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REGENERATION OF BONE MARROW CELLS IN RATS FOLLOWING CYCLOPHOSPHAMIDE OR TOTAL BODY IRRADIATION

by

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In a previous study (HØST 1966 a), differences were disclosed in the recovery pattern of granulocytes in rats, after single doses of cyclophosphamide, a nitrogen mustard derivative, and, respectively, after total body irradiation. Bone marrow studies were initiated in order to throw light on the underlying mechanism of the differences observed in the peripheral blood. The present paper reports comparative studies on the proliferation rates of the different types of marrow cells after injection of cyclophosphamide and after exposure to total body irradiation. Proliferation rates of the marrow cells were studied by a modified colcemid method (HØST 1966b).

Material and Methods. Hooded, male rats, of 200 to 280 g bodyweight, 70 to 94 days old, were used. Standard laboratory rations and water were supplied ad libitum.

A total of 148 animals were used in the experiments, 74 in the cyclophosphamide series and 74 in the irradiated series. Each of the two series comprised

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4 untreated, 14 sham-treated and 56 treated animals: cyclophosphamide-injected or irradiated. The four untreated animals were sacrificed and examined on day 0. On days 2, 4, 6, 8, 10, 12, and 14 after treatment, two sham-treated and eight treated animals were sacrificed and examined. Animals were selected at random as regards type of treatment (no treatment, sham treatment, real treatment) and day of sacrifice.

The treatment procedures have been described in detail previously (HØST 1966a). Cyclophosphamide (Sendoxan, Pharmacia, Uppsala) was injected into the exposed vena femoralis during ether anesthesia. Sham-treated animals were injected with physiologic saline. Total body roentgen irradiation was carried out at 200 keV (half value layer 1.05 mm Cu). The animals were irradiated individually in a cylindric container. Sham treatment was performed by placing the control animals in the container for the same duration as the irradiated animals.

Preliminary studies disclosed that 50 mg/kg bodyweight of cyclophosphamide and 350 R total body irradiation produced an almost identical initial depression of the total nucleated marrow cells. These doses were therefore used in the comparative studies.

Total nucleated marrow cells were counted and estimated in number per mg bone marrow (HØST 1966 b).

Smears for differential counts were taken from the middle part of the femur by the brush method (BURKE, BROTHERSTONE & HARRIS 1955) and stained by May-Grünewald and Giemsa. The smears were interpreted without knowledge of previous treatment. Differential counts were done on one thousand cells and the absolute number of each cell type was expressed per mg of bone marrow. Classification of cell types and stages of mitosis was performed as previously described (HØST 1966 b). The number of cells in prophase and metaphase per 1 000 cells capable of division was estimated on the basis of counts of 500 cells in each cell line. About 90 to 95 per cent of the mitotic cells were classified as either myeloid or erythroid. The others were difficult to place; they were possibly reticular or lymphocyte-like cells in mitosis and were not included in the study on proliferation rates and cell renewal.

The measurements of proliferation rates were made as follows. At successive time intervals after treatment (irradiation or cyclophosphamide) equal numbers of animals, selected at random, were injected subcutaneously with colcemid (Ciba, Basel) or physiologic saline. The rats were sacrificed 2 1/2 hours later, and marrow samples for total counts, differential counts and mitotic counts were taken. The numbers of mitoses arrested by colcemid were compared with those in animals injected with physiologic saline, and proliferation

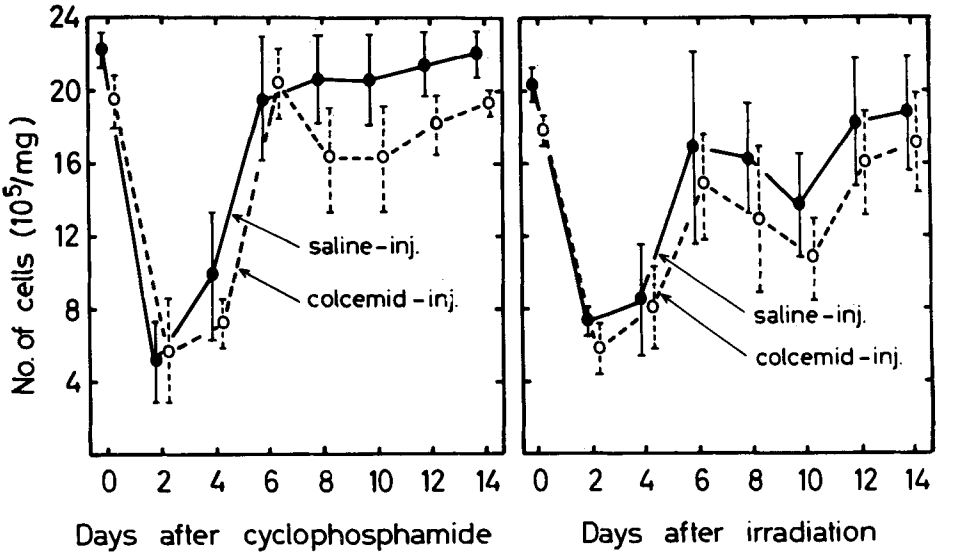

Fig. 1. Number of nucleated cells in bone marrow at various times after cyclophosphamide and total body irradiation: ●———● saline injected, ○ - - - - - ○ colcemid injected. The 95 % confidence limits are indicated.

rates were estimated (Høst 1966b). Colcemid was injected in doses of 1 mg/kg bodyweight. The injections were performed at the same time of the day (between 8 and 9 a.m.).

