Urethane-Caused Blood and Bone Marrow Changes in Agranulocytosis and Panmyelopathy of the Cat

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In previous investigations of the influence of urethane on the normal blood picture we noted that the most prominent result of the drug in man was a fall of the lymphocytes. This effect was also present in certain animals to a varying degree.

It is interesting to note that the rat showed, in this respect, the least sensitivity, and the cat a very striking sensitivity. In the latter animal, granulocytosis and thrombopoiesis were markedly affected, so that leukopenia and thrombocytopenia resulted. In the present experiments, the behavior of the bone marrow in this urethane aleukemia of the cat were investigated more closely, and, in addition, tests were made to determine if the urethane agranulocytosis could be prevented or made less severe by the simultaneous administration of various growth factors.

Technic of the Experiments

Ten cats were used: 7 as experimental animals, and 3 as controls. All 10 animals were kept free in the same room for one month before the beginning of the experiments. Blood was taken daily from the animals by entering the vein of the ear with a needle. Bone marrow punctures were made in the tibia or at the lower end of the femur. (Both these bones contain active marrow in the cat.) From the puncture material, both smears and histologic sections were made. The punctures were carried out under ether anaesthesia, and were made alternately in the several bones, in order to exclude possible effects of previous punctures (right tibia, left tibia, right femur, left femur). If it became necessary to puncture the same bone again, a lower site than the site of the previous puncture was chosen.

Studies of the blood picture were made during a control period of at least one week prior to the commencement of urethane therapy, since wide variations in the normal numbers of blood cells in cats may occur physiologically. The bone marrow was also examined in all animals before the start of the experiments. The differential enumeration of the marrow cells was made according to the technic of Rohrer, in which the numbers of nucleated red cells and reticulum cells per 100 white cells are counted separately. A total of 300 to 500 cells was counted in each marrow study. Other details, and the differential counts, are depicted on the accompanying graphs.

During the agranulocytic phase, all animals received penicillin and small doses of sulfonamides in order to protect them as much as possible from the infections which regularly occur in this phase. In spite of these measures, however, some of the animals died because of such infections, or because of the general damage caused by the urethane.

Administration of Urethane: On the basis of previous experiments we chose the following method of administration as the most suitable. The necessary amount of urethane was given daily, mixed in a small amount of raw chopped meat. Only when the animals no longer took the urethane in this form because of lack of appetite was the urethane injected subcutaneously as a 5 per cent solution. The dose administered daily was between 0.05-0.1 Gm. Kg. body weight. The urethane was generally stopped immediately on the appearance of aleukemia, as otherwise the animals died (see graphs). When urethane is administered

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as a solid, it must be re-weighed daily, since there are considerable losses through sublimation. This fact came to our attention for the first time in the present experiments, and led to accurate experiments on this point elsewhere.\textsuperscript{35}

\textbf{Results}

\textbf{I. Blood Changes}

In all 7 cats (in all 9 animals, if we include the 2 animals investigated previously)\textsuperscript{23} there appeared, under the influence of urethane, a marked to almost complete fall in the number of granulocytes and a marked reduction in the number of lymphocytes. In 6 of the 7 animals a severe thrombopenia also appeared. The rate of fall was the same for both series of blood cells.

\textit{Latent Period in the Effect}: This differed somewhat, and varied, depending on the dose and animal, between a minimum of two days (Animal III) and three days (Animal IV), and a maximum of twenty days (Animal I). The lowest point of the cytopenia was reached, depending on the dose and individual sensitivity of the animals after a period of from six and seven days (Animals III and IV), to two to three weeks, and even as late as ten weeks in the relatively unsensitive Animal I. In this latter, the leukocytes first sank to a third of the initial value with the small daily dose of 0.025 Gm./Kg. body weight, and then rose again beyond the initial value in spite of the resumption of the urethane (fig. 1). The dose had to be increased a number of times, and only when the dose was increased to 0.1 Gm./Kg. did the agranulocytotic reaction appear. It is possible that there was a certain tolerance in this case: differences in the absorption, excretion and catabolism of the reagent appear to us to enter less into the question.

\textit{Renewed Rise of the Cells}: This could be followed accurately only in 4 of the animals, because of the death of the other 3 at the lowest point of the agranulocytosis. As can be seen from the curves, the rise of the cells in Animals III and IV began two days after cessation of urethane and reached the initial value within five days. Animals III and IV had received the smallest total dose. In Cats I and V, the rise of the cells did not begin for fourteen and five days, respectively, after discontinuance of the urethane: these 2 animals had received considerably higher total doses. The initial cell values were not reached before thirty-four and fourteen days respectively.

