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Effects of Cyclophosphamide on Leukocytes in Peripheral Blood and Bone Marrow

(cyclophosphamide effects/leukocytes/bone marrow)

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Peripheral leucocyte counts and bone marrow examinations were made after intraperitoneal administrations of 10 mg Cyclophosphamide into guinea pigs for five consecutive days. The leucocyte counts were lowest on the 2nd day after the total dose of 50 mg had been injected, and returned to normal on the 8th day. Of the leucocytes component, the neutrophil counts most markedly decreased to 10% of their preinjection level, returned to the normal level on the 6th day, and revealed overshooting on the 8th day. Unlike the neutrophils, the lymphocytes decreased to 50% on the 2nd day and failed to recover fully even on the 8th day, showing a delay in recovery. Bone marrow examination in the diaphsis and epiphysis of the os longum after administration of Cyclophosphamide revealed similar findings as seen in peripheral leucocytes. The marrow megakaryocytes decreased remarkably on the lst day after injection, and returned to normal on the 10th day. Myelocytes also decreased markedly on the lst day, and the value became fairly normal by the 5th day. But there was a delay in recovery in the erythroblasts which showed a value of only 60% of the normal level even after 10 days. The fatty tissue of the bone marrow revealed changes which were contrary to that seen in other bone marrow cells.

Cyclophosphamide is an alkylating agent, and as it acts upon actively dividing cells, it is widely used as an anti-tumor drug. The side effects reported with clinical use include anorexia, general malaise and hematological changes, particularly decrease in leukocytes. However, these disappear rapidly with discontinuation of the drug. A study was made of the course of these effects from the standpoint of peripheral leukocyte count and differential count together with histopathological review of the marrow.

MATERIALS AND METHODS

All experimental animals were white male guinea pigs weighing 250—300 g. Cyclophosphamide in 100 mg vials was used after dissolving in physiological saline.

The leukocyte count and differential count were studied by giving 10 mg of Cyclophosphamide intraperitoneally into 20 guinea pigs for five consecutive days. Leukocyte count was performed and smear samples were prepared, stained by May-Giemsa stain and classified based on venous blood drawn from the hind leg immediately prior to injection, 2 and 4 days after initiating the series of injections (after injection of 20 and 40 mg respectively) and 6, 8, 10 and 12 days after discontinuation of injections (2, 4, 6 and 8 days respectively after discontinuation).

The bone marrow of os longum was studied after dividing a separate batch of 20 guinea pigs into 4 groups : Group 1—given 10 mg injections of Cyclophosphamide for 5 days after which the marrow was collected from the femur of the right hind leg on the lst day following discontinuation of injection ; Group 2—collection was performed on the 5th day after discontinuation ; Group 3—collection was made on the 10th day ; Group 4—a non-injected control group from which the marrow was collected in the same manner. Two slides were prepared from each specimen and stained with H-E stain. Three densely populated cell areas were selected from each slide and observed using a 10 mm^2 ocular micrometer and observation was made over the optical field.

The number of megakaryocytes within the optical field of the micrometer (×400) were counted and the myelocytes were observed to determine the degree of maturation and density of proliferation (×400).

Erythroblasts were counted when there was a cluster of more than 3 cells within the field of vision (×400). The degree of fatty tissue was determined by the number of areas of fatty cells within 100 squares of the micrometer (×100). Also, the degree of vessel dilatation in the marrow was also observed.

RESULTS

Changes in Leukocyte Count and Differential Count

The leukocyte count in the 13 animals in which it was possible to make continuous determinations showed a decrease with increased dose, but from the 8th day (4 days after administration was discontinued), it gradually increased, and returned to its original value on the 12th day (8 days after discontinuation) (Table I).