Results

The results of the marrow cell counts in the untreated and the sham-treated animals were subjected to an analysis of variance, to test possible systematic variations with time, and the results indicated no consistent trend. It therefore seems justified, in the following figures and tables, to simplify the presentation of these data by depicting them all at zero time.

Effects on total nucleated marrow cells. Both cyclophosphamide and total body irradiation, in the applied doses, depressed the number of total nucleated marrow cells to about 25 to 30 per cent of the initial value (Fig. 1). Maximum depression occurred on the second day after treatment and was followed by recovery. The latter was more rapid and complete in the cyclophosphamide-treated animals. In the irradiated groups of rats, a slight secondary drop was observed during recovery at about 8 to 10 days after treatment.

The general trend was essentially the same for the saline-injected and the colcemid-injected animals; in the latter, however, the marrow cell counts remained at a lower level than in the former. This is in agreement with

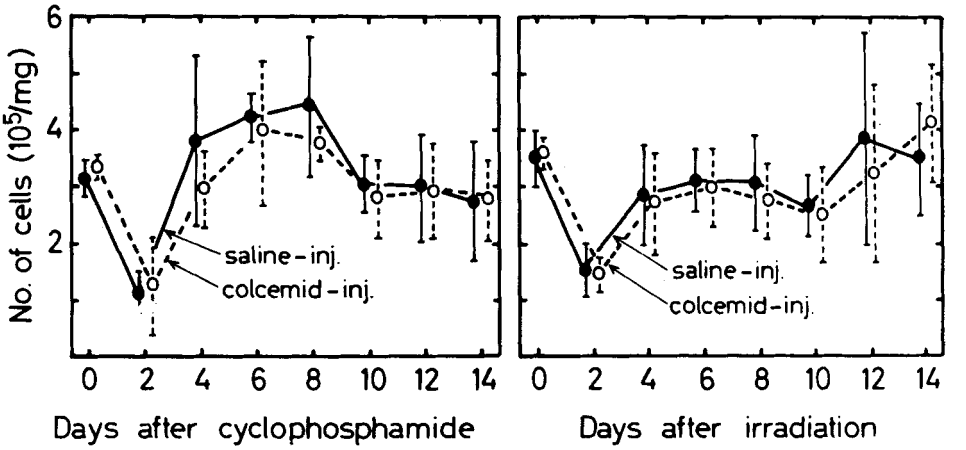

Fig. 2. Number of dividing myeloid cells in bone marrow at various times after cyclophosphamide and total body irradiation: ●——● saline injected, ○ - - - - - ○ colcemid injected. The 95 % confidence limits are indicated.

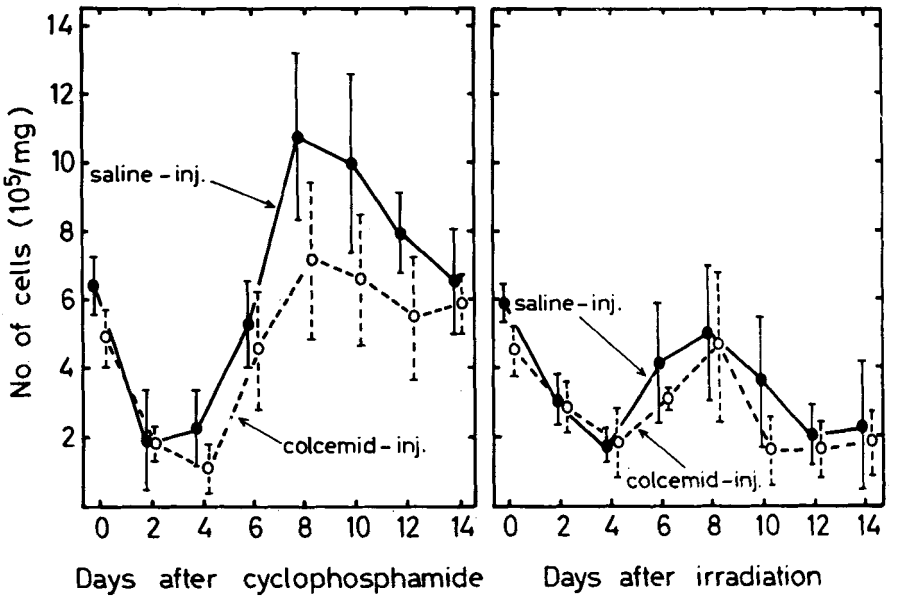

Fig. 3. Number of non-dividing myeloid cells in bone marrow at various times after cyclophosphamide and total body irradiation: ●——● saline injected, ○ - - - - - ○ colcemid injected. The 95 % confidence limits are indicated.