\textit{Behavior of the Eosinophiles}: Although the neutrophilic granulocytes and the lymphocytes showed a fall in all the experimental animals, the eosinophiles showed little change. Thus, the eosinophiles in Cat II showed no change at all, in spite of a decided fall in the remaining granulocytes to a minimal value of 200 per cu.mm. In Animal VI, the eosinophiles showed a fall only after a fall of the neutrophiles shortly before death. As we shall see, this persistence of the eosinophiles is often even more striking in the bone marrow. We shall return to this peculiar behavior of the eosinophiles in the discussion of the bone marrow findings.

\textit{Monocytes}: In the cat these cannot be differentiated with certainty from the large lymphocytes, so that a separate study of them could not be made in these experiments. But, since the lymphoid and monocytoid cells together always
showed a marked fall, the monocytes probably follow the same fate as the neutrophiles and the lymphocytes.

Lymphocytes: The lymphocytes showed, in 6 animals, a fall to very low values. The lymphocyte fall paralleled the fall in the granulocytes, but the lymphocytes never disappeared completely from the blood. Only in Cat VII did the lymphocytes fall somewhat faster than the granulocytes. The rise of lymphocytes after

![Graph showing changes in blood counts over time.](image)

**Fig. 1.** Urethane panmyelopathy in protracted urethane-administration (cat 1). *Upper curve:* behavior of the blood cells, *below:* graphic presentation of the myelograms. Here, too, there is first a distinct shift to the left in the bone marrow, with cell impoverishment of the bone marrow at the same time. Note here the relatively very strong increase of the reticulum cells and the eosinophiles, as well as of the erythroblasts, since, to a certain extent, only these cell forms remain. After stopping the urethane, very slow recovery, and gradually normalization of the bone marrow and blood findings. Through the longer lasting effect there was to be clearly noticed here also the effect on erythropoiesis, as shown by a fall of the erythrocytes (small curve in the upper part of the picture). The agranulocytotic phase could be overcome only by the aid of antiinfectious therapy (penicillin and sulfadimethylypyrimidin).

the initial fall ran, in 2 of the 4 animals in which it could be followed, parallel to the rise of the other leukocytes. In Animals II and III, however, lymphopoiesis recovered distinctly more slowly than granulocytopenia.

Thrombocytes: It is interesting to note that the thrombocytes in the peripheral blood stream fell approximately at the same time as the other cell forms, and the minimum thrombocyte level corresponded to the most marked point of the
agranulocytosis. Of the 7 animals, only one (Cat V) showed a different behavior, in that the thrombocytes showed no distinct change at all. In one case the fall of the thrombocytes continued even after discontinuance of the urethane, in spite of the rise of the granulocytes after their initial fall (Animal III). The rise of the thrombocytes after an initial fall paralleled, in 2 of the 3 animals which could be controlled, that of the granulocytes; in the third animal (Animal I), thrombocytopenesis recovered a little more rapidly.

**Erythrocytes:** As can be seen from the curves, the erythrocytes showed, in contrast to the other cell forms, no striking fall. This fact will be discussed later on together with the bone marrow changes of erythropoiesis.

**Morphologic Changes:** During the falling phase, which preceded the actual agranulocytosis, the blood cells showed no qualitative changes. At the low point of the leukopenia and, above all, in the regeneratory phase, after cessation of the urethane, the neutrophiles showed a remarkable foamy structure of the protoplasm, with increased basophilia and often with numerous vacuoles in the cytoplasm. There was a tendency towards hypersegmentation of the nuclei. The chromatin structure of the nuclei was occasionally of strikingly large granulation, analogous to the chromatin changes observed in Pelger's anomaly. The lymphocytes often showed small vacuoles in the plasma. The thrombocytes showed, in the regeneratory phase, a tendency towards the occurrence of giant platelets.

II. **Changes in the Bone Marrow**

The myelograms obtained by repeated punctures are presented graphically (figs. 1, 2, 3). The observed changes can be subdivided into two groups, with somewhat different behavior according to the dose of urethane administered.

