PROLIFERATIVE ACTIVITY OF BONE MARROW CELLS AFTER BILLROTH I SUBTOTAL GASTRECTOMY

K. A. Zufarov,* K. R. Tukhtaev, and R. K. Kurbanov

UDC 616.33-089.87-07 : 616.419-018.1-076.5

The effect of the Billroth I subtotal gastrectomy on proliferative activity of the bone marrow cells was studied in experiments on rats injected with thymidine-H^3. By the 30th day after gastrectomy hypochromic anemia was observed, accompanied by a marked decrease in the index of labeled nuclei (ILN) of the bone marrow erythronormoblasts to 44.9±5.5% from 80.8±3.9% in the control. Parallel with the increase in the blood levels 90 days after the operation, there was some increase in ILN of the erythronormoblasts. DNA synthesis in the granulocytes of the bone marrow showed no significant change after subtotal gastrectomy.

Subtotal and total gastrectomy, operations widely used in clinical surgery, frequently lead to disturbances of hematopoiesis [1, 2, 6]. Although the pathogenesis of B_{12}-deficient megaloblastic anemia developing after gastrectomy has now been well-investigated [2, 3], this is not true of the postgastrectomy hypochromic and hypoplastic anemias, and contradictory results have been described. Only a single investigation has included determination of the mitotic activity of the bone marrow cells in these states [7].

The object of the present investigation was to study the effect of the Billroth I subtotal gastrectomy on proliferative activity of the erythroid and myeloid series of bone marrow cells.

EXPERIMENTAL METHOD

Experiments were carried out on 40 male rats weighing 100-120 g. Half of the animals underwent subtotal gastrectomy by the Billroth I method under general ether anesthesia, and the rest acted as the control. Twenty animals (10 gastrectomized and 10 control) were sacrificed 30 days after the operation and another 20 animals 90 days thereafter. Both control and gastrectomized animals received an intraperitoneal injection of thymidine-H^3 before sacrifice in a dose of 0.5 μCi/g body weight. The animals were killed 1, 2, 4, 6, 8, 12, and 24 h after administration of the thymidine-H^3. Forms of the femoral marrow were fixed in absolute methanol and then coated with type M (Research Institute of Photographic Chemistry) liquid radiosensitive emulsion by the usual method [4]. After exposure for 20-25 days the films were developed in amidol developer and stained with azure-eosin by Romanovsky's method. The index of labeled nuclei (ILN) was determined by counting the number of labeled and unlabeled bone marrow cells. When ILN of the erythronormoblasts was calculated, all the cells of the erythroid series were divided into two groups. Group I consisted of young cells, including erythroblasts, pronormoblasts, and basophilic normoblasts, while group II included polychromatophilic and oxyphilic normoblasts. ILN of the myeloid series was calculated for hemocytoblasts, myeloblasts and promyelocytes, metamyelocytes and stab cells, and a also for polymorphs. Altogether 500 cells of the erythroid and 500 of the myeloid series were counted. The hematological indices of the peripheral blood and bone marrow (red cell count, hemoglobin concentration, color index, myelogram, mitotic index, etc.) were determined in all animals simultaneously. The numerical results were subjected to statistical analysis by the Fisher–Student method.

* Academician, Academy of Sciences of the Uzbek SSR.

Laboratory for Biophysical Problems, Department of Histology, Tashkent Medical Institute. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 76, No. 8, pp. 114-116, August, 1973. Original article submitted December 25, 1972.

TABLE 1. ILN of Bone Marrow Erythronormoblasts (in %) before and after Billroth I Subtotal Gastrectomy (M±m)

Time after injection of thymidine-H^3 (in h)	Time of investigation					
	before operation		30 days after operation		90 days after operation	
	I	II	I	II	I	II
1	73,4±5,2	14,1±2,2	41,6±4,8	1,9±1,1	58,4±5,7	11,1±3,4
2	80,8±3,9	29,1±2,4	44,9±5,5	12,8±3,2	69,4±5,7	23,5±4,5

Legend: I) young erythronormoblasts (erythroblast, pronormoblast, and basophilic normoblast), II) more mature normoblasts (polychromatophilic and oxyphilic normoblasts).