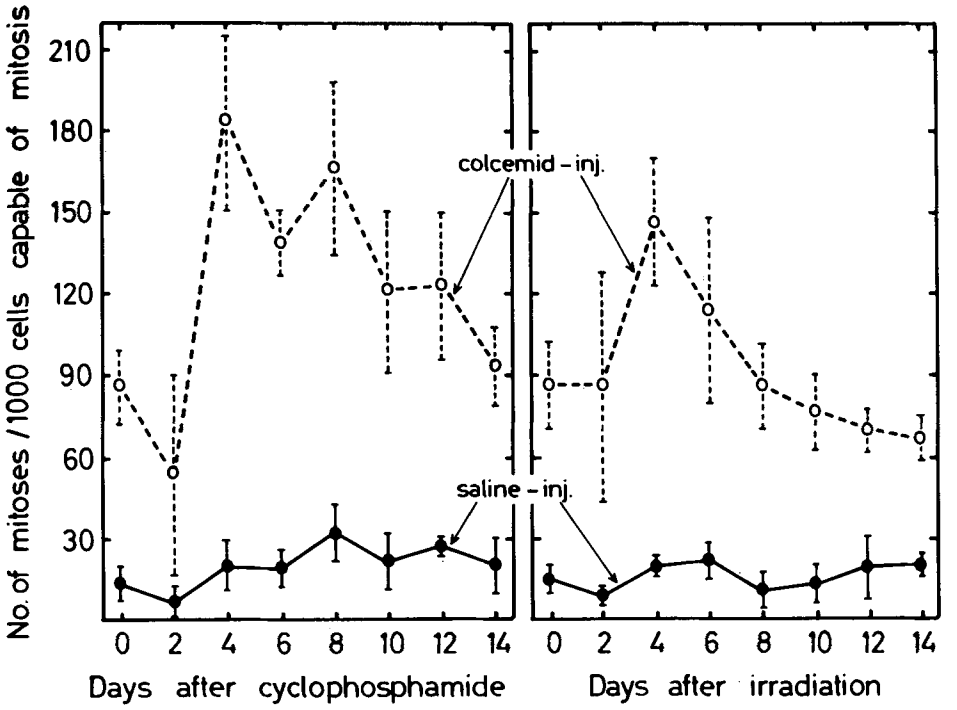

Fig. 4. Mitotic index of myeloid cells at various times after cyclophosphamide and total body irradiation: ●——● saline injected, ○ - - - - - ○ colcemid injected. The 95 % confidence limits are indicated.

previous findings (Høst 1966b) which indicated that colcemid reduces the number of total nucleated marrow cells.

Effects on myeloid cells. The pattern of changes in the number of dividing myeloid marrow cells after treatment with the two agents is given in Fig. 2. On the second day the counts were reduced to about one-third of the initial value; recovery then occurred in the course of two days. In the cyclophosphamide-treated animals the number remained at the upper limit of the normal range during the following 4 to 5 days, whereas in the irradiated animals the number stayed in the lower range for a similar period of time.

The initial depletion of the non-dividing myeloid cells ran almost parallelly in the cyclophosphamide-injected and the irradiated animals (Fig. 3). Minimum values, about one-third of the pre-treatment values, were registered on the 4th day, or two days later than in their precursor cells. Recovery was evident in all the treatment groups in the course of days 6 to 8. In the cyclo-

Table 1

Mitotic time and mitotic rate in marrow cells at various times after cyclophosphamide (50 mg/kg) and total body irradiation (350 R)

Days after treatment	Myeloid cells				Erythroid cells			
	Cyclophosphamide		Irradiation		Cyclophosphamide		Irradiation	
	Mitotic time in hours	Mitotic rate*	Mitotic time in hours	Mitotic rate*	Mitotic time in hours	Mitotic rate*	Mitotic time in hours	Mitotic rate*
0	0.41	34	0.44	33	0.48	50	0.49	61
2	0.27	22	0.26	35	0.11	43	0.25	76
4	0.26	73	0.32	59	0.27	78	0.44	91
6	0.34	56	0.49	45	0.45	69	0.50	94
8	0.46	67	0.32	33	0.30	67	0.45	82
10	0.45	49	0.42	31	0.46	62	0.50	76
12	0.59	46	0.68	28	0.50	62	0.65	65
14	0.54	36	0.81	26	0.34	91	0.52	73

* Average number of cells which complete their mitosis per hour per 1 000 cells capable of cell division.

phosphamide-saline-injected animals, values significantly above normal were observed on days 8 to 12. In the irradiated animals, on the other hand, the initial recovery of the non-dividing myeloid cells was followed by a second drop which was most accentuated at about the 12th day.

The patterns of myeloid cell response to the two different treatments agree well with previous observations. Thus temporary normalization of the myeloid marrow cell population has been described during recovery after total body irradiation both in guinea pigs (HARRIS 1956, 1959) and in rats (HULSE 1961). Likewise, an overshooting production of myeloid cells has been found after treatment with nitrogen mustard derivatives (ELSON, GALTON & TILL 1958, PLIESS & FASSBENDER 1961, BIERBRAUER 1961).

Proliferation rates of myeloid cells. As demonstrated in Fig. 4, the mitotic index, i. e. the number of mitoses per 1 000 cells capable of mitosis, was slightly below the pre-treatment values two days after cyclophosphamide as well as after irradiation. During the following days, the mitotic index in the irradiated animals rose to about normal levels and in the cyclophosphamide-treated animals to levels in the upper range of normal. Concurrently, the changes in the number of mitoses arrested during the action of colcemid were more marked, especially in the cyclophosphamide-treated animals (Fig. 4).

