EXPERIMENTAL LEUKOCYTOSIS. THE INEFFICACY OF P-CHLOROXYLENOL AND METHYL ACETAMIDE AS BONE MARROW STIMULANTS

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INTRODUCTION

In 1943, Zondek and Bromberg produced leukocytosis in 25 human subjects with intramuscular injections of methyl acetamide and para-chloro-xylenol.* In this group, 17 were normal, 4 suffered from typhoid fever with leukopenia, and 4 had local infections with leukocytosis. The maximal responses were obtained by using a 70 per cent solution of methyl acetamide with 25 per cent p-chloro-xylenol (referred to as CXM). In the normal subjects, leukocytosis of 10 days' duration resulted from injecting 50 cc. of the above solution over a period of 3 days. In the typhoid fever patients larger doses were required to produce leukocytosis and this did not persist when the injections were discontinued. In the cases of local infections with leukocytosis, the response was more prompt, and reached extraordinarily high levels. In addition to the leukocytosis, there was a "distinct shift to the left" in the polymorphonuclear leukocytes with "the number of young and band forms being increased." Sternal marrow examinations during the height of the leukocytosis showed a "high percentage of band forms." The authors suggested that CXM had a selective stimulating action on the myeloid elements of the bone marrow without affecting the red cells or platelets. The graphs reveal that 5 days was the longest period of time over which the drug was given (in typhoid leukopenia). The authors also studied the effect of CXM on rats, hens and rabbits, but because of the marked fluctuations in the total number of white cells in these animals, no conclusions were reached.

METHODS

In this study the peripheral blood, bone marrow and internal organs of two groups of rats which were given doses of CXM comparable to those recommended by Zondek and Bromberg were investigated. Fifteen mature white rats (weighing about 200 Gm.) and seventeen mature gray rats, averaging about 400 Gm., were used. The animals were housed in small cages in groups of 3 or 4 and given water and Purina dog chow ad lib. Because of the fluctuations in leukocyte counts in rats as a result of manipulation or trauma, the animals were handled several times a day, and blood counts taken (by clipping the tails) at different intervals, in order to determine the highest normal count for each subject. The intramuscular dosage was 0.1 cc. for the smaller animals, and 0.3 cc. for the larger ones. The rats were injected at intervals of 12 and 24 hours, and blood counts taken at varying periods. The smaller animals received a total dosage up to 0.4 cc., and the larger ones 0.8 cc. Several animals were found dead after 2 or 3 days' administration of

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Fig. 1. —, % P.M.N.; ———, total W.B.C.

Fig. 2. —, % P.M.N.; ———, total W.B.C.
Fig. 3. ———, % P.M.N.; ———, total W.B.C.

Fig. 4. ———, % P.M.N.; ———, total W.B.C.
the drug. The other animals were killed by a blow on the head. Smears of the vertebral marrow and histologic sections of a vertebra, liver and spleen were examined.

**OBSERVATIONS**

Figures 1 to 4 indicate the typical variations in leukocyte response to single and multiple injections of CXM in two series of rats. The maximal effect was reached 12 to 36 hours after the first injection, with a slow return to normal in 24 to 48 hours. The animals receiving 2, 3 and 4 injections evidenced a leukocyte response no greater or more substantial than those receiving one dose. In all instances, the maximal percentage of polymorphonuclear cells was about twice the highest figure reached in the week prior to the injections. All the cells were of the mature variety and no "shift to the left" could be determined. It is noteworthy that the animals responding with the greatest leukocytosis showed unusually high counts before parenteral therapy began. These observations are in accord with those of Zondek and Bromberg, who noted hyperleukocytosis when CXM was injected in patients with local infections and leukocytosis. Bone marrow smears and sections in all 4 groups were normal. The myeloid elements were not hyperplastic, nor was there any suggestion of hypoplasia or atrophy of the erythroid series. A "shift to the left" did not occur and the megakaryocytes were unaffected. The sections of spleens also indicated normal histologic appearances in all 4 groups. Pathologic alterations occurred only in the livers of rats receiving single doses of CXM as evidenced by mild cloudy swelling, whereas those receiving multiple injections presented extensive and advanced hydropic degeneration. In some hepatic cells there was beginning fatty metamorphosis.

**DISCUSSION**

The increase in the number of polymorphonuclear leukocytes in the peripheral blood apparently results from a withdrawal of these cells from depots throughout the body (spleen, vascular bed and bone marrow) and not from marrow stimulation. The fact that the leukocytosis could not be sustained further suggests that there was no stimulation of granulocyte production but rather an expulsion of these cells from reservoirs. This "redistribution phenomenon" is a normal physiologic mechanism which in this instance is augmented by the injection of CXM. Apparently the capacity of the vascular recesses as storage depots for blood cells after delivery from the marrow is not exhausted by normal physiologic requirements. Examination of the bone marrow of human subjects during acute infections associated with leukocytosis usually shows an increase in the myeloid cells with a "shift to the left." Such findings were not obtained in any of the rat marrows observed.

**SUMMARY**

1. Single and multiple injections of CXM (methyl acetamide and p-chloroxylenol) have a similar action in increasing the total number of polymorphonuclear
leukocytes in the peripheral blood of a group of 32 rats. No "shift to the left" takes place.

2. There is no stimulation of the myeloid elements of the bone marrow to suggest that this leukocytosis is due to hyperplasia.

3. The evidence suggests that the mechanism for the leukocytosis produced by the CXM consists of releasing blood cells from depots in the body. This action is selective for the granulocytes as the red cells and platelets are not affected.

4. There is progressive degeneration of the parenchymal cells of the liver after single and multiple injections of these substances.

5. If less toxic combinations of methyl acetamide and para-chloro-xylenol could be obtained, they might be of value in the treatment of certain patients with leukopenia or agranulocytosis more particularly when the bone marrow is hyperplastic but the granulocytes are not released.

REFERENCE


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