The changes in differential count as observed in 11 animals were determined for neutrophils (mononuclear and polymorphonuclear), basophils, eosinophils,

TABLE I. *Changes in Leukocyte Count in Peripheral Blood Induced by Intraperitoneal Administration of Cyclophosphamide in a Dose of 10 mg/day for 5 Days*

Prior to injection	2nd day	4th day	6th day (2 days after discontinuation)	8th day (4 days after discontinuation)	10th day (6 days after discontinuation)	12th day (8 days after discontinuation)
6060	4580	2520	1720	2800	4380	6890

TABLE II. *Changes in Counts of Various Types of Leukocytes Induced by Intraperitoneal Administration of Cyclophosphamide in a Dose of 10 mg/day for 5 Days (Mean of 11 Cases)*

	Prior to injection	2nd day	4th day	6th day (2 days after discontinuation)	8th day (4 days after discontinuation)	10th day (6 days after discontinuation)	12th day (8 days after discontinuation)
Basophils	5 (0.5)	7 (6.4)	5 (0.5)	2 (0.2)	0	0	5 (0.5)
Eosinophils	13 (1.1)	0	3 (0.3)	3 (0.3)	0	0	4 (0.4)
Neutrophils (Mono.)	6 (0.5)	2 (0.2)	1 (0.1)	3 (0.3)	22 (2.0)	47 (4.3)	51 (4.6)
Neutrophils (Poly.)	493 (44.8)	616 (56.0)	319 (29.0)	51 (4.6)	300 (27.3)	520 (47.3)	671 (61.0)
Lymphocytes	510 (46.4)	427 (38.8)	736 (66.9)	1009 (91.7)	656 (59.6)	412 (37.5)	265 (24.1)
Monocytes	70 (6.0)	48 (4.4)	36 (3.3)	32 (2.9)	120 (10.9)	91 (8.3)	104 (9.5)

Figures in ( ) are percentages

lymphocytes and monocytes. As shown in Table II, since the number of basophils and eosinophils was few, it was difficult to demonstrate a specific trend attributable to the effects of Cyclophosphamide administration, but the neutrophil value markedly decreased as the drug dose increased, and a sharp increase in mononuclear neutrophils was noticed after injections were discontinued. However, the lymphocytes failed to demonstrate such a marked increase or decrease.

Changes in Bone Marrow of Os Longum

As shown in Table III, the number of megakaryocytes in the control group ranged widely from 4 to 16, with a mean count of 8.4. On the lst day after administration of a total dose of 50 mg, the mean count was a markedly decreased 0.8, but on the 5th day it increased sharply to 6.6 and returned to normal on the 10th day. There were signs of degeneration of the nucleus

TABLE III. *Changes in Bone Marrow Cells Induced by Intraperitoneal Administration of Cyclophosphamide in Doses of 10 mg/day for 5 days (Mean of 30 sites)*

	Controls	1 day after injection	5 days after injection	10 days after injection
Megakaryocytes	8.4	0.8	6.6	8.4
Erythroblasts	10.7	1.5	7.7	6.5
Fatty tissue	0.8	86.2	0.8	0.1

in many of the megakaryocytes on the lst day after injection (Figs. 1—4).

As the number of myelocytes was numerous, a study was made to determine whether or not they were densely populated. Thirty areas were reviewed in the controls, and with the exception of one area where it was only fairly dense, the remainder were densely populated. In animals one day after administration of Cyclophosphamide, myelocytes could be seen in only a few samples, and there were two areas where there were hardly any

