THE NORMAL LIFE SPAN OF THE NEUTROPHILE (AMPHOPHILE) LEUCOCYTE (RABBIT)*

THE ACTION OF BENZOL IX

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Heretofore apparently the life span of somatic cells in their normal somatic environment has not been determined accurately. This article establishes with reasonable accuracy the normal length of life in the body of the normal amphophile leucocyte from the time when it passes from the marrow into the blood and under somatic conditions which are relatively normal.

In previous articles of this series [1, 2] attention was called to the fact that after exposure to the action of subcutaneous injections of olive oil-benzol mixture (equal parts of olive oil and benzol), the leucocyte curve follows a course, a portion of which we have called "diphasic leucopenia." In an average of six days after the beginning of an average of four and a half daily injections of 1.5 to 2 cc. per kilo of body weight, the curve falls to the neighborhood of 1,000. This fall is followed by a primary rise to a normal level. Then, independently of any further injections, the curve again falls to a level as low or nearly as low as reached in the primary fall. Following this secondary fall there occurs a rise of the curve to a normal level and there is no evidence of any subsequent falls (Charts 18 and 19). To the phase of the leucocyte curve involving the first period of leucopenia we have given the name "protophase," and to that involving the second period of leucopenia the name "deuterophase."

In the fifth article [3] of this series it was shown that in the diphasic leucopenia resulting from subcutaneous injections of olive oil-benzol mixture in rabbits the amphophile and small mononuclear curves run more or less independently of each other and that the diphasic character of the leucopenia is essentially a polynuclear amphophile phenomenon.

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Histopathology

As a basis for the subsequent discussion it is proposed to consider briefly at this time those features of the histopathology of the marrow which have to do with the diphasic character of the leucopenia.

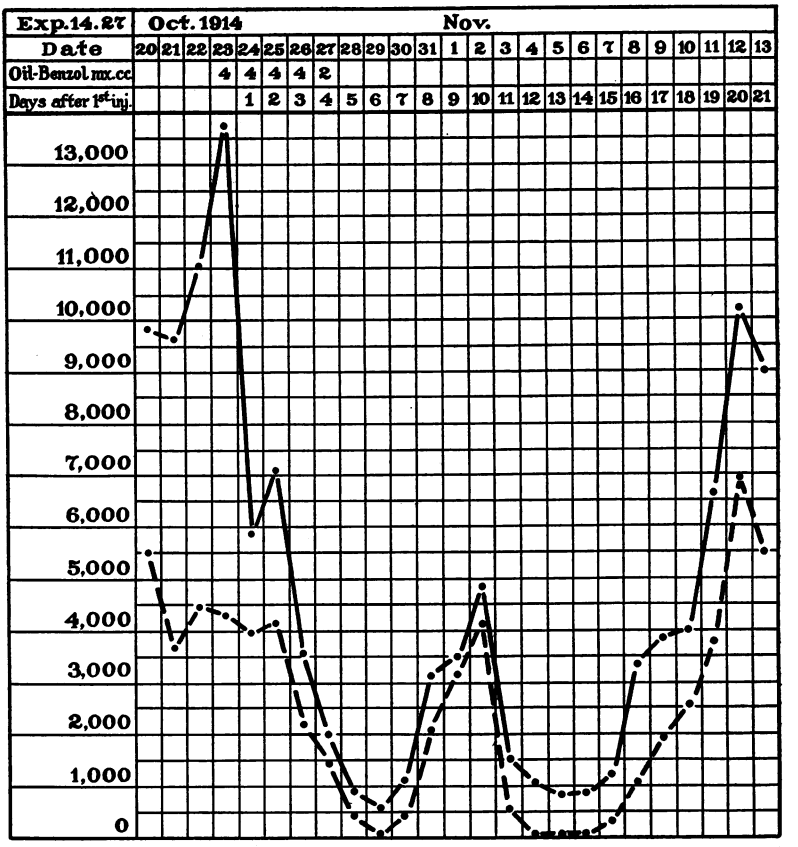

Chart 18 (Exp. 14.27). Total leucocyte and absolute polynuclear amphophile curves.

