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Effect of Urethane on Maturation of Leukocytes of Mouse Myelogenous Leukemia.*

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Urethane induces a pronounced depression in the blood leukocyte count of both human and mouse myelogenous leukemia.[1,2] In the experiments being reported, mice with myeloid leukemia were given either 1 or 2 anesthetic doses of urethane (1 mg per g of body weight intraperitoneally in 10% aqueous solution) and the alterations in the white blood cells of bone marrow and peripheral blood observed. The leukemic animals were of the second transfer generation of a myeloid leukemia of the F strain.[3] The F mice of these experiments had been inoculated with leukemic cells to effect transfer of the disease 34 to 44 days before they were placed on experiment; animals were from 6 to 8 weeks of age at the time of inoculation. They were weighed daily after treatment with urethane was begun.

Total white blood cell counts were made on the tail blood of 9 leukemic mice preceding and at 24-hour intervals (from 24 to 192 hours) after a single anesthetic dose of urethane. Differential and total blood leukocyte counts were made on nonleukemic animals preceding and 24 hours following 1, 2, 4 or 5 daily anesthetic doses. Differential counts were made on the bone marrows of 11 untreated leukemic mice, and of 12 leukemic mice within 11 to 31 hours after either a single or 2 anesthetic doses of urethane spaced at 24-hour intervals. Thin films of sternal marrow were smeared upon glass slides and stained with May-Grünwald Giemsa staining combination. In doing a differential count the myeloid cells with a single round nucleus (blast cells) were counted against those with a nucleus which had segmented. The number of mitotic figures in 40 oil immersion fields was determined on the marrow of these animals. Similar determinations of mitotic figures were made on the bone marrow of 6 nonleukemic mice which had received no treatment and 6 which had received one injection of urethane 11 hours previously.

A single anesthetic dose of urethane depressed the white counts of leukemic mice from levels of 78 to 286,000 to 23 to 44,000 within 72 hours (uniform response in 11 mice with an average drop of 106,000 in the first 24 hours). Most of the "blast" cells disappeared from the circulating blood. There was a loss of about 1 g in weight during this period (initial weight of 21 g). During the next 72 hours the counts rose, although in 6 of the 9 mice studied in this manner the counts had not reached the initial levels by this time.

The ratio of segmented to mononuclear cells in the marrow of untreated leukemic mice ranged from 13:87 to 46:54 with an average of 26:74. This ratio was altered (37:53 to 76:24 with an average of 60:40), indicative of a shift towards maturity, within 11 to 48 hours after either a single or 2 injections of urethane spaced at 24-hour intervals. These doses did not alter the white blood cell counts of normal mice, although 4 or 5 daily injections induced a depression in the white blood cell counts of nonleukemic animals. When treatment of leukemic mice with urethane was suspended (192 hours after a single anesthetic dose), mononuclear myeloid cells again pre-

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dominated in the bone marrow.

Urethane has been observed to inhibit mitotic activity in the corneal epithelium of the rat,[4] and similar effects have been noted for other cells in tissue culture.[5] Consequently counts of mitotic figures were made on the bone marrow of normal and leukemic mice before and after administration of urethane. No inhibition of mitosis in myeloid cells could be detected 11 hours after a single injection of urethane into normal mice (6 animals). Fewer mitotic figures were present, however, in the marrows of urethane-treated leukemic mice (11-24 hours after last injection) than in controls (29 mitotic figures for 40 oil immersion fields of untreated leukemic marrows—range of 12 to 42, 14 mitoses in treated marrows—range of 7 to 30). This is not interpreted necessarily as meaning that mitosis had been inhibited by the drug, since there were fewer cells in the urethane-treated marrows capable of undergoing mitotic division.

As a result of the action of urethane many mature cells appeared in the bone marrow. With the release of these cells instead of immature leukemic cells into the blood, the circulating population of white blood cells had a shorter life expectancy. Thus, a great per cent of the circulating cells were probably dying within a relatively short time after release from the marrow. This is not offered as the probable sole explanation for the depression in the white blood cell count of myeloid leukemia following urethane therapy, since other factors such as rate of release of cells from the marrow and peripheral destruction may be involved.

These animals with myeloid leukemia represent an excellent test object for determining the ability of drugs chemically related to urethane to produce similar effects on myeloid leukemia.

Summary. The administration of a single anesthetic dose of urethane resulted within 24 hours in a drop in the white blood cell count and the appearance of many mature cells in the bone marrow of leukemic mice. Since the number of mitotic figures in marrow myeloid cells was decreased, maturation may have been secondary to inhibition of mitosis in blast cells. However, in the treated mice there were fewer marrow cells capable of undergoing division, which may account for the reduced number of mitoses. The release of an increased per cent of mature cells into the circulating blood may be a factor in depression of the white blood cell counts following the injection of urethane into mice with myeloid leukemia.

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The Oxigram as a Measure of Cardiorespiratory Reserve.*

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Most of the available methods for determining the functional reserve of the cardiorespiratory system have objectionable features.[1-6] A new test was suggested by the consideration that in disease of the heart or

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