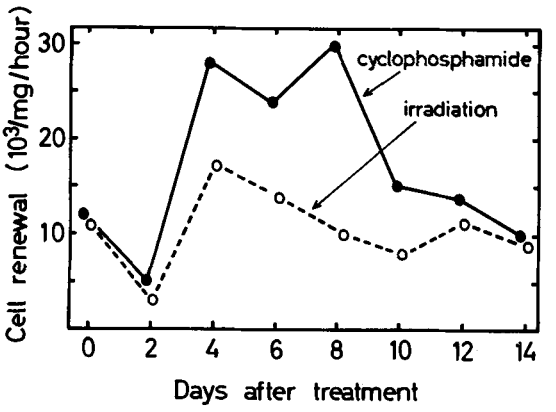

Fig. 5. Myeloid cell renewal per mg of bone marrow per hour at various times after cyclophosphamide (●——●) and total body irradiation (○- - - -○).

The number of arrested mitoses in the latter remained at a level significantly above normal for a longer time period, as compared to the irradiated animals in which counts significantly above normal were observed only on the 4th day after exposure. These findings suggest a higher mitotic activity during recovery in the cyclophosphamide-treated animals.

The data in Table 1 indicate that the mitotic time of the myeloid cells, which was calculated from the formula given by ALLEN, SMITH & GARDNER (1937), after both types of treatment, was reduced during the initial phase of regeneration. Later on, however, the mitotic time increased to levels above the pre-treatment ones, especially in the irradiated animals.

The calculated mitotic rate of the myeloid cells (Table 1) was reduced on the 2nd day while in the irradiated animals it remained within the normal range. An explanation of this may be that the recovery of the myeloid cells possibly started earlier in the irradiated than in the cyclophosphamide-injected animals. However, the speed of myeloid regeneration seemed to be faster in the last-mentioned group. Thus, in the cyclophosphamide-injected animals, the mitotic rate increased during recovery to levels above normal on the 4th day and remained at this level for about a week. On the other hand the mitotic rate of the myeloid cells in the irradiated animals exceeded the pre-treatment values only from the 4th to the 6th day.

The data on absolute myeloid cell renewal, illustrated in Fig. 5, emphasize more clearly the large difference in cell production during recovery between irradiated and cyclophosphamide-treated rats. The absolute myeloid cell renewal represents the product of the mitotic rate and the number of dividing myeloid cells per mg bone marrow. As appears from Fig. 5, the myeloid cell renewal was more than twice the normal during the 4th to the 8th day in the

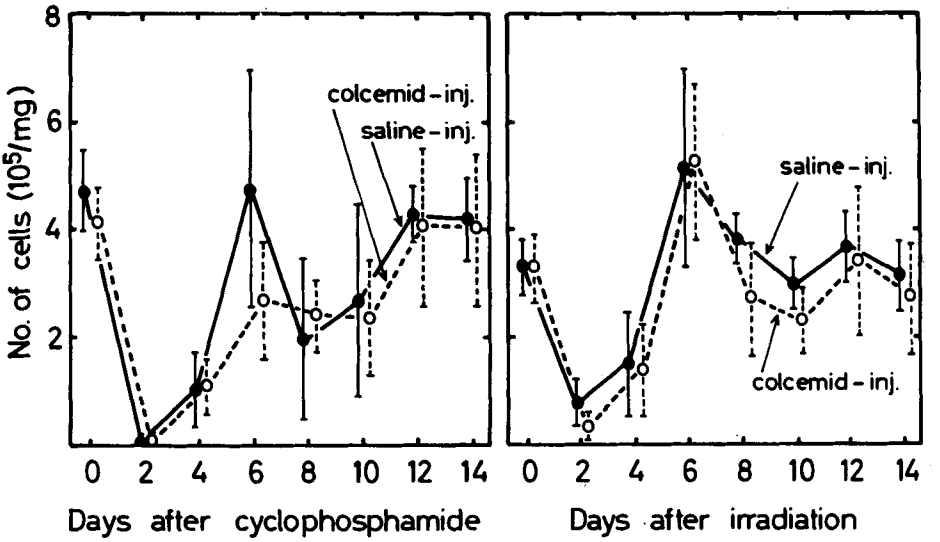

Fig. 6. Number of dividing erythroid cells in bone marrow at various times after cyclophosphamide and total body irradiation: ●——● saline injected, ○ - - - - - ○ colcemid injected. The 95 % confidence limits are indicated.

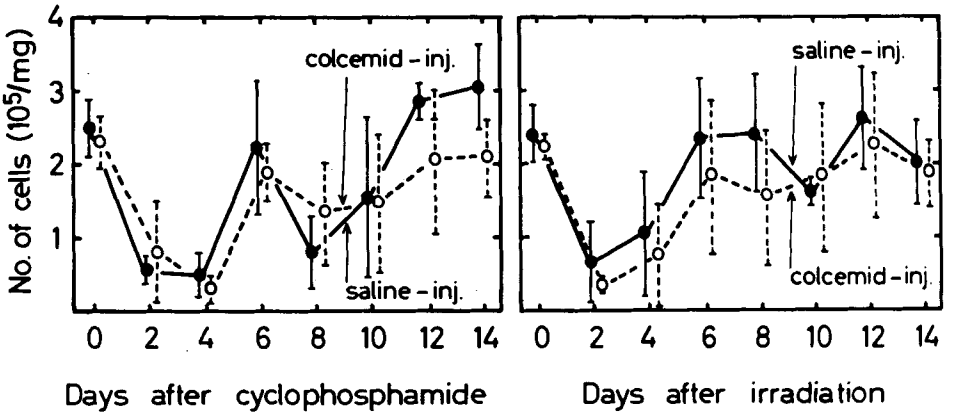

Fig. 7. Number of non-dividing erythroid cells in bone marrow at various times after cyclophosphamide and total body irradiation: ●——● saline injected, ○ - - - - - ○ colcemid injected. The 95 % confidence limits are indicated.