The histopathology is based on rabbits which died and on rabbits which were killed at various stages of the diphasic leucopenia and showed typical though incomplete diphasic leucopenia curves up to the time of death. For these reasons the histopathological pictures cannot be perfectly correlated with complete blood pictures be-

cause there are no data in regard to those parts of the blood pictures which would have occurred if the rabbits had survived. In the case of a rabbit presenting the blood picture of a typical diphasic leucopenia up to the time it died or was killed, there is no way of determining whether the remainder of the blood picture would have presented the completion of a typical diphasic leucopenia if the rab-

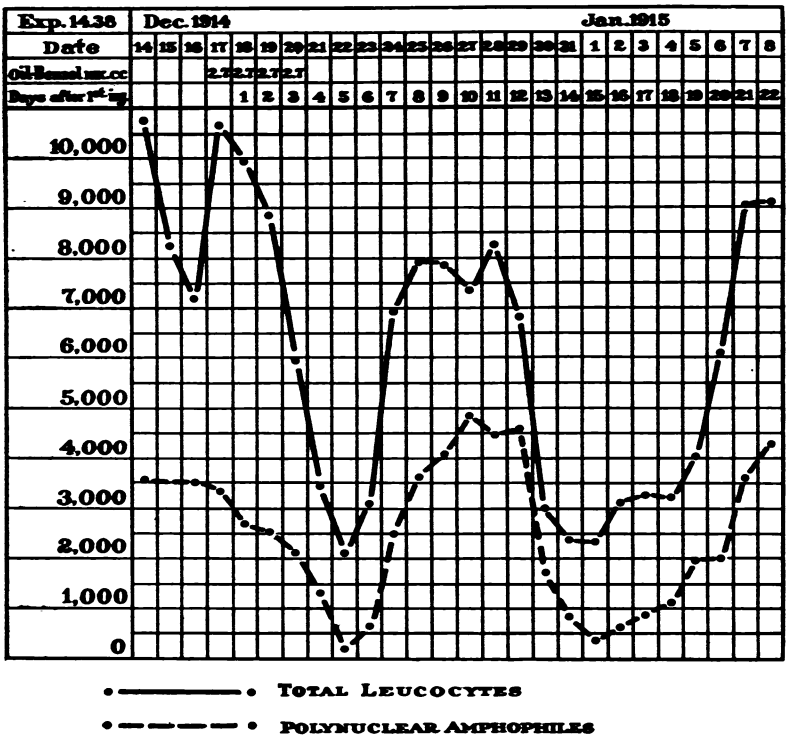

CHART 19 (Exp. 14.38). Total leucocyte and absolute polynuclear amphophile curves.

bit had survived. For these reasons considerable numbers of rabbits at stages of the diphasic leucopenia under consideration will be advantageous for the reaching of reliable conclusions. Our conclusions are based upon seven rabbits dying and two rabbits killed at the climax of the protophase; two rabbits dying and three rabbits killed during the protophase rise; four rabbits dying and three rabbits killed at the climax of the deuterophase, and fourteen rabbits killed after the completion of the phenomenon.

The injections of the benzol mixture result in necrosis and aplasia of the bone marrow. In surviving animals this is followed by resolution and regeneration. Regeneration is initiated by the development from various centers of islands of regenerating cells. These islands consist of practically pure cultures of the various bone marrow cells. In the early stages of regeneration these centers are well isolated, some consisting entirely of myelocytes (Figs. 1 and 2), others of megakaryocytes (Figs. 3 and 4), and still others of nucleated red cells. Later as regeneration progresses the various centers tend to intermingle in their growth and are accompanied by the mature cells which they produce. Observations bearing upon this matter were made by Selling,[4] and have been confirmed by MacCallum.[5]

In relation to the diphasic amphophile leucopenia, we have confined the marrow studies to amphophiles and amphophilic myelocytes, these latter being perhaps the only cells of the marrow parenchyma that can be certainly identified in fixed and stained tissues as being forerunners of amphophiles in the blood. In the sections we distinguish between original and regeneration myelocytes. As mentioned above the regeneration myelocytes appear to be characteristic in morphology and environment.

The regeneration myelocytes make their earliest appearance at the climax of the deuterophase; the earlier ones probably differentiating from more immature forms.

All other amphophilic myelocytes in the marrow are like those in normal marrow. For this reason we designate them as original myelocytes.

Examination of the marrows of rabbits dying or killed at the climax of the protophase, shows marked necrosis and aplasia. However, practically all show certain numbers of amphophiles and original amphophilic myelocytes but no regeneration myelocytes. As will be emphasized later, it is these surviving amphophiles and surviving original myelocytes which bring about the protophasic rise.

The marrows of rabbits killed at the end of the protophasic rise show practically complete aplasia with very few, if any, amphophiles or original amphophilic myelocytes, and no regeneration myelocytes. The marrow appears practically completely aplastic.

