Effect of Urethane (Ethyl Carbamate) on the Mitotic Activity in the Bone Marrow of Normal Mice*

By A. Rosin, M.D.

Following observations of Haddow and Sexton1 on the retarding effect of urethane (ethyl carbamate) on animal tumors, attempts were made to influence the course of leukemia in both human beings and animals with the aid of this drug. Patterson, Haddow et al.2 succeeded in demonstrating a fall in the total white cell count with a return towards a normal differential pattern in cases of myeloid and lymphoid leukemias in man. Many other authors confirmed these results.3–10

A similar effect of urethane was revealed in experiments done with the myeloid and lymphoid leukemias of mice.11–15

The mechanism by which urethane leads to a reduction of the number of immature cells in the peripheral blood in leukemia is not known. Observations on bone marrow of urethane treated human beings and animals suggest the possibility that urethane has no arresting effect on the mitotic activity of normal bone marrow16,17 but selectively inhibits the multiplication of leukotic cells.18,19

In the present study the effect of urethane on the mitotic activity of bone marrow cells of normal mice was investigated.

Material and Methods

Fifty-two healthy young mice (stock mice and mice from strain R3)† were injected intraperitoneally with a single dose of urethane (0.02 Gm. per 20 Gm. body weight). The animals were killed with ether after varying periods ranging from one to one hundred ninety-two hours. Smears from the bone marrow of the femur were prepared and stained with May Gruenwald-Giemsa stain. The mitotic index, i.e., the percentage of mitoses was determined by counting in consecutive fields the mitoses in 1,000 cells of the erythroid and myeloid series normally capable of undergoing mitosis. The percentage distribution of mitotic phases was tabulated and the structure of the mitoses was studied.

Differential counts of at least 500 cells were made from each animal in order to learn whether an increase occurs in the maturation rate of the white cell series. The myeloid-erythroid ratio was determined. Sixteen mice (8 stock mice and 8 R3 mice) served as controls.

Results

The mitotic index of the bone marrow cells showed a considerable increase in urethane treated mice. In untreated mice the mitotic index ranged from 0.8 to 2.5 per cent, whereas in the experimental animals it ranged from 1.6 to 8.2 per cent. The rise in the mitotic index occurred from ten to forty-four hours after

---

* This work was aided by a grant from the United States Public Health Service, The National Cancer Institute, Bethesda, Md.

† These mice were raised in our laboratory from breeders obtained from Dr. S. Graff, College of Surgeons and Physicians, Columbia University, New York.

From the department of Experimental Pathology, (Cancer Laboratories), The Hebrew University, Jerusalem, Israel.

Submitted March 28, 1950; accepted for publication January 24, 1951.
the administration of the drug. Later, from forty-four to one hundred and ninety-two hours, the mitotic index returned to approximately normal values, the number of mitoses ranging from 1.9 to 3.1 per cent. Chart 1 shows the number of mitoses in the bone marrow of urethane treated mice in percentage of the number of mitoses in the bone marrow of untreated mice.

Average percentage distributions of mitotic phases in normal mice were as follows: prophases 40 per cent, metaphases 34 per cent, anaphases 22 per cent and telophases and reconstruction phases 4 per cent. In urethane treated animals the percentage distribution of the mitotic phases was altered. There was a considerable fall in the number of prophases and a rise in the number of metaphases, especially during the period when the mitotic index was increased, i.e. from ten to forty-four hours after the administration of the drug. In this period the average percentage of prophases was 29, and of metaphases 47. The proportion of anaphases and telo- and reconstruction phases was nearly unaltered, the average percentage being 21 and 3 respectively (Chart 2).

From forty-four to one hundred and ninety-two hours after the injection, the percentage distribution of mitotic phases showed a tendency to approach normal values, the percentage of prophases being only slightly decreased and that of metaphases only slightly increased. The average proportions of the different phases during this period were: prophases 32 per cent, metaphases 38 per cent, anaphases 26 per cent and telo- and reconstruction phases 4 per cent (Chart 3).

