

# *Acta Medica Okayama*

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*Volume 23, Issue 5*

1969

*Article 3*

OCTOBER 1969

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Stem cell in peripheral blood: a study by  
parabiosis of irradiated and non-irradiated  
animals. II. Hematopoiesis of irradiated rat  
having a non-irradiated parabiont

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# Stem cell in peripheral blood: a study by parabiosis of irradiated and non-irradiated animals. II. Hematopoiesis of irradiated rat having a non-irradiated parabiont\*

Toru Kawai

## Abstract

For the purpose to confirm the existence of the stem cells of the myeloid and erythroid cells in the circulating blood and to have the information of its morphologic entity, the author conducted morphologic observations on the peripheral and bone marrow cells of the x-irradiated parabionts having non-irradiated partners joined by the vascular parabiosis devised by the author, and the following results were obtained. 1. The control rats exposed to 1000R x-ray did not show any sign of recovery of hemopoiesis in bone marrow even 8 days after irradiation. 2. In the bone marrow and spleen of the lethally irradiated animals having non-irradiated partner in parabionts, precursor cells of granulocyte and erythrocyte appeared first 4 days after conjugation irrespective of the days after irradiation, and the hemopoiesis was restored completely on the sixth to the seventh day. The results have indicated that the circulating blood has the stem cells which can dedifferentiate and transform into the precursor cells of the myeloid and erythroid cells within 3 days under adequate conditions. 3. On the basis of the morphologic observations on the peripheral blood of the parabiosis and non-irradiated rats revealed non-specific cells, a discussion was made on the possibility that some atypical lymphoid cells can serve as the stem cells of the myeloid and erythroid cells.

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Acta Med. Okayama **23**, 377—386 (1969)

**STEM CELL IN PERIPHERAL BLOOD: A STUDY BY  
PARABIOSIS OF IRRADIATED AND NON-  
IRRADIATED ANIMALS**  
**II. HEMATOPOIESIS OF IRRADIATED RAT HAVING  
NON-IRRADIATED PARABIONT**

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*Received for publication, July 5, 1969*

In the previous paper (1) the author reported on the vascular parabiosis which serves as a useful tool in hematologic research, especially as a method for an approach to the stem cell problem in circulating blood.

It is generally accepted that mature lymphocytes can dedifferentiate toward less mature blastform cells by the action of PHA (2) (Phytohemagglutinin) and in some experiments there have appeared a possibility that the mature leukocytes in circulating blood may also dedifferentiate themselves acquiring the characteristics of stem cells, though no actual processes of dedifferentiation have been observed yet (3, 4).

The existence of stem cells of granulocytes and erythrocytes in the peripheral blood have been inferred from the observations of the lethally irradiated parabiotic animals having non-irradiated partner (5), or the observation on the lethally irradiated animals having shielded leg or spleen (6).

These animals survived through the heavy irradiation, restoring the hemopoietic tissue in bone marrow and spleen suggesting that the circulating blood contain the stem cells.

Besides these, in man it was demonstrated that the granulocytes from the circulating blood cultured *in vitro* synthesize DNA (7) and divide themselves (8), thus producing the cells of various morphologic types. The fact also indicates the possibility that some mature cells in the peripheral blood are settled in the bone marrow and dedifferentiate to be transformed to the stem cells of the granulocytes and erythrocytes. To settle this problem the author tried to reconfirm the restoration of hemopoiesis of the lethally irradiated animals by the parabiosis with non-irradiated partner. But the experiment on the coarctation-anastomosis, which is generally used for such observations, showed no appreciable establishment of humoral communication between the parabiotic rats (1).

In the present experiment, the author examined the hemopoiesis of the lethally irradiated animal having a non-irradiated partner in vascular parabiosis with the parallel anastomosis of aorta devised by the author (1). By this method a complete exchange of blood can be established between the parabionts, and the severely damaged femur bone marrow of the irradiated animal is directly irrigated with the blood from the non-irradiated animal. In the present paper the restoration process of hemopoiesis of the lethally irradiated rats is undertaken by the irrigation of the femoral bone marrow with the normal rat blood by vascular parabiosis.

#### MATERIALS AND METHODS

In this experiment, 75 male litter mate Wistar rats weighing 250–300g were used. Parabiotic partners were chosen from those having nearly the same body weight, the weight difference being less than 10 g.