The marrows of rabbits dying and killed at the climax of the

deuterophase show extreme aplasia with here and there colonies of regenerating cells such as described above. The sections indicate active regeneration occurring in practically completely aplastic marrows.

The marrows of rabbits killed some time after the completion of the cycle apparently differed in no way from the marrow of rabbits which had not received injections.

The Amphophile Curve

I propose now to examine certain aspects of the amphophile curve and to give a correlative interpretation with certain prominent features of the histopathology of the marrow. Perhaps the most important result will be to show that low levels of the amphophile curve are apparently entirely dependent upon two factors, either one sometimes acting alone, or at other times both acting together:

1. The extent of the destruction or death of amphophiles going on in the blood.

2. The number of amphophiles in the marrow available to the blood.

This will mean that low levels are independent of any alteration in the potential balance of physicochemical or other biological reactions, such as amphophilotropism. The amphophile curve remains at or below the normal level depending upon the available supply of amphophiles.

As illustrated in Charts 18 and 19, there is a period of about two days after the beginning of injections and before the beginning of the protophase, during which the amphophile curve shows no effect of the injections. During this period, if there is any destruction of amphophiles in the blood, it is at once compensated for by access of marrow amphophiles into the blood.

During the protophasic amphophilic fall, destruction of amphophiles in the blood cannot be fully compensated for on account of the effective amphophile poverty of the marrow. This accounts for the occurrence of the protophasic amphophilic fall.

The first evidence of the protophasic amphophilic rise appears about at the end of the third day after the last injection. At this time it is likely that initiation of amphophile destruction, that is, attack upon additional amphophiles and amphophilic myelocytes in

the blood and marrow has discontinued, because return of the urinary phenol curve to the level existing before injections indicates that the injected benzol has been eliminated.[6] The end of the protophasic amphophilic rise occurs about two days later. The protophasic amphophilic rise is the result of access from the marrow to the blood of surviving mature amphophiles and of amphophiles differentiating from surviving original amphophilic myelocytes. The two processes of access and differentiation may be assumed to go on more rapidly with the cessation of the action of injected material on the involved cells. The rapidity would probably be accentuated as a reactive result of the existing leucopenia.

Examination of the average amphophile curve in Chart 9,[3] shows that the amphophile level at the end of the protophasic amphophilic rise is the same as that existing before injections. Examination of the amphophile curve in Chart 19 shows that it may remain at this level for over four days. This period of constant level which occurs in a considerable number of cases is of fundamental interpretative importance. During this period elevations and depressions in the curve are due to daily variation in the amphophile count. In cases in which there occurs such a period of constant normal level at the end of the protophasic rise it means that the supply of marrow amphophiles (from original myelocytes) available to the blood is large enough, not only to bring about the protophasic amphophilic rise to the level existing before injections, but also to maintain it at that level for a time by compensating for the disappearance from the blood of those which perish as a result of reaching the end of their life history.

In cases in which no such period of constant level (Chart 18) occurs it means that the supply of marrow amphophiles available to the blood is exhausted in bringing about the protophasic amphophilic rise. As a matter of fact such supply of amphophiles is in some cases insufficient to bring the protophasic rise up to the original normal level.

Next, the exhaustion of the supply of marrow amphophiles available to the blood results in the deuterophasic amphophilic fall. The deuterophasic amphophilic fall is directly dependent upon the disappearance from the blood of those amphophiles which perish.

At the beginning of the fall, practically the total potential supply of amphophiles is represented by those in the blood. Among them

all ages are represented. At the end of the fall they have nearly all disappeared. This means that the youngest of them disappear within this period. The maximum duration of life of nearly all of the amphophiles in the blood is thus not more than the interval between the beginning and the end of the deuterophasic fall. The average duration of this interval computed from twenty-six cases of diphasic leucopenia is three and a half days.

Perhaps the main complicating factor in connection with this argument is the increasing amphophile leucopenia which exists during the fall. This may be conceived of as placing an abnormal functional strain upon the amphophiles present in the blood and thus shortening their lives. Nevertheless the conditions approach vastly more nearly normal than those involved in *in vitro* studies of this problem. I know of no proof that such unusual functional cell activity shortens the lives of somatic cells.

The initiation of the deuterophasic amphophilic rise depends on access to the blood of marrow amphophiles differentiating from regeneration as distinguished from original amphophilic myelocytes. As the supply gradually increases with progressive amphophilic marrow regeneration, the rise continues until the level existing before injections is reached, as in the case of the protophasic amphophilic rise, and then this level is maintained.