The structure of the mitoses after urethane treatment was often abnormal. Minor alterations like chromosomes bridges (figures 1 and 2), aberrant chromosomes (figure 3) and clumping of chromosomes (figure 4) occurred frequently. In some rare instances scattering of chromosomes throughout the cell body was observed (figure 5). Multipolar mitoses (figure 6) in erythroblasts and promyelocytes were sometimes seen. Telophases occurred in precursors of red and white cells without division of the cytoplasm. As a sequel to these alterations multilobed or multinuclear erythroblasts and promyelocytes were observed (figures 7, 8 and 9).

In addition, mitotic figures were seen which might be interpreted as “reversion phases”, i.e. nuclei in a certain mitotic phase which do not complete the mitotic cycle but return immediately to the resting stage. In promyelocytes of urethane treated mice figures which might be interpreted as reversion phases from the anaphase stage were numerous (figures 10, 11). The anaphase plates did not separate but were held together by small or broad bands. The chromosomes were only partly visible and the entire chromatin material was often only faintly stained. There was no definite peripheral demarcation, but in some instances the impression was gained that a nuclear membrane was in the process of formation (figure 12).

It is possibly a sequel to this process that cells appeared which had nuclei resembling those of promyelocytes in size and structure, but similar to those of segmented leukocytes in their lobated and often bizarre shape (figures 13, 14). Analogous figures which may represent reversions from the metaphase stage were sometimes seen in early precursors, and these resulted in bizarre ringshaped nuclei (figure 15) or occasionally in segmented nuclei with chromatin bridges (figure 16).
The maturation of segmented leukocytes in urethane treated animals remained unchanged. The percentage of segmented leukocytes related to the total number of white cells ranged from 40 to 56 per cent in untreated mice. In the experimental animals no major deviation from these values was observed. In the majority of the experimental animals the myeloid-erythroid ratio re-
Fig. 1.—Twenty hours. Chromosome bridge in anaphase of promyelocyte. × 1700.
Fig. 2.—Thirty-three hours. Anaphase showing two chromosome bridges. × 1800.
Fig. 3.—Twenty hours. Anaphase in erythroblast with aberrant chromosome. × 1600.
Fig. 4.—Twenty hours. Erythroblast in anaphase stage showing clumping of chromosomes. × 1600.
Fig. 5.—Fifteen hours. Scattering of chromosomes in promyelocyte. × 1800.
Fig. 6.—Fifteen hours. Tripolar mitosis in erythroblast. × 1800.
Fig. 7.—Twenty hours. Erythroblast with three nuclei. × 1600.
Fig. 8.—Twenty hours. Binucleated early erythroblast. × 1600.
Fig. 9.—One hundred and twenty-two hours. Binucleated promyelocyte. × 1500.
Fig. 10.—Twenty hours. Reversion phase from anaphase stage in promyelocyte with aberrant chromosome. × 2000.

Fig. 11.—One hundred and twenty-two hours. Reversion phase from anaphase stage in promyelocyte. × 2000.

Fig. 12.—Twenty hours. Reversion phase from anaphase stage in promyelocyte. In contrast to the former figures the nucleus is sharply delimited. × 1700.

Fig. 13.—Twenty hours. Promyelocytes showing nuclei of bizarre shape. × 1200.

Fig. 14.—Twenty-two hours. Promyelocyte with bizarre shaped nucleus. × 1500.

Fig. 15.—Twenty hours. Probably a reversion from metaphase stage with formation of a ring-shaped nucleus in promyelocyte. × 2000.

Fig. 16.—Twenty-two hours. Reversion from metaphase stage with formation of a segmented nucleus in promyelocyte. × 1500.

Remained within normal limits, i.e. between 1:0 and 2:6, depending on whether the bone marrow was in a predominantly erythropoietic or leukopoietic state.
Our observations show that a single injection of urethane has a definite effect on the mitotic activity of the normal bone marrow of mice. The mitotic index rises, the distribution of mitotic phases is altered, and pathologic mitoses appear. The greatest elevation in the number of mitoses occurs during a period from ten to forty-four hours after the administration of the drug. At a later period, from forty-four to one hundred and ninety-two hours after the injection of urethane, only slight elevations of the mitotic index were observed, and the values returned slowly to normal.

