Acta Medica Okayama

Volume 19, *Issue* 5 1965 *Article* 1

OCTOBER 1965

Studies on reticuloendothelial system and hemato-poiesis, III. Relationship between differentiation of erythroblast and oxygen tension

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Abstract

For the purpose to clarify the control mechanism of erythroid cell differentiation, the author observed morphologic changes in bone-marrow cells and circulating red cells in phenylhydrazine anemia of rabbits by introducing a mass of red cells into vein at one time and reached the following conclusions. 1. After red cell transfusion in a mass to animal showing a marked hematopoietic activity, anisocytosis or macrocytosis becomes distinct with the appearance of big reticulocytes and red cells as large as four times the normal in volume. This suggests, judging from their volume, the accelerated denucleation of erythroblast as early as at the late basophilic stage. 2. Observations on bone marrow at this stage revealed the reduction in the number of erythroblasts of undifferentiated type with the increase of rather differentiated ones. In erythroid islet, undifferentiated cells are found surrounding a reticulum cell located in the center, while well differentiated ones in the outskirt area are situated near the sinusoid. Such a cell arrangement suggests that the erythroid cell requires a high oxygen tension for its differentiation. 3. From these observations and other results obtained from the studies on reticulocyte maturation and RNA synthesis of erythroblast, the author stresses that erythroid cells can differentiate as long as it is provided with a certain level of oxygen, even though it may develop m-RNA for differentiation. In other words, there should be two steps in the differentiation of erythroblast, the first is m-RNA synthesis induced by the information and the second is the somatic protein synthesis with oxygen supply. This seems to be directly connected to the control mechanism of hematopoiesis by oxygen.

Acta Med. Okayama 19, 217—225 (1965)

STUDIES ON RETICULOENDOTHELIAL SYSTEM AND HEMATOPOIESIS

III. RELATIONSHIP BETWEEN DIFFERENTIATION OF ERYTHROBLAST AND OXYGEN TENSION

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Received for publication, September 10, 1965

In the previous papers[1,2], it was described that reticuloendothelial cells or reticulum cells found in the center of erythroid islets play an important role for the differentiation of cells. The author stressed that reticuloendothelial cells should give the information to the stem cells necessary for their differentiation. According to the concept obtained from the studies on bacteria, the development of inducer should be connected directly to the release of messenger RNA (m-RNA) for the synthesis of somatic protein[3]. However, it is obvious from the cytological observations that proerythroblasts, whose nuclei are very active in RNA synthesis[4], possibly m-RNA synthesis, do not synthesize hemoglobin[5] which appears only at polychromatic stage, as revealed by the routine Giemsa staining. This discrepancy found between the stage m-RNA synthesis and somatic protein synthesis might be due to some factor that acts to inhibit hemoglobin synthesis. SENO and associates[4] have clearly demonstrated that the maturation of reticulocytes, or hemoglobin synthesis accompanied by RNA degradation, requires a high level of energy which is produced in the process of oxidative phosphorylation. By the inhibition of respiration by KCN or the ATP formation due to the uncoupling agent for oxidative phosphorylation, the maturation of reticulocytes is depressed or greatly retarded[6].

These basic experiments have led the author to seek if the differentiation of young erythroblasts could be accelerated under highly oxygenated environment. As is well known, erythropoiesis is extremely suppressed under high oxygen tension, but those that received information for differentiation may mature rapidly by synthesizing hemoglobin in their early developmental stage. In view of this, observations were carried on the bone-marrow cells and the circulating denucleated cells of rabbit previously made anemic by phenylhydrazine injection, and the animal was transfused with a mass of red cells from normal animal in one dose. In this paper, it is reported that the differentiation of erythroblasts is accelerated on early denucleation of erythroblast or on appearance of a striking

macrocytosis, with a decrease of undifferentiated erythroblasts and an increase of differentiated ones in number in the bone marrow.

MATERIALS AND METHODS

Adult male rabbits weighing 2.5—3.0 kg were used. Red cell count, hematocrit, hemoglobin content and red cell diameter were observed at certain intervals. The animals showing normal hematologic picture received subcutaneous injection of phenylhydrazine hydrochloride, 1.5 cc of 2.5% solution in distilled water, daily three to four times by the method reported in the previous paper[2]. Two to three days after the last injection when anemia reached its critical point with an activated hematopoiesis or a marked reticulocytosis, five animals were sacrificed for the observation of bone-marrow tissue. Other five animals received the intravenous introduction of red cells from a normal rabbit in a one mass dose. The red cell suspension was prepared as follows: blood 80—100 cc was taken by cardiac puncture from one non-anemic animal by using sodium citrate 1/10 volume of 3.8% solution as anticoagulant. The blood was centrifuged and the precipitated red cells were isolated by removing blood plasma and buffy coat. Then the cells were washed with physiological saline solution three times by repeated centrifugations and finally suspended in 50 cc of saline solution. Immediately after preparation the cell suspension, the total red cells obtained from one healthy animal, was introduced into ear vein of each anemic animal.

