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A Study on the Mitotic Activity in the Bone Marrow of Normal Mice Following Treatment with Urethane¹⁾

By

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(With 2 Text-figures)

Urethane attracted the attention of many investigators following the discovery by Haddow and Sexton (1946) as to the effect of this chemical in retarding animal tumors. Patterson, Haddow et al. (1946) reported a fall in the total white cell count in myeloid and lymphoid leukemias in man, and this has been confirmed by many others. Engstrom et al. (1947), Kirschbaum & Lu (1937), Weir & Heinke (1947), Law (1947), and Dustin (1947) found a similar effect of urethane in experiments with several mouse leukemias. The decrease in the number of blood cells in leukemia induced by urethane has not yet been satisfactorily explored. It was reported by Kirschbaum & Lu (1937) and Dustin (1947) that urethane did not arrest the mitotic activity of normal bone marrow, but selectively inhibited the multiplication of leukotic cells. Recently, Rosin (1951) reported a definite effect of urethane on the mitotic activity of normal bone marrow cells in mice, there being a notable increase of the mitotic index in the first several hours after the administration of the drug.

The present study was made in an attempt to examine further the effect of urethane on the mitotic activity of bone marrow cells of normal mice, with special regard to the mitotic index and the distribution of mitotic phases. The author wishes to express his sincere gratitude to Professor Sajiro Makino for his direction and for improvement of the manuscript for publication. Further cordial thanks are offered to Mr. T. Tanaka, Mrs. K. Kanô and Mr. H. Nakahara for their valuable advice, criticism and friendly assistance.

Material and method

Sixty healthy mature mice (strain S) at the age of about 50 days received

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subcutaneous injection with a single dose of urethane (ethyl carbonate): 20 mg per 20 g body weight. The mice were sacrificed at every 5-hour interval during a period of 210 hours after treatment. Squashed smears of the bone marrow of the femur were prepared. They were stained mainly with acetic gentianviolet for cytological study, and partially with Giemsa's stain for diagnostic test.

Results of observations

Most conspicuous cytological effect in the bone marrow induced by urethane in treated mice is a sudden increase of the mitotic index. Figure 1 indicates the

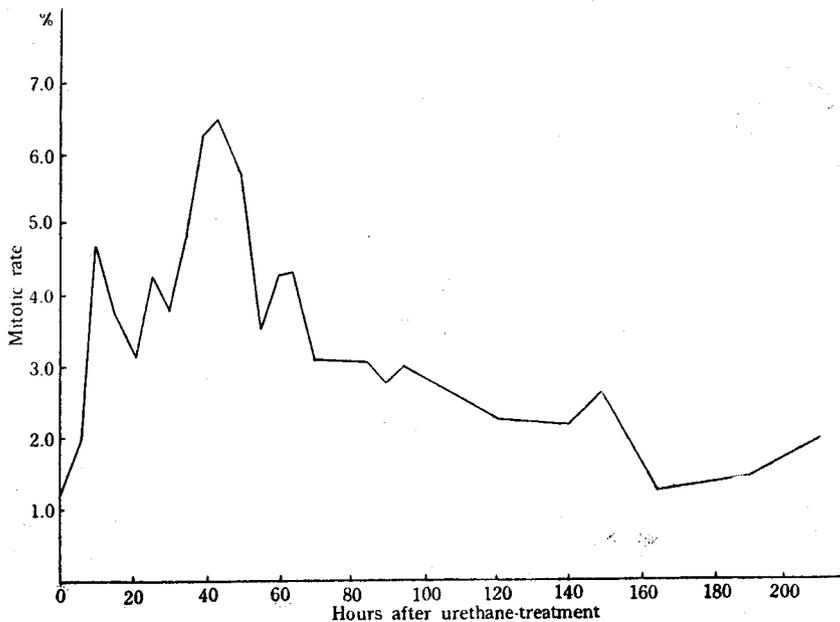


Fig. 1. Mitotic rate in bone marrow of urethane-treated mice in percentage.

change of the mitotic index at every 5-hour interval for 210 hours after treatment. In untreated mice the mitotic index was found as low as 1.16 percent on an average from eight animals. In the treated mice the mitotic index showed a remarkable increase, indicating 4.7 percent at 10 hours after treatment. A rise in the mitotic index took place with the passage of time after the administration of the drug, and showed the highest value (6.41 percent) at 45 hours after injection. Then the index showed a gradual decrease: it was 4.4 percent at 60 hours after treatment and returned to approximately normal values at 200 hours, indicating 2.0 percent.

The above observations revealed that the striking elevation in the number of mitoses occurred during a period from 10 to 50 hours after the administration of urethane. At a later period, from 60 to 210 hours after injection, a gradual decrease of the mitotic index was observed, returning slowly to normal values.

