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Effect of Urethane on Transplanted Leukemia of Ak Mice.*

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Haddow and Sexton,[1] investigating the effect of urethanes on human malignant tumors, reported that they observed a fall in the leucocyte counts. Prompted by this Paterson, Haddow, Thomas, and Watkinson[2] treated 32 cases of human leukemia, 19 myeloid and 13 lymphatic, with urethane, (ethylcarbamate). In both types of leukemia they observed a decrease in the total white blood cell count with a return towards normal of the differential pattern. This was accompanied by decrease in the size of the spleen and lymph nodes and a rise in the hemoglobin level. They considered the effects to be comparable to those obtained with deep x-ray therapy. There was no evidence that permanent benefit resulted from the therapy. Engstrom, Kirschbaum, and Mixer[3] tested the effect of urethane on the transplanted myelogenous leukemia in F strain mice. They found that just sub-lethal doses were effective in reducing the white blood cell counts to normal but did not eradicate the infiltration of the tissues. Smaller doses had a less marked effect on the counts. Kirschbaum and Lu[4] demonstrated later that a single anesthetic dose of urethane decreased the number of mitotic figures in marrow myeloid cells and reduced the total number of circulating white cells, especially myeloblasts, in transplanted myeloid leukemia of F strain mice.

Mice of the Ak strain have a very high incidence of spontaneous leukemia. About 75% develop the disease after the age of 6 months. The intravenous injection of leukemic cells from diseased older animals produces progressive leukemia in 100% of younger animals. The leukemic tissue is cut into small pieces with scissors and immersed in Tyrode's solution. The larger particles are allowed to settle leaving a suspension of cells in the supernatant fluid. The concentration of cells in the suspension is determined by counting in a hemacytometer. Dilution is usually necessary until the required concentration is reached. The injection into the tail vein of about 40,000 cells suspended in 0.10-0.15 cc of solution produces leukemia in all of the young animals in about 2½ weeks and death occurs in 3 to 5 weeks.

Twenty Ak animals† less than 5 months old were selected and divided into 3 groups. Five received the cell suspension only; 10 received cell suspension and were treated with urethane; and 5 were treated with urethane only. It had been determined previously that the maximum dose tolerated over a 5-week period by mice weighing 25-30 g was 7.5 mg injected subcutaneously twice daily. This dosage was used. White blood counts and differential counts were done once weekly for 2 weeks and then twice weekly. At autopsy sections of the liver, spleen, and bone marrow were taken for histologic study.

The white blood cell counts of the animals which were treated with urethane and were not injected with cell suspension did not show any variation from the normal. The average and individual total white blood cell counts of the other two groups are shown in Fig. 1.

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1 Haddow, A., and Sexton, W. A., *Nature* (London), 1946, **157**, 500.

2 Paterson, E., Thomas, I. A., Haddow, A., and Watkinson, J. M., *Lancet*, 1946, **250**, 677.

3 Engstrom, R. M., Kirschbaum, A., and Mixer, H. W., *Science*, 1947, **105**, 255.

4 Kirschbaum, A., and Lu, C. S., PROC. SOC. EXP. BIOL. AND MED., 1947, **65**, 62.

† The Ak mice used in this experiment were from a colony developed from breeders given to us by Dr. Jacob Furth.