**First group (smaller doses of urethane):** The first group, that is, the animals receiving the smaller doses of 0.05 Gm./Kg. per day (Cats III and IV) showed a distinct shift to the left of the granulocyte series in the bone marrow (figs. 2 and 6,c) with relative increase of the myeloblasts and promyelocytes. Besides this shift to the left there was a simultaneous decrease of the absolute number of cells in the bone marrow, but not so marked as in the second group of cats with higher doses of urethane. The reticulum cell values were increased in the myelogram to approximately three times their original value, during the low point of the agranulocytosis (figs. 1, 2, 3), from which we may conclude that the active granulocyte marrow was reduced to about one-third of the former number of cells. With the disappearance of the agranulocytotic phase the number of the cells in the granulocyte marrow rose (see Cat IV, fig. 2) at once. This can be clearly seen from the histologic sections (fig. 7,a,b) and, in the myelogram, by the return of the number of reticulum cells to normal within six days after stopping the urethane (fig. 2). In Animal III, the inhibition of the bone marrow, and therefore the relative increase of the reticulum cells, was still present six days after stopping the urethane; the animal died from a severe purulent endometritis eleven days after the urethane was stopped.

**Second group (higher doses of urethane):** In the second group (that is, in the
5 animals which received the higher dose of 0.1 Gm./Kg. there developed, under the influence of urethane, a progressive diminution of all the granulocytes of the bone marrow, the more mature cells being affected first, but, ultimately, all forms down to the myeloblast being involved. In the majority of the animals, the nucleated red cells and also the eosinophiles participated in this cell decrease but to a much lesser degree. In a few cases, the bone marrow became extremely poor in cells, with only reticulum cells and nucleated red cells (and, occasionally, eosinophiles) remaining in an otherwise depleted marrow (figs. 6, 7).

To summarize, it can be said that the effect of urethane on the bone marrow of cats depends on the dosage of urethane employed. In smaller doses (0.05 Gm./Kg. per day), its primary effect is to produce a shift to the left of the granulocyte series; that is, an inhibition of maturation. In larger doses (0.1 Gm./Kg. per day), urethane reduces the total cellularity of the marrow by decreasing the number of cells in all stages of maturation.

*Regeneration Phase:* After stopping the urethane, the bone marrow findings gradually become normal again after a latent period of varying duration. A distinct cell increase is evident in the bone marrow long before the rise of the
granulocyte values occurs in the blood. Thus, Animal I showed after the discontinuance of the urethane an approximately normal bone marrow picture as early as fourteen days, while the blood still showed a leukopenia (fig. 7).

Behavior of the Eosinophiles: In the discussion of the blood picture we have already mentioned the striking behavior of the eosinophiles, which do not always parallel the diminution of the other granulocytes. In the bone marrow, these findings were even more striking than in the blood. Of the 7 cats, 6 showed decided relative increase in the eosinophile cells of the bone marrow. It must be emphasized that this increase is only a relative one, that is, there remained in the

almost empty bone marrow only the eosinophiles, along with the reticulum cells and erythroblasts. In the histologic sections, the cell paucity of the bone marrow is very striking, in contrast with the condition of the marrow, as found on examination before the commencement of the experiment. (Animals IV and VII, figs. 2 and 3).

Of the 5 animals with marked eosinophilia of the bone marrow, all except one (Animal IV) showed a definite shift to the left of the eosinophile myelocytes, in spite of the relatively slight urethane sensitivity of these cells at the height of the neutrophile agranulocytosis. Thus this cell system also shows some inhibition of maturation under the influence of urethane.
Megakaryocytes: At the time of the decrease of the thrombocytes in the peripheral blood stream the bone marrow showed a decrease of the megakaryocytes and an increase of the more immature forms as far as this could be determined,

Fig. 4.—Changes in the distribution of the erythroblasts (basophile, solid columns; polychromat, diagonally shaded columns; orthochromat, open columns) during the administration of urethane in cats 1-8. Note the strong shift to the left, that is, the marked inhibition of maturation in cat 4. In the remaining animals it was clearly present, but less marked, with the exception of animal 8, where such a shift was altogether absent. (The dose of urethane and the urethane administration is, in each case, entered at the base of the columns.) Erythropoiesis thus also shows a distinct inhibition of maturation under the influence of urethane, although it is much less sensitive than granulocytopoiesis.

in view of the scarcity of these cells in the smears. In the regenerative phase the newly produced giant thrombocytes still point to a certain inhibition of maturation.

The reduction in the megakaryocytes of the bone marrow could be followed
better in the sections. We have pointed out elsewhere that in sections of marrow there are found on the average ten times as many megakaryocytes per a given number of granulocytes than in smears of the bone marrow. This is due to the fact that in making the smear a considerable number of these large cell forms remain in the meshes of the marrow reticulum of the smeared pieces.