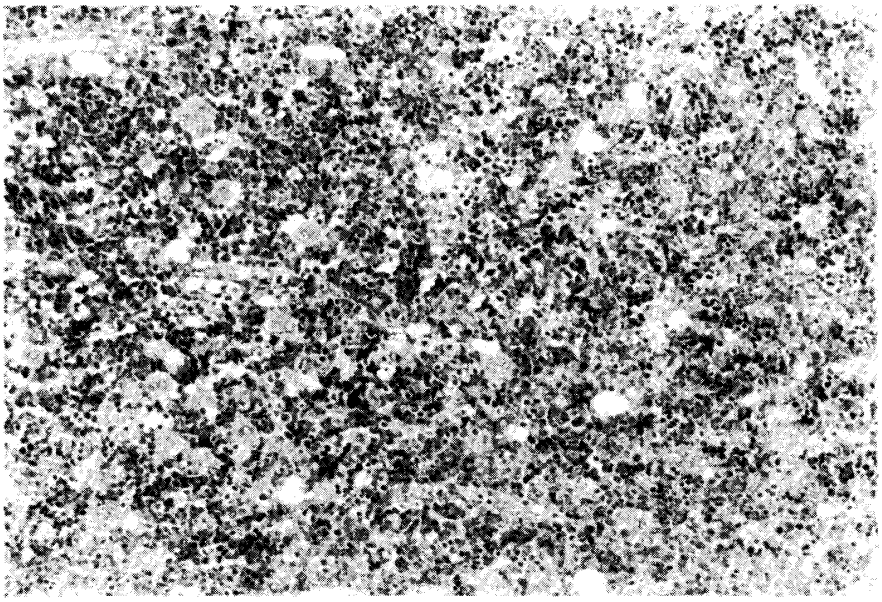

Fig. 1. Marrow of os longum-Control, H. E. stain (×80)
Megakaryocytes, myelocytes and erythrocytes.

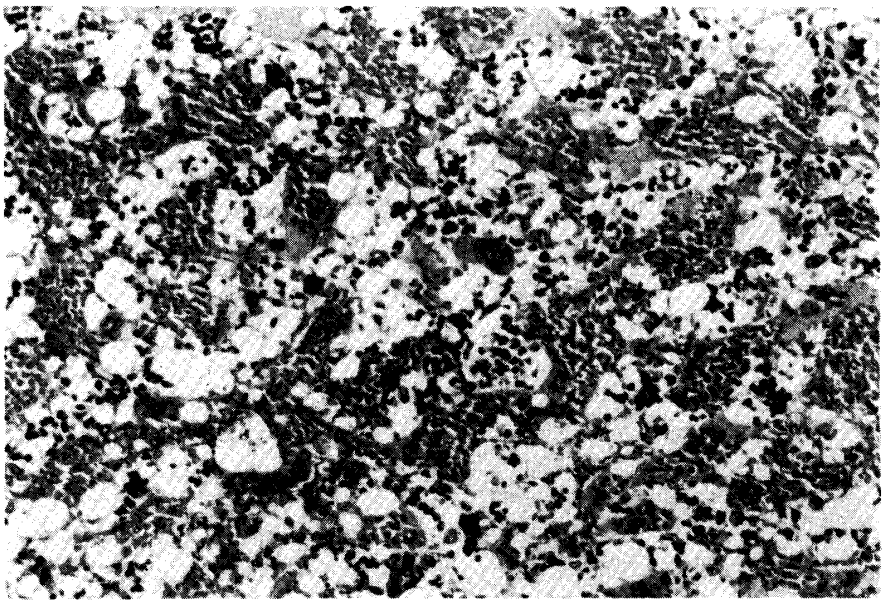

Fig. 2. Marrow of os longum-1 day after discontinuation of intraperitoneal administration of Cyclophosphamide in doses of 10 mg/day for 5 days, H. E. stain (×80)
Marked decrease in myelocytes and reduction in megakaryocytes and erythroblasts but increase in fatty tissue.