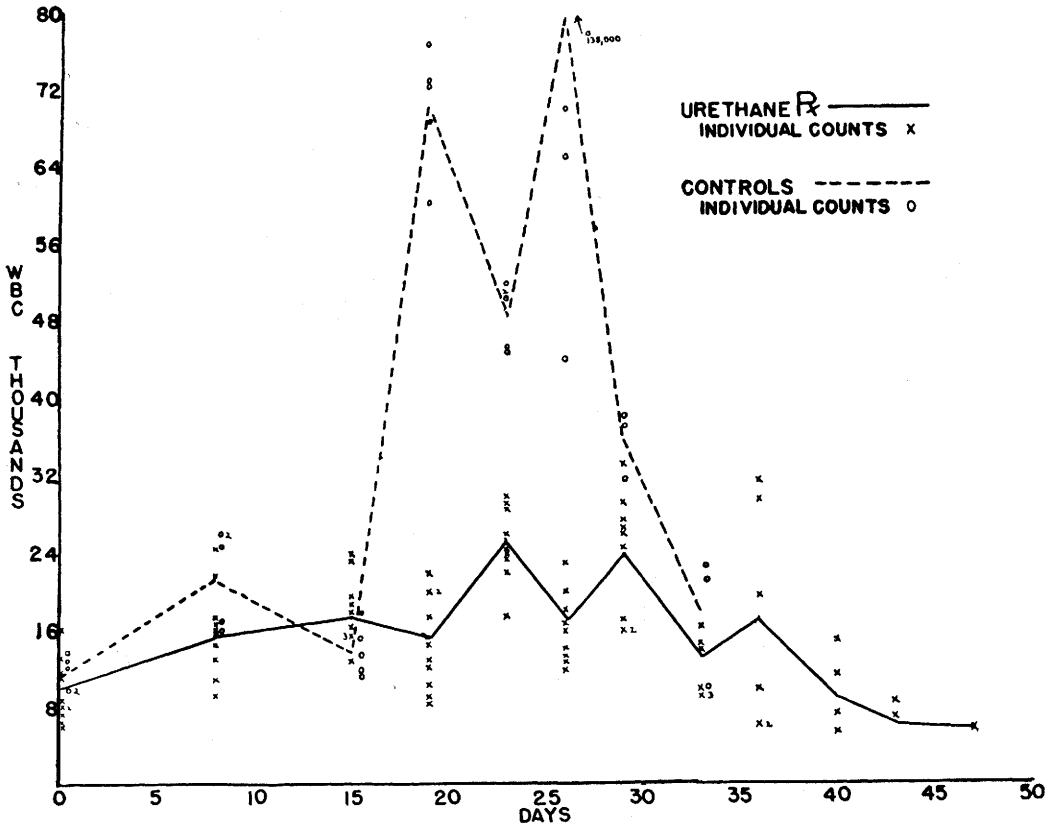

FIG. 1.

Average and individual white blood cell counts of the urethane-treated group and the control group are shown. Only a very slight rise above normal occurred in the urethane group.

In Fig. 2 the averages of the differential counts are plotted. The change in counts of the animals which were given cell suspension and not treated with urethane was entirely characteristic of the transplanted disease. Evidence of leukemia was manifest in the peripheral blood on the 19th day, at which time primitive blast cells were first seen. The leukemia was at its peak on the 26th day and all the animals were dead by the 34th day. The drop in white blood cell count shortly before death is usually seen. The treatment with urethane had a striking effect on the course of the transplanted leukemia. The total and differential counts showed no change from the normal until the 23rd day at which time there was a slight rise above normal and a few blast cells appeared. The slight rise was maintained until the 40th day at which time the total counts fell to within normal limits. A small percentage of blasts, however, persisted until the death of the last animal on the 48th day. The urethane had the effect of delaying the appearance and greatly diminishing the number of white blood cells in the peripheral blood.

The histologic changes in the control group not treated with urethane were typical of the transplanted disease. The infiltration of the organs lagged behind the development of leukemia as manifested by changes in the peripheral blood. The 2 animals which survived only 22 and 24 days showed only moderate infiltration. The 3 which survived 31, 31, and 34 days showed maximal infiltration. The animals treated with urethane also developed infiltration in the organs but this occurred later and to a less marked degree than in the untreated animals. One mouse that survived 27 days showed no infiltration.