Conclusions

1. Cessation of the supply of amphophiles from the marrow results in practically complete disappearance of amphophiles from the circulating blood in a period of between three and four days.

2. The average duration of life of amphophiles in the rabbit's blood is between three and four days.

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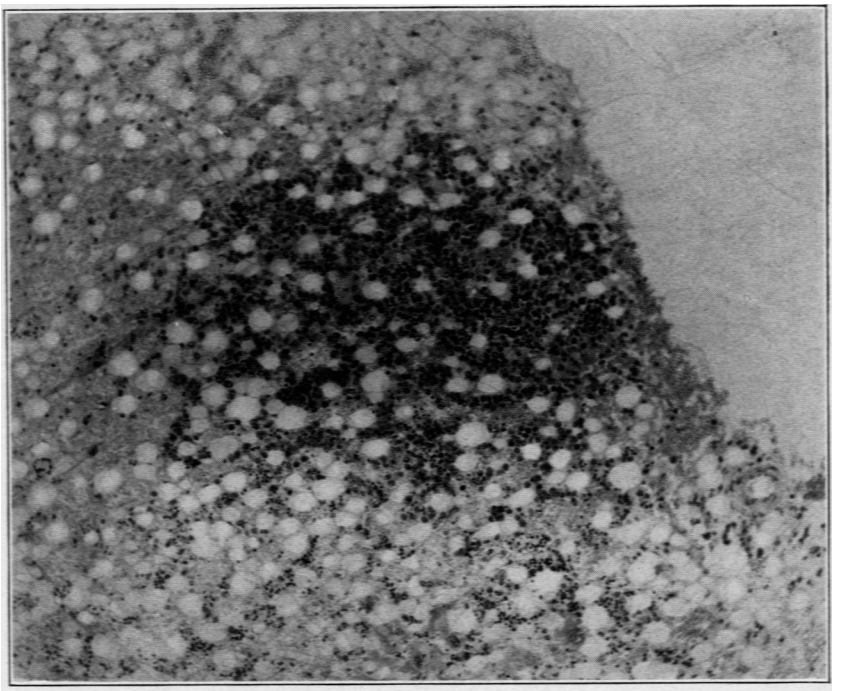
DESCRIPTION OF PLATES

PLATE 39

FIG. 1. Bone marrow regeneration. Island of regenerating myeloblasts (Exp. 14.34). × 85.

FIG. 2. Same as Fig. 1. × 1100.



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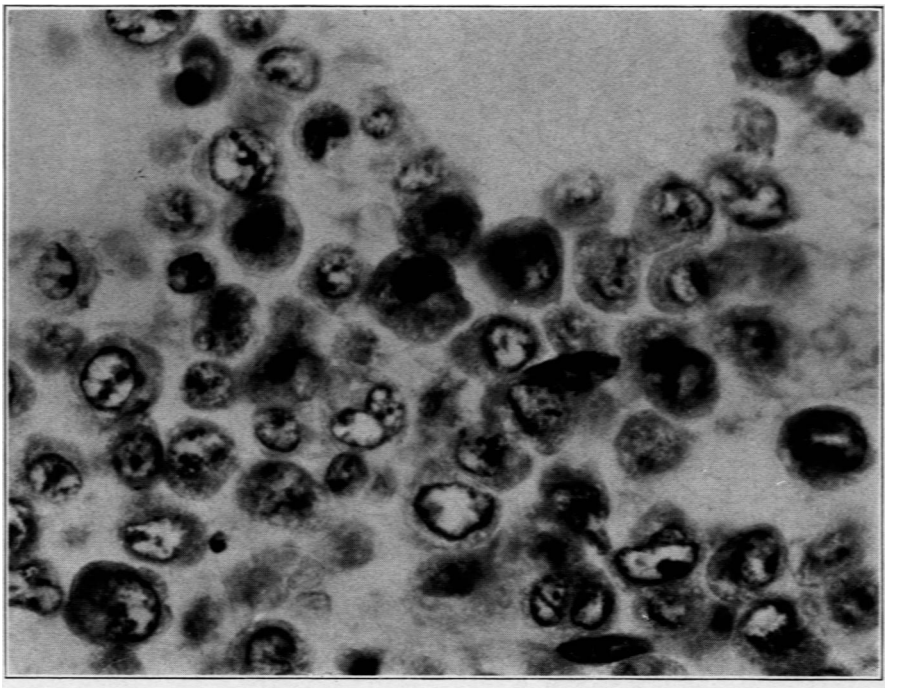