Twenty-four hours after the introduction of cell suspension, the rabbits were sacrificed for the observation of erythroid islet. The tissues of bone marrow and spleen were sectioned and stained by the routine method for observation with light microscope. Silver staining[7] was resorted to in the case of observation of reticular fibers.

Price-Jones curves were drawn on each animal at three stages, i. e. before the injection of phenylhydrazine and the red cell transfusion, and 24 hours after the red cell introduction. The curves were drawn on red cells in wet preparation; they were photographed at the magnification of 100×10 and red cells in print were picked up at random for the measurement of their diameters, taking 200 to 300 cells for one curve.

OBSERVATIONS AND RESULTS

By repeated injections of phenylhydrazine, the red cell count became lower and at the critical stage where it reached 2 to 3 days after the last injection, the count was less than 2 million per cu mm, and the red cell diameter was increased markedly and Price-Jones curve showed a right shift, a macrocytosis, which is shown by the solid thin-line in Fig. 1.

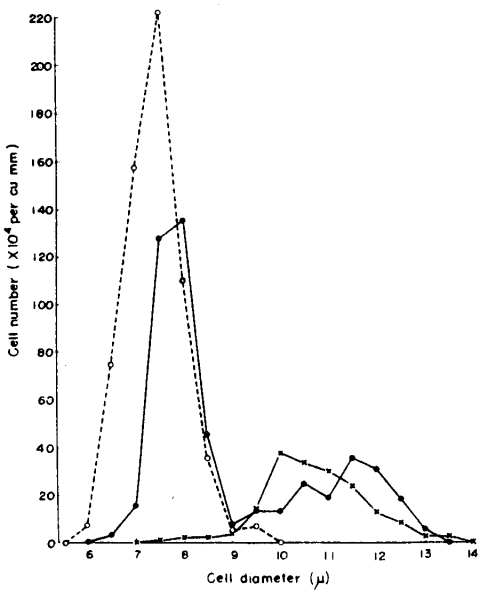


Fig. 1 Price-Jones curves of a blood-transfused rabbit after phenylhydrazine injections. Broken line: before the injection, solid thin-line: after the injection, solid thick-line: 24 hours after blood transfusion.

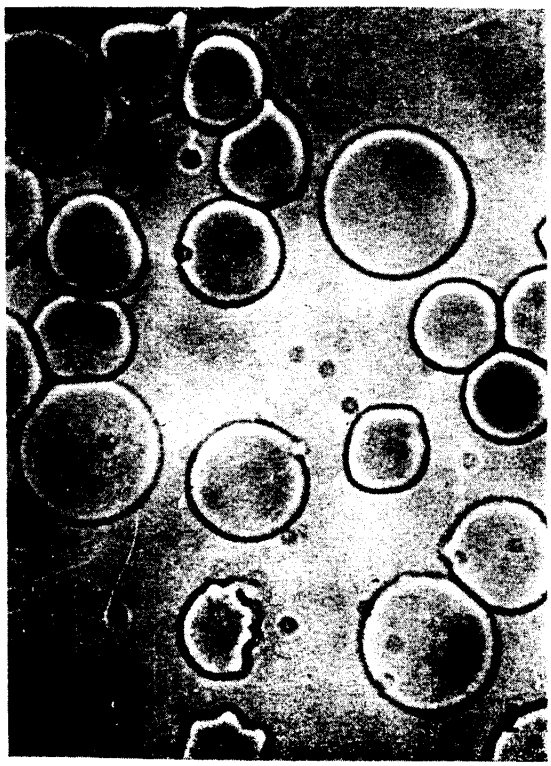


Fig. 2 Demonstration of macrocytes in the circulating blood of the same animal as in Fig. 1 and Table 1. ×1, 500

Table 1 Change of circulating-blood picture of the same animal as in Fig. 1. RBC: red cell count ($\times 10^4$/cu mm), RC: percentage of reticulocytes in the circulating erythroid cells (%), Ht: hematocrit (%), Hb: hemoglobin content (g/dl), MCV: mean corpuscular volume of circulating erythroid cells (cu μ).

Hemogram / Treatment	RBC	RC	Ht	Hb	MCV
before injection of phenylhydrazine	630	1.2	39	13.74	62
after 4 injections of phenylhydazine	175	51.4	20	3.33	114
24 hours after blood transfusion	506	6.5	40	9.60	89