Erythropoiesis: In the differentiation of the myelograms it was noted that the changes in the erythropoietic series were slight in comparison with those in the granulocytopoietic series. But a finer analysis shows distinct changes even in the erythropoietic series. In figure 4 we have presented the various maturation stages which occur per 100 erythroblasts. The shift of the erythroblasts towards the immature side is clearly evident. This shift to the left of the erythroblast series is most distinct in Animal IV, in which the basophilic erythroblasts in-

![Table](image)

**Fig. 5.—Mitotic index before and after urethane.** Exact counting of the mitoses (cats 3 and 1) shows that the mitotic index (= number of mitoses per 1000 immature cells) falls very strongly under the influence of urethane, this being true of erythropoiesis as well as of granulocytopoiesis. Thus urethane causes, besides the inhibition of maturation also a very strongly marked decrease of mitotic activity.

creased from 13 to 90 per cent after the administration of urethane, and in which only 10 per cent polychromatophilic and no more orthochromatic forms were demonstrable after urethane. Six days after discontinuance of the urethane there were still demonstrable 53 per cent basophilic forms, 33 per cent polychromatophilic, and 14 per cent orthochromatic forms. In this animal the shift to the left was accompanied by a distinct decrease of the absolute number of erythroblasts in the bone marrow. Only Animal VI had no demonstrable shift to the left.

Besides the shift to the left there was, morphologically, an increase in the macroblast forms, which also speaks for a certain inhibition of maturation. The decrease in the mitotic index will be mentioned in the section on mitosis.

To summarize, we can thus say that the effect of urethane was to cause a distinct inhibition of red cell proliferation in the bone marrow, with a decided
diminution of mitotic activity and an inhibition of maturation of the red cells. Despite these marrow changes many cases showed no distinct changes in the early phase of the peripheral blood stream.

Behavior of the Reticulum Cells: As we have already mentioned, the reticulum

![Image](https://example.com/image)

**Fig. 6.** Smears of the bone marrow puncture fluid (tibia) from cat 1 (magnification 1000X).

1. *Before urethane:* Note the abundance of cells, and the numerous myelocytes and band cells.
2. *At the height of urethane panmyelopathy after a total of 3.9 gm. urethane:* Here there are to be found only very few cells, and the myelocytes and band forms have completely disappeared.
3. *Ten days after stopping the urethane:* Regenerating marrow with numerous myelocytes, metamyelocytes and band forms.
cells were relatively increased in the marrow smears: such relative increase is good evidence for a reduction in the granulocytes in the bone marrow. There was no absolute increase in the number of reticulum cells as was shown by the histologic sections in which, in the most severe cases, only reticulum cells and erythroblasts were present in an otherwise empty marrow.

![Histologic sections of the imbedded bone marrow puncture fluid from cat I (magnification 500X)](image)

**Fig. 7.**—Histologic sections of the imbedded bone marrow puncture fluid from cat I (magnification 500X). Here the cell-impoverishment of the marrow after the administration of urethane is still more distinct.

a. *Before urethane-treatment:* A marrow very rich in cells, with numerous myelocytes and neutrophiles with band-formed nuclei.

b. *After total 3.9 gm. of urethane:* A marrow very impoverished in cells, in that only reticulum cells, erythroblasts and occasional myeloblasts are found.

The plasmacellular reticulum cells showed a distinct increase during the agranulocytic phase, with the exception of 2 animals (in Cat IV they were practically unchanged in Cat III the increase did not occur until the regenerative phase). There is evidence that this increase was not merely relative, since the ratio of these to the other types of reticulum cells increased (Cat VI) from two to as
much as twenty times. This increase persisted in many cases far into the regenerative phase.

Behavior of Mitosis: Morphologically, a distinct disturbance in the course of the various stages of mitosis was present in all marrow preparations at the beginning, and above all, at the height of the peripheral agranulocytosis. Thus we saw, in both erythroblast and granulocyte mitoses, striking clumping and sticking-together of the chromosomes (fig. 8). Furthermore, there was often a splintering off of chromosomes and, especially, many cell divisions, which appeared to remain in telophase (fig. 8,c) (accumulation of binucleated forms).

In order to gain insight into the quantitative relations of the mitoses before and after urethane, we counted, in 2 animals (I and III) the number of mitoses per 10,000 cells and then determined the mitotic index (the number of mitoses per 1,000 immature cells which are still capable of division). The results are shown graphically in figure 5. There was a distinct fall of the mitotic index for granulocytopoiesis as well as for erythropoiesis.