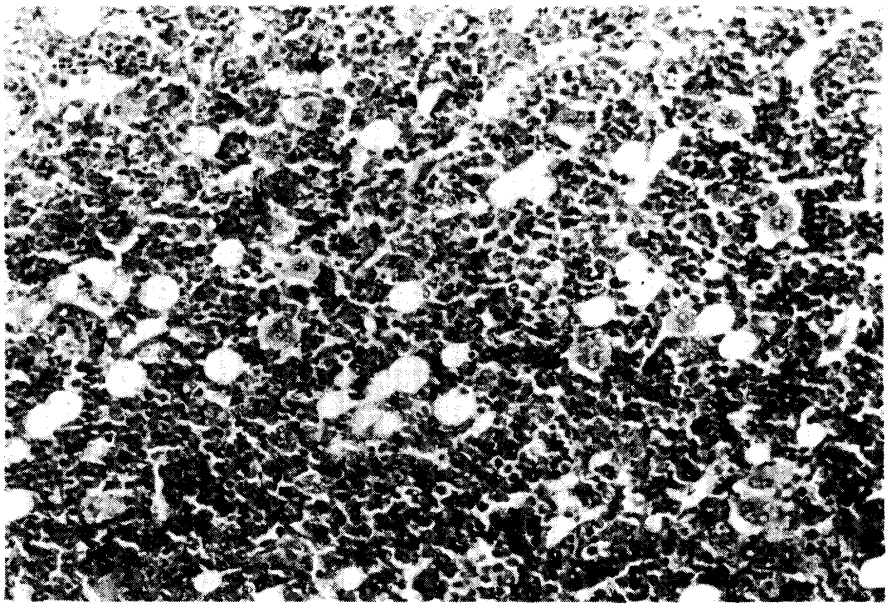

Fig. 3. Marrow of os longum-5 days after discontinuation of intraperitoneal administration of Cyclophosphamide in doses of 10 mg/day for 5 days, H. E. stain (×80)
Increase in megakaryocytes, myelocytes and erythrocytes.

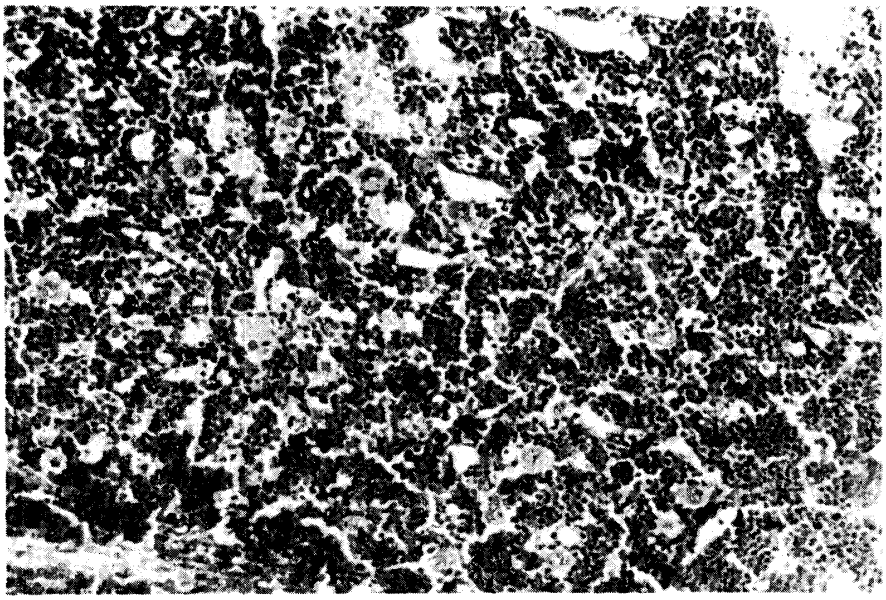

Fig. 4. Marrow of os longum-10 days after discontinuation of intraperitoneal administration of Cyclophosphamide in doses of 10 mg/day for 5 days, H. E. stain (×80)
In comparison to the controls, a decrease in fatty tissue and increase in myelocytes.

cells. On the 5th and 10th days after injection, the density of myelocytes was similar to or even more densely populated. The main cells seen in the bone marrow on the lst day after administration included erythrocytes, plasma cells histiocytes and lymphocytes. Further, on the 5th day after injection, cells of the marrow series, particularly proliferation of immature cells were

evident. Similarly, on the 10th day after injection, many blast-like cells were observed (Figs. 1—4).

The mean number of erythroblasts in the controls was 10.7, but on the lst day after administration of 50 mg of Cyclophosphamide, the number dropped drastically to 1.5. However, there was a gradual increase on the 5th and 10th days (Figs. 1—4).

