

**TREATMENT.** The i.p. route of application was used throughout the experiment. Urethan solution was delivered by a Hamilton microsyringe with a 30-gauge needle, 0.005 ml (0.5 mg)/gm body weight. All treated groups received 6 injections of urethan except Groups 2 and 3 which received 3 and 5 injections, respectively. The animals were less than 24 hr of age at the time of the 1st injection. The injections were given at 3-day intervals to Groups 2, 3, and 4. The time interval between the 3rd and 4th injection for Groups 5 and 6 was 9 and 21 days, respectively. The total administered dose/gm of body weight therefore was 3.0 mg for Groups 4–6 and 1.5 or 2.5 mg for Groups 2 and 3, respectively. The leakage through the needle puncture was minimized as already described (9).

The observations were terminated for all groups at 50 weeks of age in order to allow a between-group comparison before differential mortality could occur. Other neoplasms developed due to the urethan treatment at a later age but for clarity of this presentation are not presented here.

**Results**

Table 1 summarizes the results. Because of the fact that both sexes were equally distributed within the groups and that no significant sex difference was observed in the incidence of leukemia, the data for each sex were not presented separately.

The 2nd column under the heading “effective number” gives the number of mice that reached the weaning age (30 ± 3 days). The acute toxicity effects of urethan resulting in the death of animals were slight and without significant group variations.

In all instances of malignant lymphoma, the thymus has been...
TABLE 1
THE EFFECT OF THE NUMBER OF URETHAN INJECTIONS AND THE INTERRUPTION OF TREATMENT ON THE LEUKEMOGENESIS

<table>
<thead>
<tr>
<th>C57BL x C3H F1 MICE</th>
<th>ADMINISTRATION OF URETHAN</th>
<th>MICE WITH LEUKEMIA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Effective No.</td>
<td>Age at 1st injection (days)</td>
</tr>
<tr>
<td>----------------------</td>
<td>--------------</td>
<td>---------------------------</td>
</tr>
<tr>
<td>1</td>
<td>73</td>
<td>&lt;1</td>
</tr>
<tr>
<td>2</td>
<td>59</td>
<td>&lt;1</td>
</tr>
<tr>
<td>3</td>
<td>49</td>
<td>&lt;1</td>
</tr>
<tr>
<td>4</td>
<td>95</td>
<td>&lt;1</td>
</tr>
<tr>
<td>5</td>
<td>79</td>
<td>&lt;1</td>
</tr>
<tr>
<td>6</td>
<td>74</td>
<td>&lt;1</td>
</tr>
</tbody>
</table>

* Both sexes were equally distributed within the groups.
* 0.005 ml of 10% solution of urethan was given/gm body weight at each i.p. injection, which were spaced at 3-day intervals in Groups 2-4.
* Animals alive at weaning.
* Only if period between 3rd and 4th injection exceeded 3 days periodicity.
* Age at recognition of leukemia—clinically and/or at post-mortem examination.
/ Single animal.

Third column for Group 1 indicates age at the inception of the experiment.

enlarged and showed neoplasic morphology. Involvement of other organs has been similar to the description presented recently (9).

Nontreated controls (Group 1) were free of malignant lymphomas. Group 2, which received only 3 injections of urethan, developed leukemia in 2%, manifesting thus only a threshold effect of such a treatment. Five urethan injections (Group 3) were only slightly more leukemogenic (an incidence of 10%). Group 4, however, which received 6 injections of urethan with the same periodicity of 3 days without an interruption, developed malignant lymphoma in significantly higher proportion of mice (32%) with an average age of 21.7 weeks. However, as the treatment-free interval between the 3rd and 4th injection of urethan was increased to 9 or 21 days (Groups 5 and 6) a progressively lower incidence of leukemia was observed (13 and 4%) and at a later average age (28 weeks). This decrease in the incidence of leukemia was highly significant even when the time interval between the 1st and 2nd triad of urethan treatments was only 9 days (x² = 7.6405; P < 0.01). In Group 6, in which treatment was interrupted for 21 days, the incidence of leukemia did not exceed the one observed in Group 3 that received only 3 injections of urethan, indicating the inefficiency of the last 3 treatments given to the former group. Similar treatment, however, delivered after only 9 days of intermission was still additive (Group 5 vs. Group 3; x² = 6.7854; P < 0.01). The above points have been illustrated in Chart 1.

Discussion

The presented experiments showed that when the periodicity of urethan treatment of newborn mice was either interrupted or discontinued early, the incidence of leukemia has been significantly lower than if such a treatment was given continuously.

Kaplan and Brown demonstrated that in X-irradiation leukemogenesis the distinctly greater incidence occurred when a given total dose was delivered in a series of fractionated exposures separated by intervals of a few days, rather than in a single massive exposure or in consecutive daily fractions (6). Therefore, it was established that a sustained period of thymic impairment was an essential prerequisite for lymphoma development by X-irradiation (5). Fiore-Donati and Kaye (2) gave single large doses of urethan (1.25 mg/gm body weight) to young adult C57BL/6J mice and observed 3–4 days later that thymic cortex was practically devoid of mature lymphocytes and consisted almost entirely of immature (large, rounded) cells. On the 7th day, thymuses had normal histologic appearance. Similarly, Haran-Ghara and Kaplan (3) pointed out that the histologic pattern following a single urethan treatment to newborn and adult C57BL mice was similar to that observed after a single X-ray exposure.

The outer zone of thymic cortex in newborn mice is mainly composed of large and immature lymphoid cells (1). The number of these cells decreases sharply during infancy. The period of greatest susceptibility to the Gross’ Passage A virus coincides with the period when the greatest number of large cells are proliferating and differentiating. The susceptibility decreases at the time when the number of these cells diminish (1). Kaplan also suggested that the abundance of undifferentiated cells in thymuses of newborn C57BL mice might be the reason for their susceptibility to virus (4). It is thus likely that, in general, a necessary condition for susceptibility to leukemogenic viruses, X-ray, or chemicals is the presence of immature multiplying cells at the time of the action of the carcinogen.

Single exposures of adult mice to urethan or X-irradiation result in a short-lasting impairment of thymus, the transient appearance of the large and immature lymphoid cells and in no or low incidence of leukemia. Apparently, repeated and periodic urethan treatments from the newborn through the infant age...
of mice are leukemogenic because of concurrent existence of immature cell population in the thymic cortex. At the same time such urethan treatment might delay normal maturation of these cells which would further enhance leukemogenesis. It is postulated that if periodicity of treatment was interrupted before the critical amount of urethan was delivered, the number of immature lymphoid cells might diminish from the thymus before the resumption of the treatment resulting in a lower responsiveness of mice to leukemogenesis. Preliminary histologic studies of the thymus confirmed the latter speculation (unpublished data). However, this assumption and others are currently being investigated more extensively.

The rate of catabolism of urethan in mice following a single injection increases during the 3rd and 4th weeks of life (7, 8) so that a lower incidence of leukemia might be causally related to this latter phenomenon as well. However, presently it is not known whether metabolic maturation was affected by damage inflicted upon the liver through repeated urethan exposures. Also, the effect of urethan on leukemogenesis in previously X-irradiated adult mice is not mediated through its delayed catabolism (6).

Both hypotheses are not mutually exclusive and are apparently complementary. However, the 1st discussed hypothesis better fits the existing indirect evidence of being of crucial importance.

Acknowledgments

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References

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