

# The Strain Difference in the Induction of Leukemia by Urethan<sup>1</sup>

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## SUMMARY

The objective of the present investigation was to find out the trends in leukemogenesis in several strains of mice when exposed to identical, leukemogenic, urethan treatments.

C3H, C57BL, and (C57BL × C3H)F<sub>1</sub> mice were exposed to repeated i.p. injections of urethan. The injections were given at 3-day intervals, the first when mice were less than 24 hours of age. Animals received either 3 or 6 treatments (0.7 mg/gm body weight each) totaling 2.1 or 4.2 mg of urethan per gram body weight. The experiment terminated when the mice were 40 weeks of age.

All three strains developed leukemia, although in different incidences. The C57BL were most responsive (49% and 82% for two dose levels), while C3H were the least responsive (12% and 52% for two dose levels). Their F<sub>1</sub> hybrids developed leukemia in an intermediate fashion.

Several factors which might be associated with the strain difference in the development of leukemia are discussed.

## INTRODUCTION

The significance of newborn age, dose, and periodicity of urethan treatment in leukemogenesis was recently presented and discussed (15, 16). It was demonstrated that leukemogenesis was most successful when treatment was begun within the first 24 hours after birth and then continued at 3-day intervals for a total of 6 times (15). When 0.5 mg per gm body weight of urethan was given for only 3 times, a threshold leukemogenic effect was observed (15). Similarly, a low effectiveness was observed when the periodicity of urethan treatment was interrupted for various periods of time (16). These experiments were conducted on (C57BL × C3H)F<sub>1</sub> mice as part of an integrated study on the neoplastic potentiality of various tissues in newborn and infant mice (17). These particular mice were chosen because of their hybrid vigor, longevity and low incidence of spontaneous tumors of any type within the first 2 years of life.

Studies in leukemogenesis were extended recently to both maternal (C57BL) and paternal (C3H) strains. The main objective was to find out the trends in leukemogenesis in those inbred strains when exposed to identical, leukemogenic, ure-

than treatments. The use of multiple urethan exposure in the present experiments has been rewarding, as malignant lymphomas developed in both strains but with significantly different incidences.

## MATERIALS AND METHODS

**Mice.** The experimental animals were C3H, C57BL, and (C57BL × C3H)F<sub>1</sub> mice raised in our laboratory since 1961. They originally came from Dr. Tannenbaum's laboratory at Michael Reese Hospital. Pregnant 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, the mice were weighed and inspected periodically. Following death, the animals were autopsied and specimens were taken from all thymuses, lymph nodes, kidneys, lungs, spleens, and the livers regardless of gross pathology. The tissues were fixed in 10% formalin, processed and stained with hematoxylin and eosin.

**Urethan.** White, crystalline urethan (ethyl carbamate) reagent grade was utilized. A solution of 14% concentration was always made in redistilled water shortly before use.

**Treatment.** The i.p. route of application was used throughout the experiment. The urethan solution, 0.005 ml (0.7 mg)/gm body weight, was delivered by a Hamilton microsyringe with a 30-gauge needle. The animals were less than 24 hours of age at the time of the first injection, and the subsequent treatments followed at 3-day intervals. Each strain of mice received urethan at two-dose levels for a total of 3 injections (2.1 mg/gm body weight) and 6 injections (4.2 mg/gm body weight). The leakage through the needle puncture was minimized as already described (15). The control animals were untreated.

The observations were terminated for all groups at 40 weeks of age in order to allow a between-group comparison before differential mortality could occur.

## RESULTS

In the present experiment all strains of mice developed malignant lymphomas at both dose levels of urethan used. The actual incidence of leukemia and the latent period of its development is presented for each strain of mice in Table 1. Sexes were combined and data were not presented for each

<sup>1</sup>This investigation was supported by Contract PH 43-65-67 from the National Cancer Institute, NIH, USPHS.

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Received October 16, 1968; accepted May 8, 1968.

Table 1

| Group | Strain            | Mice <sup>a</sup>             |                     | Treatment <sup>c</sup>            |                         | Animals with leukemia |         |                     |
|-------|-------------------|-------------------------------|---------------------|-----------------------------------|-------------------------|-----------------------|---------|---------------------|
|       |                   | Effective number <sup>b</sup> | Age at start (days) | Number of injections <sup>d</sup> | Total dose <sup>e</sup> | Number                | Percent | Average age (weeks) |
| 1     | C3H               | 80                            | 1                   | 0                                 |                         | 0                     |         |                     |
| 2     | C57BL             | 82                            | 1                   | 0                                 |                         | 0                     |         |                     |
| 3     | CxHF <sub>1</sub> | 84                            | 1                   | 0                                 |                         | 0                     |         |                     |
| 4     | C3H               | 83                            | 1                   | 3                                 | 2.1                     | 10                    | 12.0    | 28                  |
| 5     | C57BL             | 84                            | 1                   | 3                                 | 2.1                     | 41                    | 48.8    | 21                  |
| 6     | CxHF <sub>1</sub> | 81                            | 1                   | 3                                 | 2.1                     | 21                    | 25.9    | 23                  |
| 7     | C3H               | 84                            | 1                   | 6                                 | 4.2                     | 44                    | 52.4    | 25                  |
| 8     | C57BL             | 90                            | 1                   | 6                                 | 4.2                     | 74                    | 82.2    | 19                  |
| 9     | CxHF <sub>1</sub> | 83                            | 1                   | 6                                 | 4.2                     | 60                    | 72.2    | 21                  |

Leukemogenic response of several strains of mice following neonatal exposure to urethan. CxHF<sub>1</sub> stands for (C57BL × C3H)F<sub>1</sub>.