The alteration in the percentage distribution of the mitotic phases consisted of a considerable decrease in prophases and an increase in metaphases. The percentage of anaphases and telophases was maintained practically unchanged. The deviation from the normal were especially evident when the mitotic index was high, i.e. in the period from ten to forty-four hours following treatment. Later, from forty-four to one hundred ninety-two hours after administration of the drug the decrease of prophases and increase of metaphases persisted, but to a lesser degree.

Without knowledge of the duration of the whole course of the mitotic process and of the time relationships of the various mitotic phases, it is not possible to decide if the rise of the mitotic index in our experiments is due to a stimulation of the mitotic activity of the bone marrow cells or to a retardation of the whole mitotic process or of a single phase leading to an accumulation of mitotic figures. The high number of metaphases after treatment with urethane can be due either to a real increase of mitoses or to a retardation of the metaphase stage. The low value of prophases on the other hand must not necessarily indicate that few cells enter the mitotic cycle but can be due to a shortening of the prophase stage.

Moellendorff\textsuperscript{16a, b} and Bucher\textsuperscript{17a, b} found in fibroblast colonies in vitro, treated with urethane, a considerable rise of the mitotic index in the first hours after the application of the drug, and later a decrease. They assumed that urethane has a temporary stimulating effect on the mitotic activity of fibroblasts cultivated in vitro. The same authors showed by motion picture documentation on fibroblast colonies treated with urethane that the whole course of the mitotic process was prolonged. The duration of prophase was shortened, there was a marked retardation of metaphase and often a prolongation of the later phases, especially the telo- and reconstruction phases. These authors as well as Bastrup-Madsen\textsuperscript{18} observed in counts on fixed cultures a decrease in the percentage of prophases, a rise in the percentage of metaphases and, in some instances, even a rise in the number of anaphases\textsuperscript{19b} or of telo- and reconstruction phases\textsuperscript{16a, 17a, b}. Moellendorff pointed out that weaker solutions of urethane affect primarily the telo- and reconstruction phases whereas with stronger solutions both the metaphase and the later phases are retarded.

Our experiments clearly demonstrate that there was a decrease in prophases...
and an increase in metaphases, whereas the number of the later phases was practically unaltered. If we assume that there is a retardation of metaphase, we should expect a drop in the number of the later phases. That this did not occur, could be due to the fact that there is also a retardation of the later phases and therefore an accumulation of mitoses of this stage. This is in accordance with Moellendorff's\textsuperscript{16a,b} and Bucher's\textsuperscript{17a,b} observations on fibroblasts.

Observations on the influence of urethane on the mitotic activity of normal bone marrow are very scarce. Moeschlin\textsuperscript{16b} noted no changes in the mitotic activity of bone marrow cells of rabbits treated with urethane. Kirschbaum and Lai\textsuperscript{12} found no inhibition of mitosis in the bone marrow of normal mice eleven hours after treatment with a single dose of urethane. Dustin\textsuperscript{19} observed a very low percentage of prophases and a high percentage of metaphases three and twenty-four hours after a single administration of the drug to normal adult mice. Baldini\textsuperscript{20} recorded a rise in the mitotic index of the red cell precursors, which reached its peak within the first twenty-four hours after the administration of a single dose of urethane to normal mice. The same author also observed an increase in prophase two to four hours after the injection, an increase in metaphases and decrease in prophase in the first twelve hours after the injection and an increase of the later phases twenty to twenty-four hours after the beginning of the treatment.

As mentioned above, abnormal mitoses were observed in the bone marrow cells of mice after treatment with a single dose of urethane. Clumping of chromosomes, chromosome bridges, aberrant chromosomes and sometimes multipolar mitoses were observed in both red and white cell precursors. In some instances telophases without division of the cytoplasm were seen. As a result, multilobed and bi- or multinucleated erythroblasts and promyelocytes were observed. Moellendorff\textsuperscript{16b}, Bucher\textsuperscript{17a,b} and Bastrup-Madsen\textsuperscript{18} noted similar anomalous mitotic structures in vitro in fibroblast colonies following the addition of urethane. Osgood\textsuperscript{20} observed double nuclei in granulocytes of human bone marrow treated with urethane in vitro.