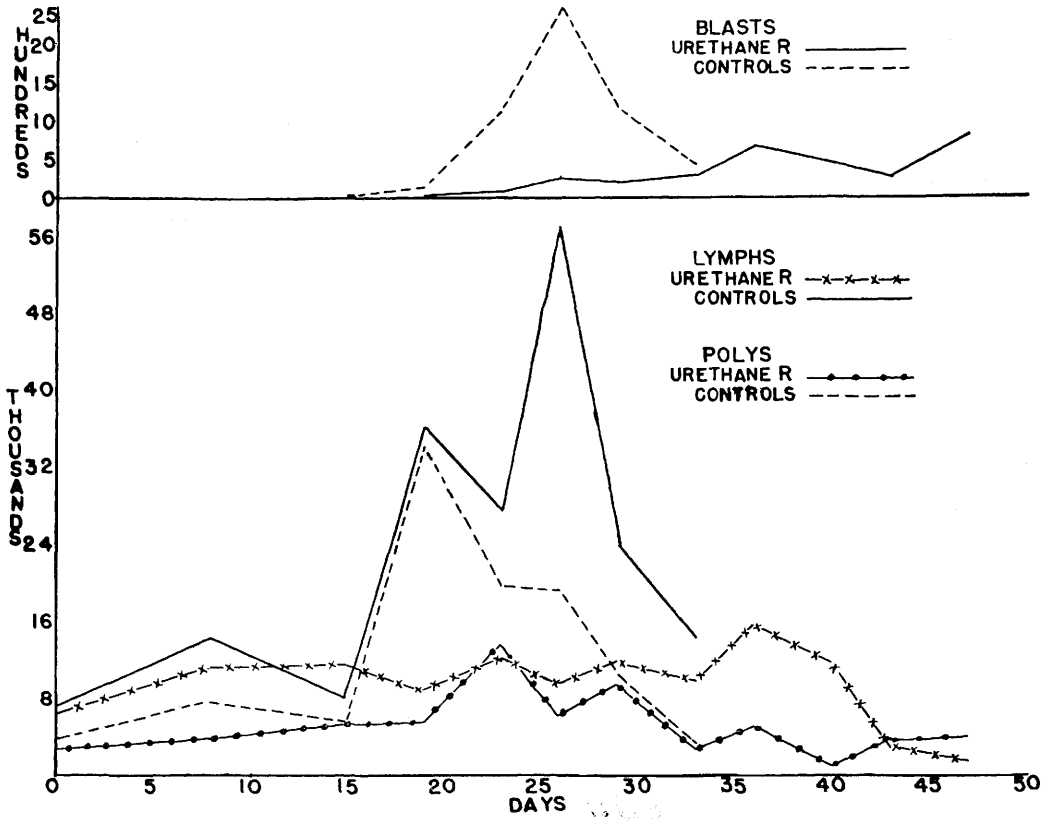

FIG. 2.

Average differential counts of the urethane-treated group and the control group are shown. Primitive blast cells appeared later and in smaller numbers in the urethane group.

Another that survived 29 days showed minimal infiltration in the spleen and one small focus of leukemic cells in the liver. In the animals that survived 32 to 48 days there was moderate infiltration which was much less extensive than that present in the untreated animals surviving 31 to 34 days. There was a general tendency to weight loss which was not extreme in any one group and was not more or less marked in any one group.

The urethane treatment increased the survival time of the animals injected with cell suspension. The average survival time of those not treated with urethane was 28.4 days, of those treated with urethane 36.0 days. This is statistically significant. P equals 0.05.[5] Of the 5 animals which were treated with urethane and not injected with cells one died on the 19th day and one on the 41st. The other 3 were killed on the 50th day. It is probable that the dose of urethane was toxic and was approaching a lethal dose.

Conclusions. The administration of urethane to Ak mice with transplanted leukemia caused a delay in the appearance of immature cells in the peripheral blood and prevented a marked increase in total white blood cell count and numbers of immature blast cells. Infiltration of organs occurred later and to a less marked degree. The average survival time of the animals was increased and this increase was statistically significant. In order to secure these effects a dosage of urethane was used which was just sub-lethal. It is probable that the drug itself was partly responsible for the death of the treated animals.

[5] Fischer, R. A., *Statistical Methods for Research Workers,* Oliver and Boyd, Edinburgh, 1934.