Fig. 8.—Morphologic changes of the mitoses in the bone marrow of the cat after urethane.

Upper row: Granulocytopoiesis (magnification 1500X).

a. Chromosomes of a myeloblast mitosis (monaster) swollen and partly clearly stuck to each other.

b. Chromosomes completely stuck together (diaster).

c. Bi-nucleated myeloblast which arose through arrest of mitosis in telophase.

Lower row: Erythropoiesis (magnification 1200X).

d. Mitosis of a basophile macroblast. Note the bizarre figures, which arise through the sticking together of the chromosomes.

e. The same changes with beginning fragmentation and splitting off of chromosomes.

f. Basophile macroblast, with the typical foamy structure of the cytoplasm.
III. Attempts to Influence the Effect of Urethane by the Administration of Folic Acid or Vitamin B₆,*

On the basis of the consideration that urethane perhaps inhibits some growth substances, it appeared to be of interest to test whether the effect of urethane could be abolished by the administration of large doses of certain vitamins which are important for metabolism. We were able to study only folic acid and vitamin B₆.

Cat V received 10 mg. of folic acid daily injected subcutaneously at the same time that it was given 0.1 Gm. of urethane/Kg. orally each day (fig. 3). There was a severe agranulocytosis, with fatal termination seventeen days after urethane administration was begun. The bone marrow also showed no differences as compared to that seen in the other experimental animals (fig. 3). There was thus no effect of folic acid under these circumstances.

The same holds for the experiments with vitamin B₆ (Animals VI and VII): in spite of the daily subcutaneous injection of 50 mg. of vitamin B₆ ("Benadon") at the same time that urethane was given, the animals died after twenty-four and seventeen days with the picture of a very severe aleukia with almost complete disappearance of the granulocytes in the bone marrow (fig. 3).

DISCUSSION

Exclusion of Infectious Agranulocytosis of the Cat: For a long time we have been familiar with an infectious agranulocytosis in the cat which is probably transmitted by a virus. After an incubation period of a few to twelve days, there rapidly appears the picture of a fully developed agranulocytosis. Histologically there are found, besides inflammatory changes of the mucous membrane of the intestine and the disappearance of mature forms in the bone marrow, typical “acidophile intracellular inclusion bodies” in certain cells of the intestine, bone marrow and spleen.

In the experiments reported in the present paper, the possibility that the blood changes reported might be due to such a virus disease rather than to urethane, or that a latent virus was “mobilized” by the urethane, can be eliminated for the following reasons:

1. Infectious agranulocytosis is a pure agranulocytosis, in contradistinction to the disorder described in this report in which leukocytes, thrombocytes, and erythrocytes are shown to have been involved.
2. Inclusion bodies could not be found at autopsy in the experimental animals.
3. The 3 control animals, which were kept together with the urethane animals, showed no blood changes or clinical disease.
4. An attempt to transmit the disease from a urethane animal with panmyelopathy to a control animal, by means of heart blood was unsuccessful.
5. Discontinuance of the urethane led to a gradual normalization of the blood and bone marrow picture in animals in which the changes were not yet too

* We wish to thank the firm of Hoffmann-La Roche, Basel, for its kind gift of the necessary amounts of the drugs for these experiments.
advanced. Renewed administration of urethane led promptly to the same changes again. This behavior would hardly be expected in the case of the mobilization of a virus by urethane, since infectious agranulocytosis of the cat leaves behind a permanent immunity after successful recovery from the disease.

6. For one month previous to the start of the experiments, the observed animals which were left free in a common room showed no agranulocytosis in the blood pictures.

**Blood and Bone Marrow Findings**

**Blood:** Our experiments show that a severe aleukia can be regularly produced in cats by means of urethane: The granulocytes generally showed a greater fall than the lymphocytes. Granulocytopoiesis in the cat is thus more sensitive to urethane than lymphopoiesis. This slight sensitivity of the lymphocytes is in contrast to the behavior of the lymphocytes in man and in the mouse where lymphocytopoiesis is more sensitive than granulocytopoiesis. The fall of the thrombocytes in all except one case shows that, in the cat, thrombocytopoiesis is also inhibited by urethane.

Contrary to former observations in other animals and in leukemias a distinct excitatory phase due to the urethane before the onset of the depressing effect, was not demonstrated in these experiments. The spontaneous fluctuations of the leukocytes in the cat are often large, however, as can be seen in the curves of the control period preceding the experiments.