Twenty-five rats were selected to be the x-ray irradiated partners of which 16 received whole body irradiation of 1000R just before parabiosis and then were conjugated with the non-irradiated partners. Other 8 animals were also exposed to 1000R x-ray by whole body irradiation but they had their non-irradiated partners conjugated 48 hours after x-ray irradiation.

The remaining 9 animals served as controls and received 1000R x-ray whole body irradiation, were left single without parabiont.

After the operation the animals were sacrificed daily for eight days. Two pairs of parabionts conjugated immediately after irradiation and one pair conjugated 48 hours after irradiation and one irradiated control were sacrificed per day.

Just before sacrifice, the red cell and white cell counts and the hematocrit values were estimated with the blood from the orbital sinus of the animals. Smears of blood and femur bone marrow were also prepared. They were stained with May-Grünwald-Giemsa and served for the morphologic observations: formation of hemogram and myelogram. Several organs and tissues, spleen, stomach, intestine, thymus, mesenteric nodes, testis and bone marrow were fixed with 10 % formal and Hematoxylin Eosin stained paraffin sections were made for histologic observations.

#### RESULTS

The rats receiving 1000R x-ray irradiation showed severe devastation of the bone marrow. Already one day after irradiation, leukocytes have rarely been detected in the circulating blood, but in the bone marrow a fairly large number of cells were seen, though most of them were of mature cells. Younger precursors of erythroid and myeloid cells have been markedly reduced in number, and the reticulum cells and plasma cells

became predominant. On the third day both myeloid and erythroid cells were no longer visible; 7—8 days after irradiation, almost all of the bone marrow became completely fatty and appearing sparsely under the microscope; almost all hemopoietic cells disappeared from the bone marrow, leaving only the proliferated plasma cells and reticulum cells, which reflects the severely damaged bone marrow hemopoiesis, and the granulocytes were hardly encountered in the smear of peripheral blood. In the animals conjugated with normals immediately after the x-ray irradiation, 1000R at one time, the damaged bone marrow hemopoiesis showed a recovery tendency 4 days after irradiation and younger precursors appeared already 5 days after irradiation. Of course, in the circulating blood of the irradiated parabionts, "post irradiative leukopenia" did not develop, and white cell counts remained in the range of normal values, 4000 to 10000 per cu mm, and it kept the same level with that of the intact normal partner throughout the experiment. (Fig. 1)

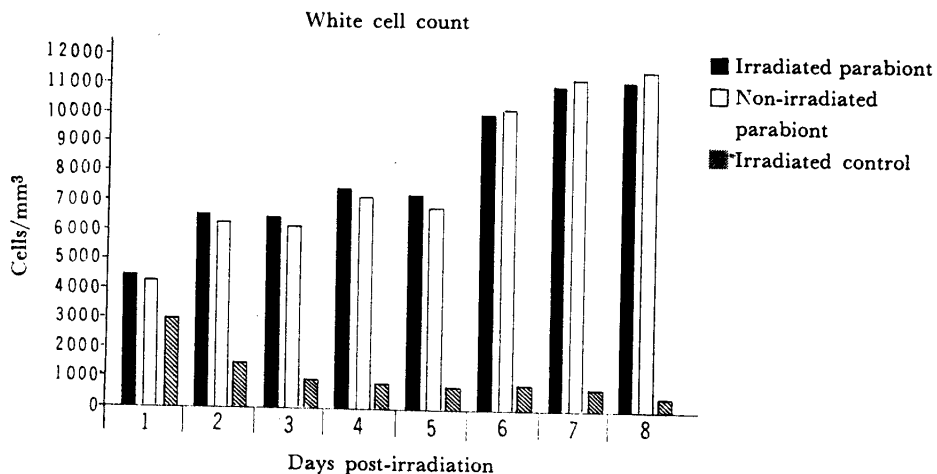


Fig. 1 Changes of white cell count in the circulating blood from parabiotic and control rats