<sup>a</sup> Both sexes were equally represented.

<sup>b</sup> Animals alive at weaning (30 days of age).

<sup>c</sup> 0.005 ml of 14% urethan solution (0.7 mg) per gram body weight was given i.p. at each treatment. First injection was delivered when mice were <24 hours of age, and the subsequent treatment followed at 3-day intervals.

<sup>d</sup> Total number of injections.

<sup>e</sup> Total amount of urethan in mg given per gm body weight.

sex separately as no significant difference was observed in their incidence.

The data show that even three injections of urethan (a total of 2.1 mg/gm body weight) were quite leukemogenic, apparently because a relatively high concentration of urethan (14%) has been used. The C57BL mice were the most responsive, as 48.8% of the mice developed leukemia at an average age of 21 weeks (Group 5). The other strains were significantly less responsive. The C3H mice (Group 4) developed leukemia in only 12% after an average latent period of 28 weeks ( $P < 0.001$ ). The first generation of (C57BL × C3H)F<sub>1</sub> hybrids, however, responded with a significantly higher percent than the paternal C3H mice (Group 6 vs. Group 4;  $P < 0.05$ ) and in a significantly lower incidence than the maternal C57BL mice (Group 6 vs. Group 5;  $P < 0.001$ ).

The same difference in trends of leukemogenesis has been also observed at higher dose levels of urethan (6 injections, a total of 4.2 mg/gm body weight). Thus 82.2% of C57BL mice (Group 8) had leukemia at an average age of 19 weeks, while C3H mice (Group 7) developed leukemia in 52.4%. At this dose level of urethan, hybrids were practically as responsive as the maternal strain. The above difference between C57BL and C3H mice was also statistically significant ( $P < 0.001$ ).

## DISCUSSION

The presented results demonstrated that urethan was able to induce leukemia in all three strains of mice utilized. They showed, however, the existence of a significant difference in their readiness to develop this neoplasia following urethan treatment. This positive observation is in contrast to one reported earlier where leukemogenesis did not take place in any of the strains tested, apparently because only single doses of urethan were given to newborn mice (13). Our original ex-

periments pointed to the significance of periodicity of urethan treatment and the total dose administered for efficient leukemogenesis (15, 16). Single exposures to urethan were not leukemogenic in strains used in the present study (unpublished data).

The rate of leukemogenesis by urethan in newborn mice of different strains is governed apparently by a number of endogenous factors irrespective of dose of urethan delivered (15-17). Thus the variation in the rate of urethan catabolism might modify the effective tissue dose of urethan (2, 6, 11), while the difference in pattern of the thymic cell morphology (1, 3, 10, 14-16) may play a role in the process through variation in the number of susceptible cell population.

Cividalli *et al.* (2) indicated that newborn mice of various strains have apparently similar ability to catabolize urethan. We observed, however, certain differences between C57BL and C3H mice in their tolerance to urethan treatment as indicated by their survival rates, gain in weights, and the duration of the anesthetic effect. Thus C57BL mice survived administration of urethan better, gained more weight, and showed shorter anesthetic effect than C3H mice. Presently it is not known in which manner and if at all these differences had any bearing on the higher responsiveness of C57BL mice to urethan leukemogenesis. These observations suggest, however, that metabolism of urethan might differ in these two strains. This is in agreement with findings of Kaye (6) that the adult C3H mice catabolize urethan more efficiently than the Swiss strain of mice.

Morphologic studies (unpublished data) indicated similarity in the dynamics of thymic cell morphology in C57BL and C3H mice during the first 16 days of life. Thus both these strains, as well as their F<sub>1</sub> hybrids, showed two cortical thymic zones at birth, with the outer zone consisting of a pure culture of the large and immature lymphoid cells. By the end of

the second week, only occasionally could such cells be found subcapsularly in any of these strains. However, the injurious effect of urethan on thymus (pyknosis and karyorrhexis) has been more pronounced in C57BL mice.

The relative importance of the above factors in explaining the strain difference cannot yet be evaluated. However, further studies might clarify to what extent also the genetic determinants (5, 9) and probably other factors such as presence of leukemogenic viruses (4, 8, 12) and/or the degree of immunologic competence (7) might have contributed to differential rate of leukemogenesis.

#### ACKNOWLEDGMENTS

The author wishes to thank Dr. P. Shubik for his interest in the work and Dr. N. Mihailovich for his capable assistance in the performance of the experiment. The competent technical assistance of A. Davis, C. Montalvo, and B. Romero is greatly appreciated.

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