At all events a leukocyte rise at the beginning of urethane administration was absent in 4 of 7 animals.

**Bone Marrow:** To summarize, it can be said that urethane, in smaller doses, caused in the bone marrow of the cat, besides a decrease of the absolute cell number, a shift to the left of the cell series (that is, an inhibition of maturation) and in larger doses a decided diminution of cells through a decrease of cells in all stages of maturation.

The relative increase of the myeloblasts and promyelocytes was so marked in one cat (Animal IV, fig. 2) that it simulated the hematologic picture of pyrimido-granulocytosis of man, with the almost complete absence of the granulocytes in the peripheral blood stream, and the enormous shift to the left of the granulocytic cell series of the bone marrow. Just as in the case of human agranulocytosis, the strongly marked tendency towards lobulation on the part of the nuclei of the myelocyte and promyelocyte forms is to be emphasized here, as reflecting in a sense a dissociation of the maturation of the plasma and nucleus. Together with the appearance of strikingly large and basophile inhibition-forms, this is an indication of the delayed maturation of these cells. We see, of course, very similar changes in other cases of inhibition of maturation, as, for example, in pernicious anemia, toxic granulocytopenia, etc.

As can be seen from the above findings (figs. 1 and 3) the eosinophiles showed a much less pronounced fall than the other granulocytes, this being the case in the blood as well as in the bone marrow. As can be seen from the histologic
bone marrow sections, there was actually a relative increase of these cells in comparison with the other cells, which are more sensitive to urethane. Figured absolutely, however, these cells also showed a distinct fall.

However, from the behavior of Animals III and IV, in which the fall in the eosinophiles of the bone marrow ran approximately parallel to the fall in the other cells, we see that even in urethane-agranulocytosis, the eosinophiles do not necessarily show a behavior different from that of the remaining granulocytes. It is hard to say on what this difference in behavior depends. In any case, it may be said that it is most probable that the presence of intestinal parasites does not enter into consideration here. Such parasites are very frequently found in cats, but in our experiments, we found them only in Animal III, which showed no increase in the eosinophiles.

It is interesting that this predominance of the eosinophiles has been observed also in numerous agranulocytoses of man (for example, in aminopyrine agranulocytosis). Tobler speaks, in this sense, of a "dissociated granulocyte reaction." Bock looks upon this predominance of the eosinophiles above all as a reaction to the increased cell-destruction. But, as this predominance of the eosinophiles can also occur occasionally in cases of benzol panmyelopathias (as we ourselves have observed), where the effect of the toxic agent often goes back several months, it appears to us that a different explanation is needed.

It is possible to conclude from this behavior of the eosinophiles that these cells form an independent system among the granulocytes. It can, therefore be assumed that these cells differ functionally from the other granulocytes. It seems improbable to us that the relative increase of these cells could be due to a life-span longer than that of the neutrophile granulocytes (two to three days, according to Osgood and others). Some few authors have also considered the eosinophilia of the bone marrow in agranulocytoses as an indication of the anaphylactic nature of this disturbance of the bone marrow. This is certainly not the case in urethane agranulocytosis, in which there is a toxic action. We are, therefore, of the opinion that this eosinophilia in certain agranulocytoses of man is merely an indication of the differences in sensitivity among the several myeloid cell systems.

**Erythropoiesis:** The changes in the erythropoietic system were mild in comparison with those in the granulocytopoietic series, but a finer analysis showed, as we have already pointed out, distinct changes (fig. 4). Thus, we noted a mild but definite inhibition of red cell proliferation in the bone marrow, a decrease of the absolute number of erythroblasts, a shift to the left, an increase of the macroblasts, and a diminution of the mitotic index.

These changes of erythropoiesis are little reflected in the peripheral bloodstream. This is partly true because the urethane must, in most cases, be stopped too early, on account of the severe changes of the white blood picture, so that erythropoiesis returns to normal quite rapidly (Case I). In addition, the life-span of the erythrocytes is long (120 days) in comparison with that of the granulocytes which is only 2-3 days and does not allow long range changes to be recog-
nized quickly. Like our former investigations, the present work shows that, of all the normal blood cell systems, the erythropoietic is the least sensitive toward urethane.