During 1 to 3 days after irradiation, however, no sign of restoration of the damaged hematopoietic tissue was observed in both bone marrow and spleen. Therefore, the peripheral white cell counts must have been kept by the cells from the non-irradiated partners. On the fourth day some younger precursors appeared in the bone marrow of the irradiated animals and became predominant and 6 days later the cells in various maturation stages appeared in the bone marrow in which erythropoiesis predominated. Granulopoiesis was rather accentuated in the intact normal partner, especially in the later stage of parabiosis, 6—8 days after irradiation. The

induction mechanism of the accelerated granulopoiesis in normal partner is unknown, but the marked increase in white blood cell number in the circulating blood in the later stage would greatly be due to the white cells from the normal partner. No remarkable changes were observed in red cell counts of both parabiotic partners throughout the experimental period (Fig. 2). This should be due to the long life-span of the erythrocytes,

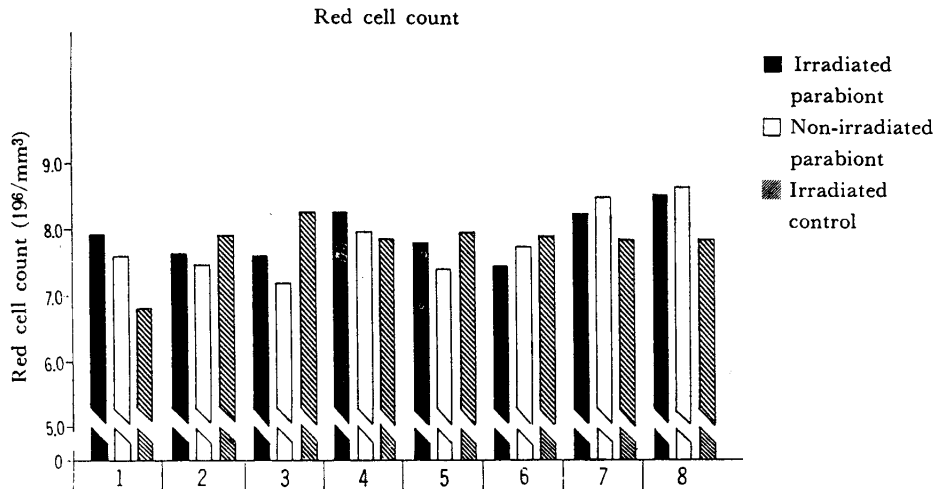


Fig. 2 Changes of red cell counts in the circulating blood from parabiotic and control rats

60—70 days in rats, while the mature granulocytes survive only several days. Erythropoiesis in the bone marrow was severely damaged with complete disappearance of the erythroblasts from the bone marrow already 2 days after irradiation of 1000R. The younger precursors of erythroid cells did not appear in the irradiated single animals, but in those having parabiotic normal partners, the erythroblast appeared 4 days after the irradiation and became marked on the fifth day. This erythropoiesis in the bone marrow grew often extremely marked on the seventh to the eighth day after irradiation accompanied by the poor restoration of granulopoiesis.

The hematocrit value decreased slightly during 2 to 3 days after irradiation but it stayed within normal range, giving no distinct information about the damage of erythropoiesis.

The relatively high values in the hematocrit of the single irradiated controls on the first to the fourth day indicate the severe dehydration after irradiation, irrespective of hematopoietic activity. (Fig. 3)

In the animals conjugated with normal rats 4 days after irradiation,

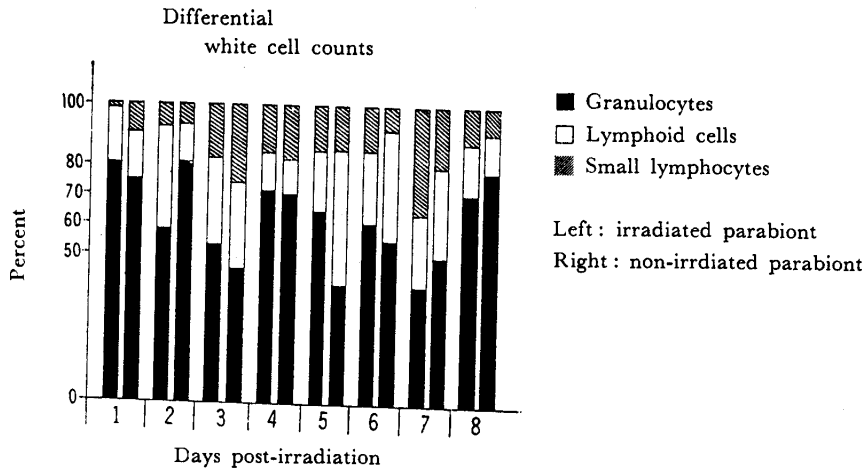


Fig. 3 Graphic representation of hematocrit of blood from parabiotic and control rats

similar results were obtained; namely, the restoration of the bone marrow hemopoiesis began 4 days after parabiosis and became marked 5 days later. The morphologic observations on femoral bone marrow revealed the same processes as in those conjugated with normal rats immediately after irradiation. Hemogram of the circulating blood was nearly the same as that in the former experiments.