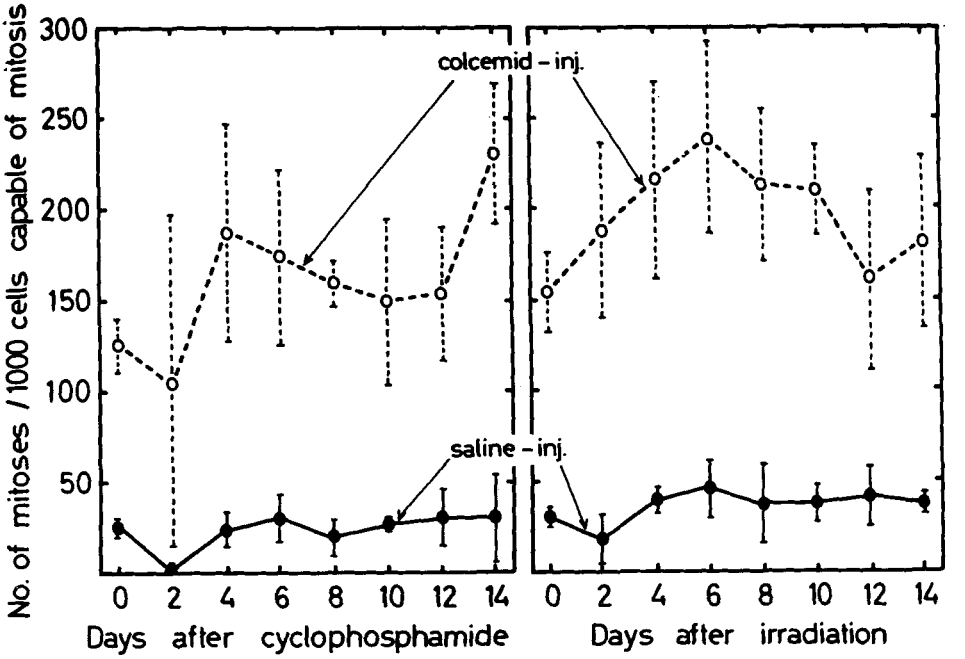

Fig. 8. Mitotic index of erythroid bone marrow cells at various times after cyclophosphamide and total body irradiation: ●——● saline injected, ○ - - - - - ○ colcemid injected. The 95 % confidence limits are indicated.

cyclophosphamide-treated animals, while in the irradiated animals the cell renewal was only slightly increased.

Effects on erythroid cells. The data presented in Figs 6 and 7 indicate that the erythroid precursor cells were very sensitive to radiation as well as to cyclophosphamide. On the 2nd day, the number of dividing erythroid cells was lower in the cyclophosphamide-treated than in the irradiated animals (Fig. 6), while the number of non-dividing erythroid cells (Fig. 7) was about the same in the two treatment groups. The dividing erythroid cells thus seemed to be more sensitive to cyclophosphamide than the non-dividing cells. As far as the data go, they seem to indicate a similar response pattern in the irradiated groups.

Recovery was evident on the 4th day, and normal values were regained two days later. A secondary drop, most marked in the cyclophosphamide-injected animals, was observed on days 8 to 10. The recovery pattern was nearly the same both in the dividing and in the non-dividing erythroid precursors

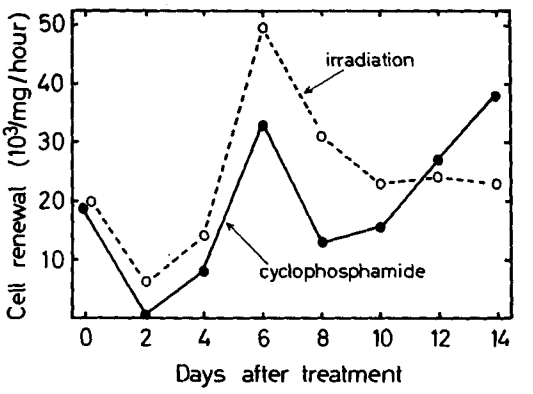

Fig. 9. Erythroid cell renewal per mg of bone marrow per hour at various times after cyclophosphamide (●——●) and total body irradiation (○- - - - -○).

after the two types of treatment. This is in contrast to the mode of myeloid cell regeneration after irradiation and after cyclophosphamide, where the two treatments led to different recovery patterns.

Late, acidophilic, normoblasts occurred frequently in the bone marrow in both series on the 2nd day, on which these cells comprised the major part of the non-dividing erythroid cells. Acidophilic normoblasts are seldom observed in the bone marrow of rats, under normal conditions, as the cytoplasma of erythroid precursors in this species retains an element of basophilia much later than the corresponding cells in man (HULSE 1964). Occurrence of late, acidophilic, normoblasts has previously been reported by LAMERTON, BELCHER & HARRISS (1956) after irradiation of rats with non-lethal doses. They suggested that irradiation produced a hold-up in the release of the mature cells from the marrow.