**Plasmacellular reticulum cells:** As already noted, we found a distinct increase of these cells at the time of the severe pancytopenia caused by urethane. This was also noted by Rohr in the bone marrow in pyrimidon-agranulocytosis of man. He believed that this increase must be attributed to the anaphylactic phenomena (antigen antibody reaction) in agranulocytosis. Since such an anaphylactic reaction does not occur in urethane agranulocytosis, it must be assumed that the increase of these cells is related to the infections which always are superadded to agranulocytic conditions. Gormsen and Fagraeus have shown that these cells play a decisive role in the production of antibodies and that the latter are not produced by lymphocytes.

It is interesting that the normal plasmacellular reticulum cells of the cat, are, to all appearances, not inhibited by urethane, whereas the pathologic neoplastically degenerated plasmacellular reticulum cells of human myeloma occasionally show a very marked sensitivity towards urethane. This is, in analogy to the sensitiveness of certain neoplastic cells in contrast to the behavior of their normal counterparts.

**Mitosis:** The disturbance of the morphology of the various stages of mitosis was striking in all marrow preparations. This is in agreement with the observations made by us in leukoses treated with urethane and with the investigations reported by Bock, and Storti. These morphologic changes would not in themselves be proof of a disturbance of cell division. But, the distinct fall of the mitotic index for granulopoiesis as well as for erythropoiesis shows that in the cat this drug has a marked inhibitory effect on cell division in the bone marrow. This is analogous to what we have emphasized for chronic leukoses.

Very probably the extensive hypocellularity of the bone marrow is also partially due to this inhibition of mitotic activity in that the maturing cells pass into the bloodstream, but are not replaced because of an insufficient formation of new cells (see figures 5–8).

We cannot, of course, say from our experiments if this inhibition of mitotic activity is a direct or indirect one. The marked shift to the left in the bone marrow observed in the animals treated with smaller doses of urethane indicates above all, an inhibition of the maturation of the cells, while the general cell impoverishment, without significant shift to the left, in the animals treated with larger doses is due largely to a diminution of the number of mitoses. Probably not only the diminution of mitoses plays a role here, but also the appearance of pathologic mitoses, which lead to the formation, as an end product, of cells that are no longer capable of life because of injury of the chromosome apparatus. This has been demonstrated for other mitotic poisons. Thus, the final result is an even greater diminution of effective cell division, since probably only a small percentage of the mitoses lead to the formation of cells capable of life.

On the basis of the changes here determined experimentally, it can be seen that urethane, in smaller doses, causes above all, an inhibition of maturation, but that in larger doses or when acting over a longer period, it causes above all
a far reaching inhibition of mitotic activity. Both effects naturally merge into one another and are probably due basically to the same alteration in cellular metabolism.

We cannot present here all the theories of the mechanism of action of urethane. We ourselves believe that urethane has the power to block certain cell enzymes or ferments which are vital for cell growth and cell division. Especially involved, probably are the enzymes which are of importance for intracellular respiration.

Recovery of the Bone Marrow: It is clearly evident from our findings that the recovery of the bone marrow depends largely on the total dose of urethane administered. With smaller doses over a short period of time the bone marrow recovers relatively rapidly, whereas with larger doses of urethane administered over a longer period the depressive action of the drug persists considerably longer. In the bone marrow a distinct cell increase is evident long before the rise of granulocytes in the blood (Animal 1). This is due, on the one hand, to the continued decline of the granulocytes in the blood, because of the infections which appeared during the agranulocytic phase. On the other hand, there is a prolongation of the effect of urethane, in the sense of an inhibition of the proliferation and maturation of the bone marrow cells. As a matter of fact, the shift to the left of the bone marrow is, in most of the cases, still demonstrable relatively long after the urethane is stopped, this shift being an expression of an inhibited and delayed maturation. This persistent shift to the left of the bone marrow was not dependent upon the secondary infections which often occur in this late stage of agranulocytosis despite the use of antibiotics and was also demonstrable in our cats without infections.

The after-effect of urethane clearly appears to depend on the duration and dosage of the urethane administration as well as on individual sensitivity (Animal 1). As we have pointed out elsewhere we have to deal here, probably not with a continued action of urethane (which is eliminated rapidly), but with the almost complete blocking or destruction of ferments or enzymes important for cell growth caused by urethane, these ferments being only very gradually replaceable. This can perhaps be compared with the long inhibitory effect of streptomycin on the growth of tubercle bacilli in spite of its rapid elimination from the body.