There was marked variation in the size of the area occupied by the fatty tissue and on the lst day after injection, the marrow appeared to be almost totally replaced by fatty tissue. In the controls, the number of fatty tissue areas was few and these areas were fairly uniformly distributed throughout. On the 5th and 10th days after administration, the amount of fatty tissue was markedly reduced, particularly so on the 10th day (Figs. 1—4).

There was practically no vascular dilatation on the controls, but vessels in animals on the lst day after injection showed marked dilatation, but by the 5th and 10th days, the vessels were only slightly dilated (Figs. 1—4)

The above mentioned findings were similar in both the diaphysis and epiphysis.

DISCUSSION

Injection of Cyclophosphamide has been found through animal experiments to cause reduction in leukocytes, but there is rapid recovery with its discontinuation (1—9), and similar findings have been observed in man in connection with use of the drugs for treatment of malignant tumors (10—14). Recently, efforts are being made to enhance recovery of leukocyte reduction following Cyclophosphamide injection by pre-treatment with complete Freund's adjuvant (15). In this experiment, maximum decrease in count was noted on the 2nd day after drug discontinuation, and fairly normal values were reverted to by the 8th day, but the changes, decreases and increases, primarily involved the neutrophils. However, the lymphocytes are also fairly affected, e. g., the mean leukocyte count on the 6th day was 1,720 and the lymphocytes accounted for 91.7% (1,577) which was half that of the pre-injection count of 3,062 or 46.4% of the total leukocyte count of 6,060. Further, on the 12th day when the leukocyte count was practically restored, the lymphocyte count was only 1,160, which is a great delay when compared with the neutrophils. Review of literature in which follow-up was continued beyond 10 days after drug discontinuation showed that in mice administered 250 mg/kg of Cyclophosphamide, the overshooting observed from the 10th day was due to regeneration of granulocytes while the recovery of lymphocytes was delayed (4). De Wys *et al.* (6) similarly injected 275 mg/kg into mice, and the leukocyte, granulocyte and lymphocyte counts dropped to their respective lowest levels on the 4th day, but the counts excluding the lymphocytes recovered on the 6th day, while 21 days passed before the lymphocytes reached their original level. Host (3) also gave 50 mg/kg of Cyclophosphamide intravenously to rats and noted a similar tendency.

Hunninghake and Fauci (9) administered a single dose of 100 mg/kg of Cyclophosphamide intraperitoneally into guinea pigs and observed the animals for 5 days. They found a gradual decrease in neutrophils which became marked on the 5th day, but no such decrease was seen in the number of lymphocytes.

As the type of experimental animal used, method of drug administration, number of injections and dosage differed between our study and those reported above, direct comparisons cannot be made, however, since the effects of Cyclophosphamide on the granulocytes and lymphocytes tended to be similar, there are probably no great differences.

In view of the marked decrease and overshooting of granulocytes as a result of Cyclophosphamide administration, the possibility of an effect upon the bone marrow, the major hematopoietic organ, was considered, and a comparison of the peripheral blood findings was made by carrying out histology of the marrow. The changes noted in the marrow mainly involved the megakaryocytes and myelocytes, that is, they both decreased considerably from immediately after the injection of 50 mg of Cyclophosphamide, but almost completely recovered by the 5th day and were back to normal count by the 10th day. Among the various reports on myelocyte count after the administration of Cyclophosphamide, the observation of nucleated cells made by Ito *et al.* (4) showed that there were hardly any cells on the 3rd day, but signs of recovery were noted on the 5th day and overshooting was seen on the 10th day. In view of the changes in the bone marrow and leukocyte count in relation to time, it is considered that a definite relationship exists between the two. At the same time, the state of the marrow erythroblasts were studied, and there was a marked reduction from immediately after administration of the drug, and by the 10th day, the recovery stood at only 60%. A similar trend has been reported (4) in mice, in which 15 days were required for full recovery.

Further, since the amount of fatty tissue in the marrow and the completeness of tissue composition are inversely related, the fact that there is a marked decrease 10 days after administration reflects the tendency of overshooting, particularly by the myelocytes.

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