The results clearly indicated that 2 to 3 days' lag phase is required for the appearance of the precursor cells of granulocytes and erythrocytes.

In the peripheral blood of the animals x-ray irradiated and conjugated with normal ones by vascular parabiosis some change have been observed in the components of nucleated cells. As can be seen in the decrease of lymphoid cells, especially small lymphocytes were hardly detectable in those of the irradiated partners. On the second day the percentage of lymphoid cells increased nearly 50% and on the third day it recovered to normal range, 2 to 30 per cent. (Fig. 4)

Among these lymphoid cells were seen some unclassified cells resembling large lymphocytes but often with nuclei with indentation or of irregular type and with rather basophilic cytoplasm. They may be called lymphoblasts, atypical lymphocytes or monocytoïd cells. The smears and sections of the femoral bone marrow of the rats irradiated and conjugated with normal rats showed an increase in cellularity on the third day after conjugation but most of the cells were mature granulocytes with some lymphoid cells. On the fourth day some younger precursors of myeloid and erythroid cells appeared among them. On the fifth day, bone marrow became quite hyperplastic and was mainly composed of immature cells of

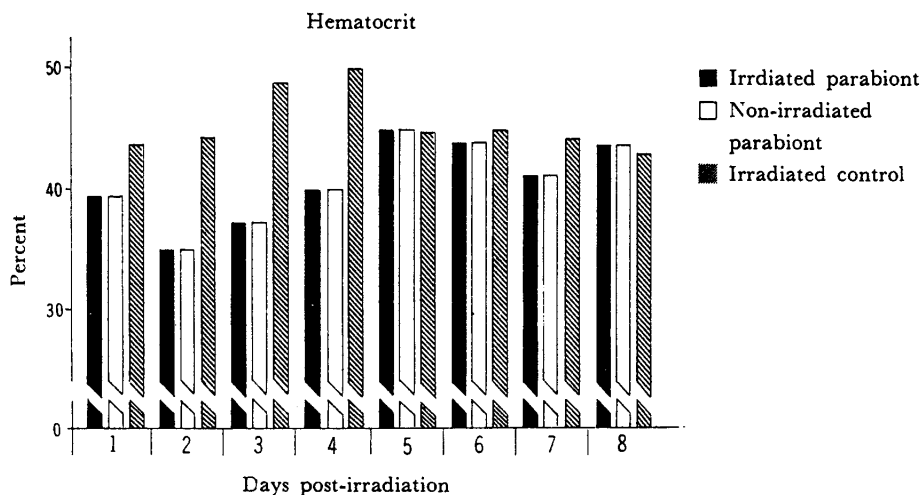


Fig. 4 Graphic representation of differential white cell counts of circulating blood from parabionts

the erythroid and myeloid series. Several mitosis were observed in cells of the erythroid series. Six days and thereafter, the cellular proliferation by erythropoiesis was dominant and during this period, erythroid islets and myeloid clones were also seen in tissue sections.

In the non-irradiated partners conjugated with the irradiated animals, granulopoiesis in the bone marrow and spleen became marked, especially

Fig. 5 A bone marrow section of the rat irradiated and conjugated with normal rat. The photograph was taken on the third days after conjugation and the marrow show a higher cellularity than those of control rat, but most of cells are mature granulocyte with some lymphoid cells

Fig. 6 A bone marrow section of control rat three days after irradiation. Myeloid and erythroid cells are no longer visible. Reticulum cells and plasma cells are predominant.

Fig. 7 Bone marrow section of the rat irradiated and conjugated with normal rat, five days after conjugation, and the marrow appear quite hyperplastic and mainly composed of immature cells of the erythroid series.