The marked diminution of these enzymes may also explain the lack of resistance towards infections on the part of patients treated with urethane which Bock demonstrated. Such infections could also be seen again and again in our animal experiments, in spite of intensive antibiotic therapy. Sandkühler could show in phagocytosis experiments that after urethane administration there also developed functional injury of the granulocytes in that such cells lost, in large measure, the ability to phagocyte bacteria.

Administration of Folic Acid and Vitamin B6

On the basis of the consideration that urethane might inhibit the activity of growth factors, it appeared to be of interest to determine whether the effect of certain urethane could be abolished by the administration of large doses of cer-
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tain vitamins which are important for metabolism. On the basis of the negative results obtained thus far, we can say that the depressing effect of urethane on granulocytopenias and lymphopenias in the cat is not lessened or blocked by high doses of folic acid or by vitamin B6. It thus also appears improbable that the action of urethane is in any way related to an inhibition of these two substances.

Although our experiments in no way show this, we believe that the therapeutic effect of these two agents on the bone marrow is very questionable even in the various other agranulocytoses, and that most of the reported successes, with the possible exception of the granulocytopenias due to sulfonamides7,34,38 were cases of pure coincidence. Recovery in these cases, we believe, merely follows removal of the offending agent (e.g., pyrimidin), after a period of 8–10 days which corresponds to the normal maturation time of the granulocytes.15

We have shown in previous experiments, the maturation time of the neutrophiles in man is 6–8 days.15 Even normally, therefore, at least this period of time is required before the output of granulocytes from the bone marrow can take place, after all mature granulocytes in the marrow have disappeared. It is evident that if this fact is neglected one may come to very false conclusions according to the time at which the therapy is applied. The interesting experiments of Kelemen12 who could abolish the leukocytotic effect of paraaminobenzoic acid in man by means of urethane were not yet known to us when these experiments were performed. By taking into account this substance and numerous other growth-substances and fermentes important for cell metabolism, it may, in the course of time, become possible to secure a better insight into the mechanism of action of urethane. This would be of great significance for further research in the field of cytotstic agents and agents inhibiting mitosis. We shall report later on the experiments we have now in progress.

Summary

The investigations reported in the present paper deal with the behavior of the peripheral blood and the bone marrow in urethane-produced agranulocytosis and panmyelopathy in the cat. The principal conclusions follow:

1. A severe panmyelopathy can be produced regularly in cats by the administration of relatively small doses of urethane: viz., 0.05 to 0.1 Gm. per Kg. per day. The panmyelopathy is still reversible if the urethane is stopped early enough and if the animal is maintained with antibacterial therapy.

2. The time required for recovery of the bone marrow after cessation of urethane administration depends on the total dosage of the drug. After small doses recovery occurs rapidly; after large doses much more slowly. Individual sensitivity of the experimental animal also plays a role, however.

3. The effect of smaller doses of urethane (0.05 Gm./Kg. day) on the bone marrow is to produce inhibition of maturation of granulocytopenias, and, to a lesser degree, of thrombocytopenias. To a still lesser extent, there is also inhibition of maturation of the red cell series.

4. The effect of larger doses of urethane (0.1 Gm./Kg./day) on the bone
marrow, the effect on maturation is overshadowed by a generalized depletion of all marrow cells. Part of this effect is due to a decrease in the number of mitoses, with reduction of the mitotic index; as well as qualitative alterations in the mitotic patterns. It is probable that the effect on mitoses results in the formation of cells which are incapable of life as well as in an overall decrease in the formation of cells.

5. In the cat, the neutrophilic granulocytes are most sensitive to the action of urethane. The lymphocytes are next most sensitive; then the megakaryocytes, finally the erythroblasts. The eosinophils were remarkably insensitive.

6. The normal plasmacellular reticulum cells are relatively insensitive to urethane. This is in contrast to the neoplastic myeloma cells in human beings which have been reported to be sensitive to urethane.

7. The administration of folic acid or of vitamin B₆ to the experimental animals had no effect on the course of urethane agranulocytosis.

8. The normal granulocytopoiesis of the cat is about as sensitive towards urethane as the pathologic granulocytopoiesis of chronic myeloid leukoses of human beings. This is in contrast to the findings in other animals, and to normal granulocytopoiesis of man. The variation in behavior possibly indicates variations in enzyme systems in the various types of granulocytopoiesis, with only certain enzymes being affected by urethane.

9. Further experiments are in progress to determine if the effect of urethane on the bone marrow can be counteracted by means of other growth substances. It is hoped that better insight may thus be gained into the mechanism of action of urethane.

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