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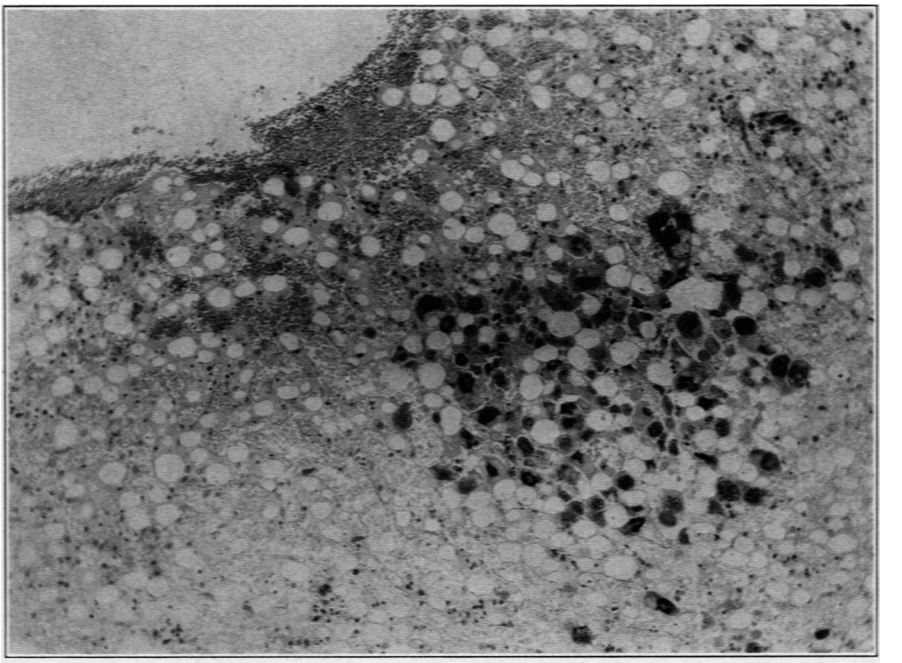
PLATE 40

FIG. 3. Bone marrow regeneration. Island of regenerating megakaryocytes (Exp. 14.34 same section as Fig. 1). × 85.

FIG. 4. Same as Fig. 3. × 1100.



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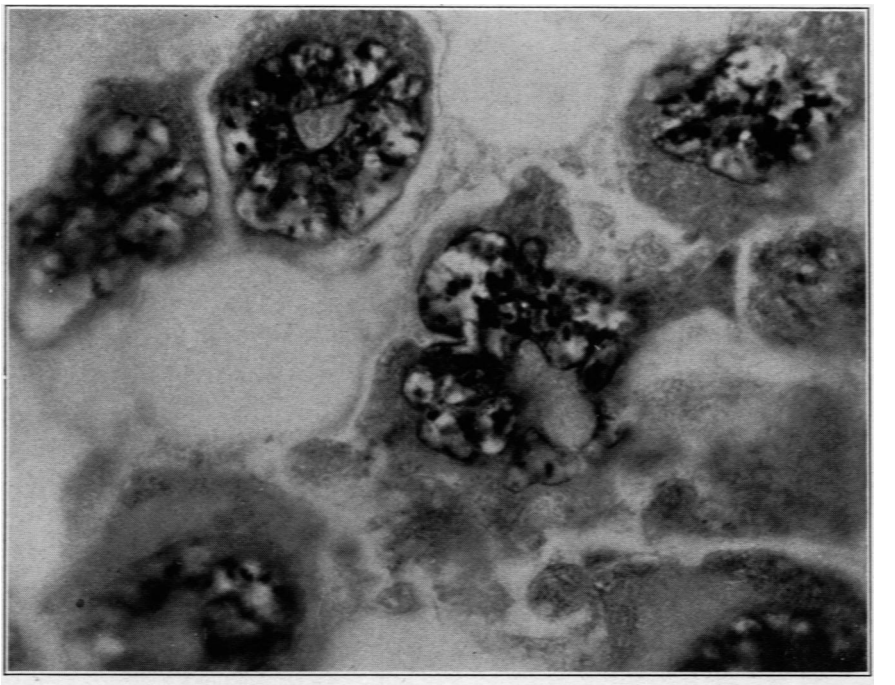

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