Proliferation rates of erythroid cells. On the 2nd day, both the mitotic index and the number of mitoses arrested during the action of colcemid (Fig. 8) were lower in the cyclophosphamide-treated than in the irradiated animals. Ionizing irradiation thus had a less depressive effect on erythropoiesis than had cyclophosphamide. Furthermore, the regeneration started earlier after irradiation than after cyclophosphamide.

The data on cell renewal in Fig. 9 indicate a higher erythroid cell renewal in the irradiated than in the cyclophosphamide-treated animals during the initial phase of regeneration, in contradistinction to the findings on the myeloid cell renewal (cf. Fig. 5). A temporary overshoot in erythropoiesis occurred during recovery in both the irradiated and the cyclophosphamide-treated animals.

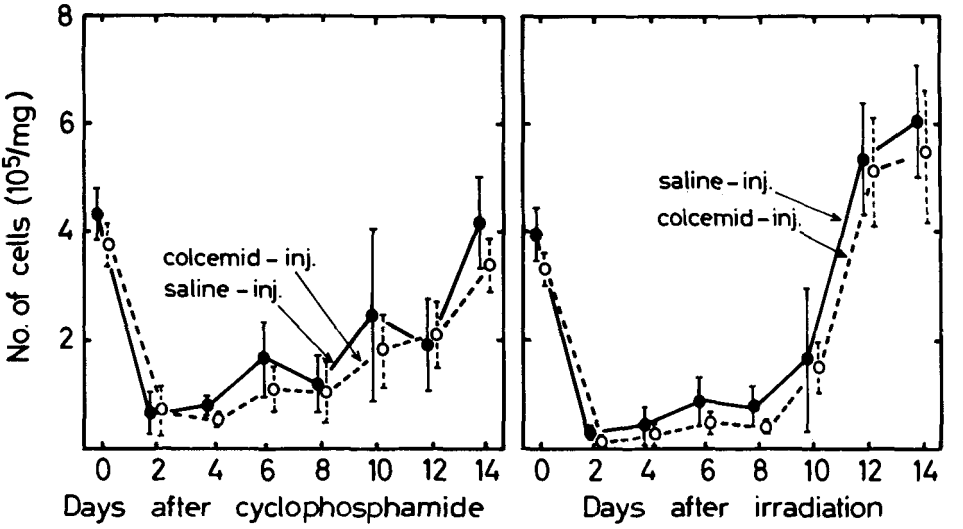

Fig. 10. Number of lymphocyte-like cells in bone marrow at various times after cyclophosphamide and total body irradiation: ●——● saline injected, ○ - - - - - ○ colcemid injected. The 95 % confidence limits are indicated.

The mitotic time of the erythroid cells (see Table 1) was reduced during early recovery and followed a pattern similar to that observed in the myeloid cells.

Effects on lymphocyte-like cells. The depletion phase and the initial recovery period of the lymphocyte-like cells occurred almost parallelly in the different groups (Fig. 10). These cells were very sensitive to treatment, the number on the 2nd day being only 10 to 15 per cent of the normal. Differences were observed in the recovery pattern from the 10th day, and onwards. The lymphocyte-like cells in the cyclophosphamide-treated animals rose only to sub-normal or normal values, these being regained later than normalization of myelopoiesis and erythropoiesis (Figs 3, 6 and 7). In the irradiated animals, on the other hand, the lymphocyte-like cells rose to levels considerably above normal. This increase in the number of such cells in the irradiated animals occurred concurrently with the second drop in number of the myeloid cells (cf. Fig. 3) but after the recovery of the erythroid cells (cf. Figs 6 and 7).

The occurrence of large numbers of bone marrow cells resembling lymphocytes has been reported in various species during recovery after total body irradiation (DENSTAD 1943, BRECHER, ENDICOTT, GUMP & BRAWNER 1948, HARRIS 1956, YOFFEY 1960). The significance of this finding has been subject

Table 2

'Reticulum' cells, plasma cells and 'damaged' cells in bone marrow at various times after cyclophosphamide (50 mg/kg) or total body irradiation (350 R) — The values represent the mean of 4 animals with 95 % confidence limits

	Cyclophosphamide			Total body irradiation		
Days after treatment	'Reticulum' cells 10^3 cells/mg	Plasma cells 10^3 cells/mg	'Damaged' cells 10^3 cells/mg	'Reticulum' cells 10^3 cells/mg	Plasma cells 10^3 cells/mg	'Damaged' cells 10^3 cells/mg
0	98 ± 41	9 ± 6	26 ± 6	77 ± 16	5 ± 3	32 ± 10
2	74 ± 48	14 ± 6	11 ± 6	74 ± 19	14 ± 6	19 ± 6
4	118 ± 76	17 ± 16	9 ± 3	61 ± 26	10 ± 3	17 ± 3
6	97 ± 29	7 ± 3	34 ± 10	84 ± 41	6 ± 6	34 ± 29
8	108 ± 70	6 ± 3	16 ± 10	78 ± 13	5 ± 3	32 ± 13
10	71 ± 22	16 ± 12	22 ± 10	84 ± 26	6 ± 3	33 ± 10
12	92 ± 62	21 ± 13	26 ± 10	111 ± 35	7 ± 6	35 ± 3
14	93 ± 41	15 ± 10	14 ± 10	88 ± 32	6 ± 3	31 ± 13

to controversial opinions (HULSE 1963). In some recent papers (HARRIS, HAIGH & KUGLER 1963, HARRIS & KUGLER 1964, 1965), it has been suggested that these cells, during recovery after irradiation, may act as stem cells.