TABLE 2. ILN of Bone Marrow Granulocytes (in %) before and at Various Times after Billroth I Subtotal Gastrectomy (M±m)

Time of investigation (days)	Time after thymidine-H^3 injection (h)	Hemocytoblasts	Myeloblasts and myelocytes	Neutrophilic myelocytes	Eosinophilic myelocytes	Metamyelocytes and stab cells	Polymorphs
Control	1	70,3±3,05	51,4±1,8	40,1±1,7	34,7±1,5	—	—
	24	33,3±3,1	43,3±2,2	48,0±2,05	43,3±1,4	35,9±1,6	36,1±0,9
30	1	68,2±3,1	55,6±4,1	54,3±1,2	38,0±1,8	—	—
	24	47,9±1,8	37,5±1,9	53,9±1,7	44,0±1,9	55,6±1,3	33,6±1,0
90	1	65,5±7,5	61,6±6,4	42,1±3,7	43,2±4,0	—	—
	24	27,7±4,8	31,1±6,8	51,9±4,9	51,4±4,3	56,6±3,7	37,8±3,1

EXPERIMENTAL RESULTS

Subtotal gastrectomy caused significant disturbances of hematopoiesis in all animals undergoing the operation. By the 30th day after the operation a marked hypochromic anemia with disturbances of maturation of the erythroid series of cells had developed. The mitotic index of the erythronormoblasts was considerably reduced.

ILN of the erythronormoblasts was considerably below the control value on the 30th day after the operation (Table 1). Whereas 2 h after injection of thymidine-H^3 ILN for the young erythronormoblasts of the control rats was 80.8±3.9%, for the analogous cells of the gastrectomized animals it was 44.9±5.5%. By the 30th day after the operation the number of erythronormoblasts synthesizing DNA was thus reduced almost by half compared with the control. Some increase in the peripheral blood and bone marrow indices was observed 90 days after the operation, correlating with the increase in ILN in the erythronormoblasts.

A decrease in ILN of the erythroid cells has been found in renal failure, when it is attributed to a decrease in erythropoietin production by the affected kidney [5]. Addition of erythropoietins to a bone marrow culture causes the number of cells synthesizing DNA to double [10]. At the same time it has been shown that the stomach is one of the main sites of erythropoietin production [8, 9]. Presumably subtotal gastrectomy leads to a decrease in the erythropoietin concentration in the blood serum and to a corresponding decrease in the level of proliferative processes in the erythronormoblasts.

ILN of cells of the granulocytic series 1 h after injection of thymidine-H^3 was not significantly different from the control on the 30th day of the experiment (Table 2). However, after 24 h an increase in the number of labeled granulocytes at the metamyelocyte stage was observed (up to 55.6±1.3% compared with 35.9±1.6% in the control). A similar increase in ILN of the metamyelocytes was also observed by the 90th day after the operation (up to 56.6±3.7%). This fact suggests that DNA synthesis in young granulocytes follows a normal course, but the accumulation of labeled metamyelocytes takes place through a disturbance of their maturation and their release into the blood stream.

It can be concluded from this description that after subtotal gastrectomy proliferative processes in the erythronormoblasts are considerably reduced. This, in turn, leads to a decrease in the number of

erythrocytes, and it is evidently one link in the complex chain of pathogenesis of the ensuing postgastrectomy anemia. DNA synthesis in the granulocytes is not significantly affected under these circumstances.

LITERATURE CITED

1. A. A. Bagdasarov, P. M. Al'perin, M. Ya. Anshevits, et al., Ter. Arkh., No. 7, 11 (1960).
2. A. I. Gol'dberg, Agastric B_{12}-Deficiency Anemias – Late Sequelae of Total Gastrectomy, Author's Abstract of Doctoral Dissertation, Tomsk (1960).
3. D. I. Gol'dberg, A. I. Gol'dberg, V. S. Lavrova, et al., Klin. Med., No. 8, 65 (1962).
4. O. I. Epifanova and V. V. Terskikh, The Method of Autoradiography in the Study of Cell Cycles [in Russian], Moscow (1969).
5. G. T. Karimova and V. Ya. Plotkin, Probl. Gematol., No. 10, 22 (1971).
6. M. G. Kakhetelidze, G. P. Moskaleva, O. V. Aksenova, et al., Pat. Fiziol., No. 2, 32 (1966).
7. V. A. Kolaev and A. E. Gel'fman, in: Proceedings of the Third Medico-Biological Session of the Academy of Sciences of the USSR [in Russian], Moscow (1968), p. 44.
8. N. A. Fedorov, M. G. Kakhetelidze, and I. K. Koryakina, Probl. Gematol., No. 11, 17 (1960).
9. N. A. Fedorov and M. G. Kakhetelidze, in: Current Problems in Hematology and Blood Transfusion [in Russian], No. 40, Moscow (1968), p. 357.
10. E. R. Powsner and L. Berman, Blood, 30, 189 (1967).