Fig. 8 A Bone marrow section of control rat, five days after irradiation. Most of cells are plasma cells and reticulum cells.

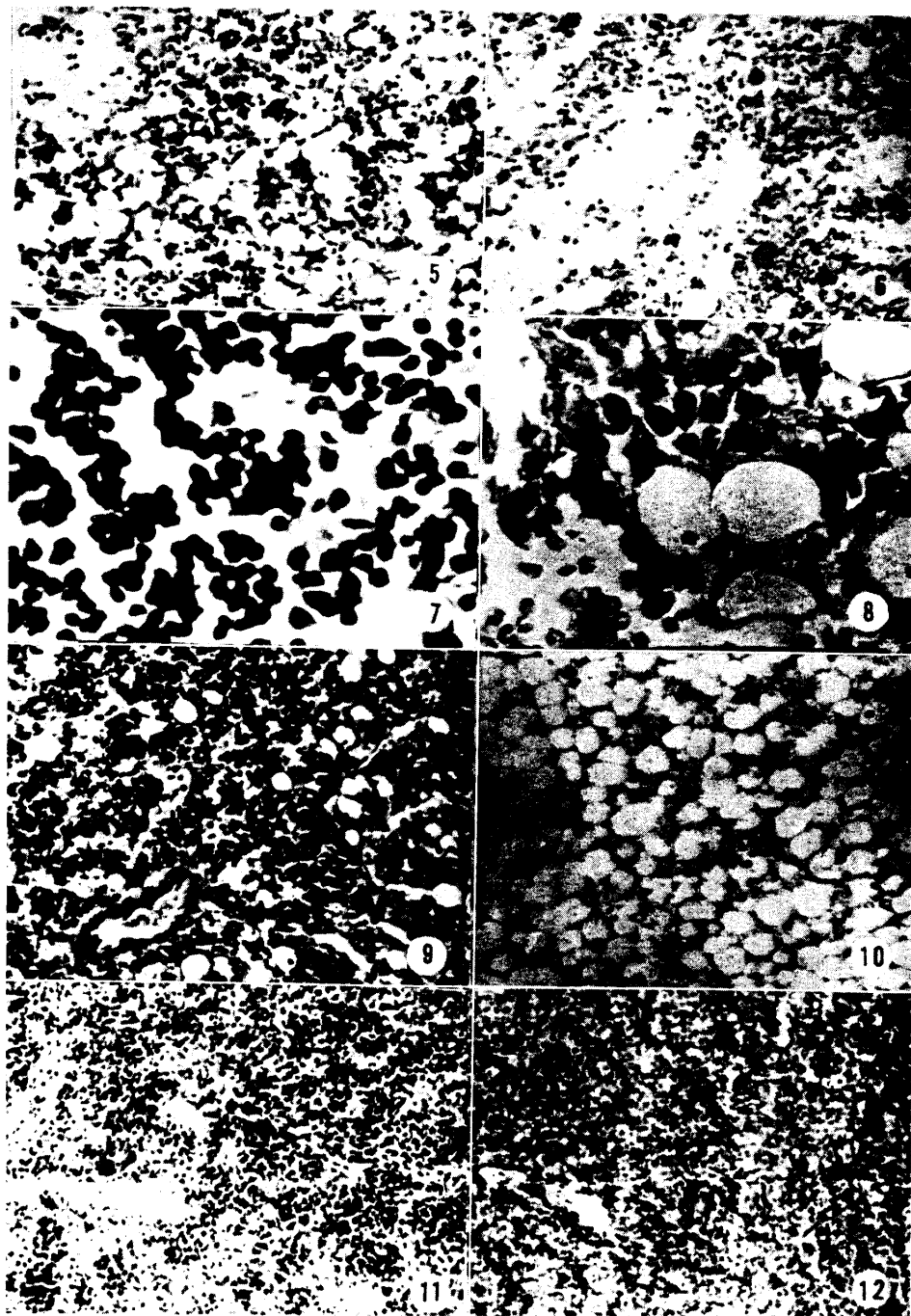
Fig. 9 A bone marrow section of the rat irradiated and conjugated with normal rat, eight days after conjugation and the marrow shows the well restored hemopoiesis. Erythroid islets and myeloid cones are seen.