Effects on other marrow cells. The data presented in Table 2 are difficult to interpret as the range of observed values is wide. It seems, however, that the 'reticulum' cells, the plasma cells, and the so-called 'damaged' cells were unaffected by treatment. The group of 'reticulum' cells comprised several different cell types. No differential counts of the cells included in this group were performed.

Discussion

In a previous study in rats (HØST 1966 a) differences were demonstrated in the recovery pattern of granulocytes in the peripheral blood after treatment with cyclophosphamide and after total body irradiation, respectively.

The present study confirmed that cyclophosphamide produces only a short-lived depression of myelopoiesis, followed by an overshooting myeloid cell production, while roentgen irradiation causes a more long-lasting suppression of myelopoiesis, interrupted by an abortive rise. It was found however that

the lymphocyte-like marrow cells exceeded normal numbers during recovery after irradiation, while the recovery of these cells in the cyclophosphamide-treated animals was more protracted. The erythroid cell precursors demonstrated an almost similar, rapid and apparent complete recovery after both irradiation and cyclophosphamide.

The similarity in the response of erythropoiesis may seem difficult to reconcile with the difference in response of myelopoiesis. However, the following explanation may be put forward. Both irradiation and cyclophosphamide will affect the reproductive integrity of the marrow cells. According to LAJTHA (1961), the irradiation effect may demonstrate itself in the following ways: (1) temporary delay in mitosis, (2) permanent inhibition of mitosis and giant cell formation, (3) death of cell before entering mitosis, and (4) death after a few mitoses. LAJTHA furthermore predicted the theoretically expected effect of a single dose of radiation on marrow cells in terms of a simplified scheme for the bone marrow cell population. The radiation-induced mitotic delay will decrease the numbers of dividing marrow cells. Later, when these cells have been depleted, the 'feed' into more mature non-dividing cells will be reduced. The latter marrow cells will also decrease in number, as their entry into peripheral blood remains unchanged.

The stem cells will continue to differentiate into the 'dividing-compartment', but as these cells also have received the same dose of radiation the subsequent mitosis may be delayed and 'interphase-death' may occur. An 'abortive' recovery may follow after cessation of the mitotic delay induced by radiation. Cells may enter mitosis but many of them, depending upon the dose of radiation received, have lost their reproductive integrity and will undergo only one or two mitoses. The result will be a suboptimal temporary rise in their numbers. After a time lag, depending upon the radiation dose, the stem cells will regenerate and increase to the normal population level, and complete recovery will follow. The pattern observed in the present study of myeloid cell response corresponds well with the above hypothesis as regards the irradiation effect on marrow cells.

On the basis of this pattern of myeloid regeneration after cyclophosphamide it would appear that no reduction in the reproductive integrity persisted after cessation of the induced mitotic delay, i. e. the damage to the stem cells was less marked than that to the 'dividing compartment'. There will thus be quick recovery with a temporary overshoot in myelopoiesis, owing probably to increased demand for granulocytes, but without any secondary failure of myelopoiesis.

Regeneration of erythropoiesis followed a fairly steady course both after irradiation and cyclophosphamide and occurred apparently without any abortive

rise. This may indicate that no reduction in reproductive integrity of the erythroid precursors occurs after cessation of the mitotic delay induced by either cyclophosphamide or radiation. Another possible explanation may be that the demand for red cell production did not exceed the capacity for erythropoiesis, even if the latter was reduced. If so, possible differences in the restitution of erythropoiesis after irradiation and after cyclophosphamide may be revealed by an artificially increased demand for red cell production. According to experimental data now in hand, such differences are indeed present (HØST, under preparation).

It has previously been shown that total body irradiation (350 R) had a more depressive effect on lymphocytes in peripheral blood than cyclophosphamide (50 mg/kg bodyweight) (HØST 1966 a). The lymphocytes did not regain pre-treatment levels within 3 to 4 weeks after irradiation, while in the cyclophosphamide-treated animals such values were obtained in 10 to 14 days. The present results, together with those reported earlier, indicate that after cyclophosphamide the recovery of the lymphocytes in peripheral blood possibly ran simultaneously with that of the lymphocyte-like marrow cells. In the irradiated animals, however, the recovery of these cells in peripheral blood and bone marrow, respectively, occurred with a 2 week interval. This observation may suggest that the cells observed in blood and marrow, during recovery after total body irradiation, are different cells with different functions but with almost similar morphologic characteristics. The marrow lymphocytes have been claimed to act as stem cells during recovery after irradiation in guinea pigs (HARRIS, HAIGH & KUGLER 1963, HARRIS & KUGLER 1964, 1965). If these cells act as stem cells, one would expect them to precede the other marrow cells in recovery. The present study indicates that in the cyclophosphamide-treated animals the regeneration of the myeloid and the erythroid cells preceded that of the lymphocyte-like marrow cells by at least 4 to 6 days. In the irradiated animals, the initial regeneration of the myeloid and the erythroid cells also occurred prior to the regeneration of the lymphocyte-like marrow cells. A secondary failure of myelopoiesis occurred, however, and the final regeneration did not take place within the period covered by the present investigation. The accumulation of the lymphocyte-like marrow cells may therefore be of significance in the final regeneration of the myelopoiesis.