Next, percentage distributions of mitotic phases were observed in both treated and untreated mice by way of comparison. The data are shown in Figure 2. Average percentage distributions of mitotic phases in untreated mice were 45.6 percent for prophase, 31.5 percent for metaphase, 14.0 percent for anaphase

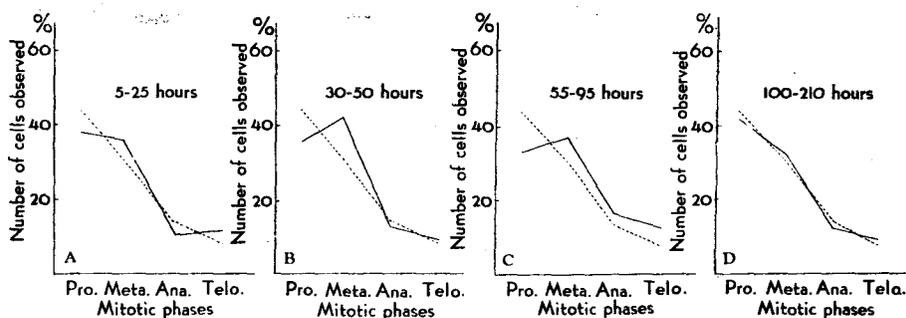


Fig. 2. Percentage distributions of mitotic phases in the bone marrow of mice. Broken lines: Average values in untreated mice. Solid lines: Average values in urethane-treated mice.

A, average values during the period of 5 to 25 hours after urethane administration, in comparison with those from untreated mice. B, the same during the period of 30 to 50 hrs. C, the same during the period of 55 to 95 hrs. D, the same during the period of 100 to 210 hrs.

and 8.0 percent for telophase, whereas those in urethane treated mice were 39.5 percent for prophase, 37.9 percent for metaphase, 10.5 percent for anaphase and 12 percent for telophase during a period from 5 to 25 hours after injection.

From 30 to 50 hours after treatment the percentage distribution of mitotic phases showed a decrease in the percentage of prophase with an increase in the percentage of metaphase as follows: prophase 37 percent, metaphase 42.4 percent, anaphase 13 percent and telophase 8.6 percent. From 55 to 95 hours after injection, the average percentages were found to be 34 percent, 37 percent, 17 percent and 12 percent for prophase, metaphase, anaphase and telophase, respectively. From 100 to 210 hours after the administration of urethane, the percentage distribution of mitotic phase showed a tendency to return to approximately normal values as follows: prophase 44 percent, metaphase 33 percent, anaphase 14 percent and the telophase 9 percent.

In urethane-treated animals the percentage distribution of the mitotic phases showed a shift as described above. It is a remarkable feature that there is a

considerable fall in the percentage of prophases and rise in the percentage of metaphases during the period when the mitotic index was increased, that is, from 30 to 50 hours after treatment.

The mitotic abnormalities induced by the application of urethane in the bone marrow were studied next. Cytological investigation showed that urethane affected markedly the cells of the granulocytic series and immature erythrocytes occurring in the bone marrow, leading to various types of abnormalities. Remarkable are the stickiness and coalescence of metaphase chromosomes, deformation of chromosomes into unusual-shaped bodies, appearance of chromosome bridges at anaphase, scattering of chromosomes in the metaphase plate and multipolar mitoses.

Discussion

The present investigation has revealed that a single dose of urethane shows a definite effect on the mitotic activity of the normal bone marrow of mice, causing the elevation of the mitotic index, alternation in the distribution of mitotic phases and the appearance of mitotic abnormalities.

The rise in the mitotic index takes place during a period from 10 to 50 hours after the injection of the drug. From 60 to 210 hours after administration, a gradual decrease of the number of mitoses occurs. It is remarkable that the return of the mitotic index to normal values is very slow. It may be assumed therefore that the action of urethane lasts a rather long time.

The percentage distribution of the mitotic phases shows a considerable decrease of the prophase cells and an increase of the metaphase cells, from 30 to 50 hours following treatment with urethane. The percentage of anaphases and telophases has remained without remarkable change from that in non-treated mice. The high percentage of metaphases following treatment is explicable as a result of one of three events, namely a real increase of mitoses, a retardation of the metaphase stage, and a prolongation of the duration of metaphase.

Moellendorff (1937) and Bucher (1937, 1949) have observed in fibroblast colonies in tissue culture a considerable rise of the mitotic index in the first hours after the application of urethane and later a decrease. They showed by motion picture study that the duration of prophase was shortened, further that there was a marked retardation of metaphase and often a prolongation of telophase and reconstruction stage. Recently Rosin (1951), in the study of urethane-treated mice, has clearly demonstrated that there is a decrease in prophase and an increase in metaphase, whereas the number of the later phases was unaltered. The results of the present investigation fall in fair agreement with those of Rosin (1951).

The author should like to consider the high number of metaphases after treatment with urethane observed in this experiment as being due to the prolongation of the duration of metaphase. In this connection the study of Makino and Nakahara (1953) on the living tumor cells is very interesting. They observed

in the rat ascites tumor the duration of mitotic phases in the successive series of a division process followed through the same cell at varying temperatures. Their observations showed that there was striking decrease in the duration of metaphase with rising temperature. From this result it can be assumed that the duration of metaphase is variable under the influence of certain experimental treatments. It seems likely that urethane has the effect of causing the prolongation of the metaphase stage.

Moeschlin (1947) reported no changes in the mitotic activity of bone marrow cells of rabbits treated with urethane. Kirschbaum & Lu (1937) noted no inhibition of mitosis in the bone marrow of normal mice following treatment with urethane. Dustin (1947) found a very low percentage of prophases and a high percentage of metaphases in normal mice after a single dose of urethane.

Summary

Normal adult mice were injected subcutaneously with a single dose of urethane, and the effect of the drug on the mitotic activity of the bone marrow was investigated. It was found that urethane in a single dose has a definite effect on the mitotic activity of normal bone marrow cells: the mitotic index increased, the distribution of mitotic phases was altered, and abnormal mitoses appeared.

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