Fig. 10 A bone marrow section of control rat six days after irradiation, the marrow shows severe devastation, almost all the bone marrow cells disappear being completely fatty with some cells

Fig. 11 A spleen section of non-irradiated partner five days after conjugation note accentuated hemopoiesis in the sinuses.

Fig. 12 Spleen section of normal intact rat shows a normal hemopoiesis in spleen





2 to 3 days after conjugations.

#### DISCUSSION

The present observations on hematopoiesis of the lethally irradiated animals with or without parabiotic normal partner by the aortic anastomosis suggested that there are stem cells of myeloid and erythroid cells in the circulating blood, indicating that the animals with non-irradiated partners restore their damaged hemopoiesis within a short period of 4 to 5 days, while the single irradiated controls died without recovery from the pan-myelophthiesic condition after irradiation. The data are consistent with that of TYLER (5) who observed the recovery of hemopoiesis of the irradiated animals by coerio-anastomosis, revealing the transport of the stem cells from normal partners to the irradiated animals through the established communication between the two animals.

Restoration of hemopoiesis of the animals lethally irradiated with shield leg or spleen may mean the same thing.

The present experiment has also indicated that the period required for the recovery of hemopoiesis is nearly 3 days and younger precursor cells of the myeloid and erythroid series appeared first on the fourth day of parabiosis regardless of the length of time after irradiation. This means that at least 3 days are required for the formation of the precursor cells from the "stem cells" in the circulating blood. As the bone marrow was irrigated directly with the blood from the non-irradiated animals, the stem cells should reach the bone marrow immediately after conjugation.

That 5 1/2 days are required for the appearance of the precursor cells in TYLER's (5) experiment is due to an incomplete humoral communication between the two animals as just pointed out in the previous paper (1).

Considering the observations of the blast formation of lymphocytes, 20 to 30 hours may be required for turning of the "stem cells" of mature cell type into the blast form and another similar period may be needed for the transformation of the stem cells to the blast form and further into the younger precursor of myeloid or erythroid cell with or without mitosis.

In this experiment there is no direct evidence that the stem cells are transferred from the normal partner to the irradiated one, but the observations of McCULLOCH and others (9, 10) and TYLER (5) and associates, demonstrate that the cells will actually move from the normal animal to the irradiated one and proliferate in the latter, especially readily in the irradiated individual where the immunologic activity is markedly reduced.

It may be supposed that some unfavorable factors for hemopoiesis are formed in the animals irradiated with x-ray, but it should be noted that unexpectedly some hemopoiesis promoting factor is liberated from the x-ray irradiated animals showing a markedly accelerated proliferation of the myeloid and erythroid cells in the bone marrow and spleen, which became most marked 3 days after conjugation with irradiated animals. The data support the observations of A. CEDERBERG (11) who observed the promoted proliferation of the myeloid cells *in vitro* after adding the serum from the irradiated animal.

Therefore, it is reasonable to deduce that there is a hemopoiesis-activating condition in the irradiated animal.

Concerning the stem cells, it does not necessarily mean the undifferentiated cell type but possibly some differentiated one such as the mature cell, because it can dedifferentiate into a blast form cell which can divide and differentiate again to the mature cells as in the lymphocytes. But in the case of the "stem cells" of the myeloid and erythroid series, we have found no precursor cells in the circulating blood. Therefore, the "stem cells" must transform into the other strain cell, and this can actually be seen in some other cell strains. For example, the well differentiated pigment cells of iris lose their pigment and transform and differentiate into the lens cells when the lens is removed, as demonstrated in the experiment with newt (12).

Therefore, it is quite probable that some mature cells in the circulating blood of other strain cells serve as the stem cells of the other strain cells.

#### SUMMARY

For the purpose to confirm the existence of the stem cells of the myeloid and erythroid cells in the circulating blood and to have the information of its morphologic entity, the author conducted morphologic observations on the peripheral and bone marrow cells of the x-irradiated parabionts having non-irradiated partners joined by the vascular parabiosis devised by the author, and the following results were obtained.

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#### ACKNOWLEDGEMENT

The author is indebted much to Professor SATIMARU SENO for valuable advices throughout this work and painstaking proof reading of the paper. Many thanks are also due to Miss HIRATA for her technical assistance and the members of Department of Pathology for their helpful suggestions.

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