In our experiments peculiar mitotic figures were observed in promyelocytes, which might be interpreted as "reversions" from the meta- and anaphase stages to the resting stage with suppression of the later mitotic phases. These reversions may thus be considered as abortive mitoses. As described above, early white precursors with bizarrely shaped, often highly lobated nuclei were seen after administration of urethane. These cells represent immature cells which had undergone premature lobation of the nucleus, probably as a result of abortive mitosis.

Reversions from the metaphase stage to a resting nucleus were seen by La Cour\textsuperscript{21} in "premyelocytes" of normal mice. Under "premyelocytes" he classifies, so far as we understand, all precursors of segmented leukocytes capable of undergoing mitosis. He assumes that the ring shaped nucleus of the myelocytes and leukocytes of the mouse originates in the following way: The metaphase which in the mouse forms a hollow sphere, goes, under suppression of the anaphase, directly into the telophase stage and the resting stage. (La Cour,\textsuperscript{21} table 2, figures 9-11.) This process takes place in the last division of the "premyelocyte." After this event the cell processes a ring shaped tetraploid nucleus and does not
divide again. This results in a ripe myelocyte which proceeds by further maturation into a segmented leukocyte.

Reversions from the anaphase stage were seen by Peters in Allium cepa after treatment with sulfanilamide. They give rise to lobated nuclei, to nuclear fragments connected by bridges or to even more abnormally shaped nuclei.

It is evident, therefore, that abortive mitosis in normal or pathologic conditions may play an important role in the future configuration of the nucleus. We saw reversions from the meta- and anaphase stage only very seldom in the white precursors of normal mice. In urethane treated mice, reversions, especially those from the anaphase stage, were numerous. Our findings differ from those of La Cour, who observed reversions only in the last myelocytes, whereas we have seen them after treatment with urethane, also in early white precursors, creating thus the described polymorphism of the nuclei of these highly immature cells.

Küster noted abortive mitoses between the meta-anaphase stages in the bone marrow of a child suffering from acute myeloid leukemia after the treatment with urethane. He mentioned the appearance of paramyeloblasts, i.e. myeloblasts with marked polymorphism of the nuclei in the peripheral blood.

Moellendorff saw "pseudoamitoses" in fibroblasts cultivated in vitro and treated with urethane.

After a single injection of urethane no increased maturation of white cells to segmented leukocytes could be observed.

No deviations from normal of the myeloid-erythroid ratio were noted after the administration of a single dose of urethane. This indicates that no unilateral increase of one series of hemic cells took place. This observation supports the findings of Balduini who noted neither increased maturation of white cells nor changes in the myeloid-erythroid ratio in the bone marrow of mice treated with a single dose of urethane.

SUMMARY

Normal young albino mice were treated with a single intraperitoneal injection of urethane, and the effect of the drug on the mitotic activity of the bone marrow was studied. A notable increase of the mitotic index was observed from ten to forty-four hours after the administration of the drug. At the same time the percentage of propliphases was decreased, while the percentage of metaphases was increased. Abnormal mitoses were frequent. Mitotic figures which might be interpreted as reversions from the meta- and anaphase stages to a resting nucleus were seen. Promyelocytes with highly lobated nuclei of bizarre shape appeared.

No changes were found in the myeloid-erythroid ratio, or in the maturation rate of the white blood cells.

CONCLUSIONS

A single dose of urethane has a definite effect on the mitotic activity of normal bone marrow cells in mice.

It does not increase the maturation of white precursors to segmented leukocytes.

Under the influence of urethane, lobated nuclei of bizarre shape probably resulting from abortive mitosis were observed in early white precursors.
REFERENCES

Effect of Urethane (Ethyl Carbamate) on the Mitotic Activity in the Bone Marrow of Normal Mice

A. ROSIN