Taken together, the present data indicate that in cyclophosphamide-treated animals the lymphocyte-like cells are of slight significance for the final recovery of the myeloid and erythroid cells. In irradiated animals, on the other hand, the lymphocyte-like cells may play a part in the final regeneration of the myeloid cells.

Acknowledgements

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SUMMARY

Comparative studies of the effect of cyclophosphamide or total body irradiation on bone marrow cells have been performed in rats. The recovery of erythropoiesis was the same after the two types of treatment, whereas differences were evident in the pattern of myeloid regeneration. It is suggested that the stem cells are less damaged by cyclophosphamide than is the 'dividing compartment', whereas the reproductive integrity of the stem cells is still reduced after cessation of the radiation-induced mitotic block.

ZUSAMMENFASSUNG

An Ratten wurde der Effekt von Cyclophosphamidverabreichung mit dem Einfluss der Totalbestrahlung auf das Knochenmark verglichen. Nach Anwendung beider Methoden war die Erholungsbereitschaft der Erythropoese die gleiche, aber es bestanden deutliche Unterschiede in der Regeneration des myeloiden Gewebes. Es erscheint, dass der Zellstiel sich nach Cyclophosphamidverabreichung rascher erholt als der Teil der Zelle, in der die Zellteilung vor sich geht; nach der Blockierung der Mitose durch Bestrahlung bleibt jedoch die Reproduktionsfähigkeit des Zellenstieles vermindert.

RÉSUMÉ

L'auteur a fait sur des rats des études comparatives de l'effect du cyclophosphamide et de l'irradiation totale du corps sur les cellules de la moelle osseuse. La restauration de l'érythropoïèse a été la même après ces deux types de traitement, alors qu'il y a eu des différences évidentes dans l'aspect de la régénération myéloïde. L'auteur pense que les cellules souches sont moins lésées par le cyclophosphamide que les cellules en division, alors que le pouvoir reproducteur des celles souches est encore réduit après la cessation du blocage mitoque dû aux radiations.

REFERENCES

ALLEN E., SMITH M. and GARDNER W. U.: Accentuation of the growth effect on genital tissues of ovariectomized mouse by arrest of mitosis with colchicine. Amer. J. Anat. 61 (1937), 321.

BIERBRAUER U.: Die Einwirkung der Cytostatica Endoxan und Trisaethyleniminobenzochinon auf das Knochenmark und perifere Blut der Ratte. Folia haemat. (Frankfurt) 5 (1961), 143.

BRECHER G., ENDICOTT K. M., GUMP H. and BRAWNER H. P.: Effects of X-ray on lymphoid and hemopoietic tissues of albino rats. Blood 3 (1948), 1259.

BURKE W. T., BROTHERSTONE G. and HARRIS C.: An improved technique for obtaining bone marrow smears from the rat. Amer. J. clin. Path. 25 (1955), 1226.

DENSTAD T.: The radiosensitivity of the bone marrow. Acta radiol. (Stockh.) (1943) Suppl. No. 52.

ELSON L. A., GALTON D. A. G. and TILL M.: The action of chlorambucil (C. B. 1348) and busulphan (myleran) on the haemopoietic organs of the rat. Brit. J. Haemat. 4 (1958), 355.

HARRIS P. F.: Quantitative examination of bone marrow in guinea pigs after gamma-irradiation. Brit. med. J. (1956), 1032.

— The correlation between bone marrow activity and blood neutrophil levels from quantitative studies in irradiated guinea-pigs. Brit. J. exp. Path. 40 (1959), 589.

— and KUGLER J. H.: The use of regenerating bone marrow to protect guinea-pigs against lethal irradiation. Acta haemat. (Basel) 32 (1964), 146.

— — An investigation of lymphocyte production in guinea-pig bone marrow. Acta haemat. (Basel) 33 (1965), 351.

— HAIGH G. and KUGLER J. H.: Quantitative studies of mitoses and DNA-synthesizing cells in bone marrow and blood of guinea-pigs recovering from sublethal whole-body gamma irradiation. Brit. J. Haemat. 9 (1963), 385.

HULSE E. V.: The recovery of myelopoietic cells after irradiation: a quantitative study in the rat. Brit. J. Haemat. 7 (1961), 430.

— Lymphocytic recovery after irradiation and its relation to other aspects cf haemopoiesis. Brit. J. Haemat. 9 (1963), 376.

— Quantitative cell counts of the bone marrow and blood and their secular variations in the normal adult rat. Acta haemat. (Basel) 31 (1964), 50.

HØST H. (a): Comparative effects of cyclophosphamide, nitrogen mustard and total body irradiation on survival and on white blood cells in rats. Radiat. Res. 27 (1966), 638.

— (b): The effect of colcemid on bone marrow cells studied by a quantitative method. To be publ. in Acta path. microbiol. scand.

LAJTHA L. G.: The effect of ionizing radiations and tumour-chemotherapeutic agents on the bone marrow. Progr. Biophys. 11 (1961), 80.

LAMERTON L. F., BELCHER E. H. and HARRISS E. B.: Discussion on clinical and experimental studies with radioactive iron. Proc. roy. Soc. Med. 49 (1956), 863.

PLIESS G. und FASSBENDER D.: Die Wirkung sublethaler Dosen von Endoxan auf die Hämopoese der Ratte. Arzneimittel-Forsch. 11 (1961), 179.

YOFFEY J. M.: Quantitative cellular haematology. C. C. Thomas, Springfield, Illinois U.S.A. 1960.