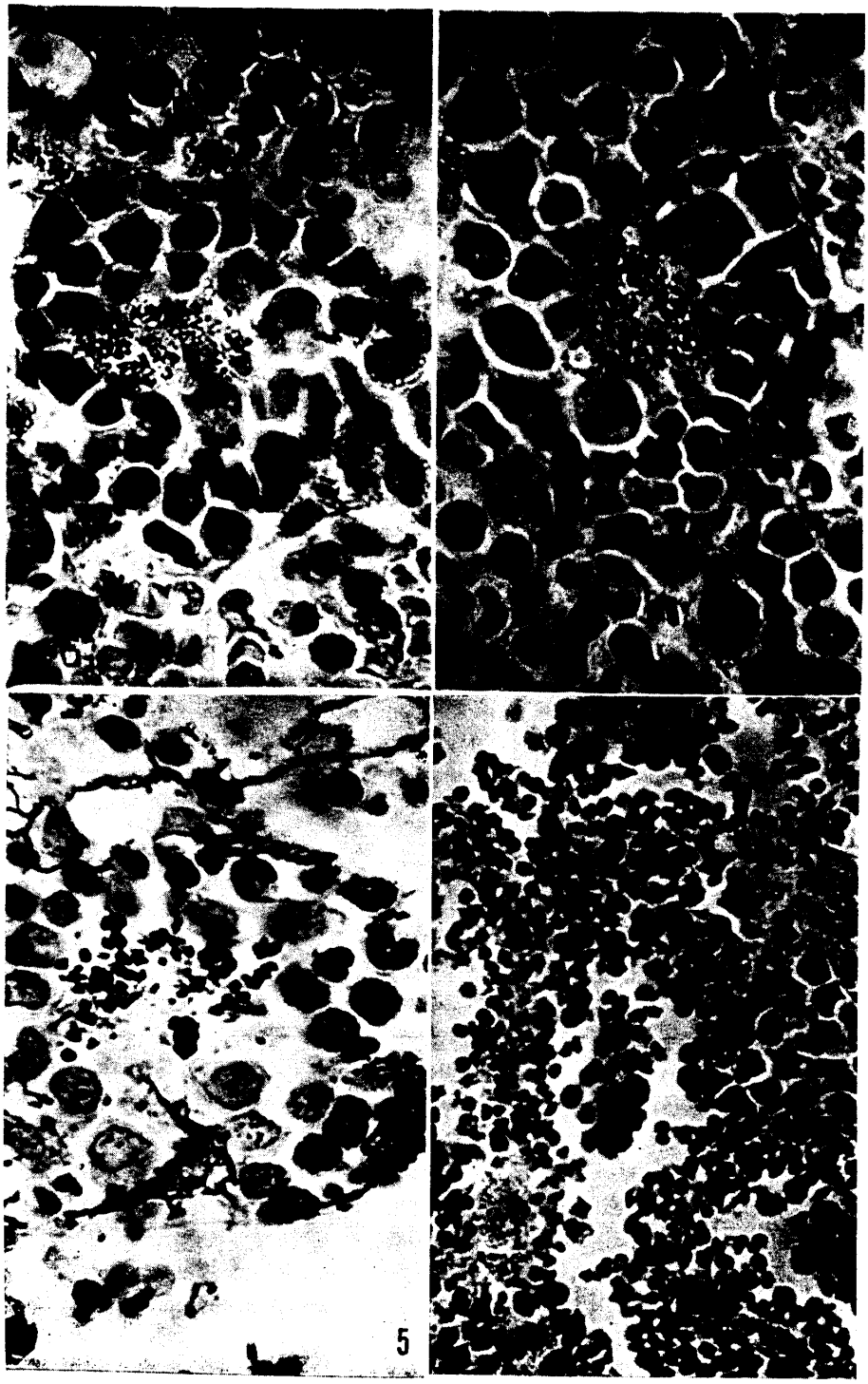
Histologic observations of bone marrow of the animal sacrificed at this stage revealed a large number of erythroblastic islets occupying almost the whole area of the bone marrow. As reported previously[2], in the center of each islet one or two reticulum cells were situated, around which were erythroblasts in various differentiation stages, arranged radially. In the center of the islet there were found many large erythroblasts with big nucleoli and basophilic cytoplasm, and these were thought to be the stem cells or proerythroblasts. Surrounding these cell groups there were more mature cells or smaller cells, probably basophilic erythroblasts, and on the margin of the islet just adjacent to the sinusoidal wall, there were very small erythroblasts with dark nuclei and weakly basophilic cytoplasm, probably polychromatic or orthochromatic erythroblasts. Some of the mature nucleated cells were in the cavity of sinusoid surrounding the islet, which was filled with denucleated cells, reticulocytes and mature erythrocytes (Figs. 3, 4). In the sections containing stained reticular fibers, the sinusoidal structures were distinct (Fig. 5). In the extramedullary erythropoietic foci of spleen, a similar cellular configuration as observable in the bone marrow was seen, but in spleen the erythroid islet was smaller than the one in the bone marrow, and the reticulum cell in the center was directly surrounded by a mature nucleated cell layer (Fig. 6).

At this stage, the other animals received intravenous injection of red cell suspension, by which red cell counts and hemoglobin contents reached nearly normal level. Twenty-four hours after this treatment a marked anisocytosis was

Figs. 3, 4 Section of bone marrow of a phenylhydrazine injected rabbit, showing erythroblastic islets. Reticulum cells are found in the center of the islets and large undifferentiated erythroblasts are situated in the central part, while small differentiated ones in the outer layer. In sinusoidal cavities more mature erythroblasts are in company with mature erythrocytes (H-E). $\times 1,000$

Fig. 5 The same section with staining for reticular fibers by Pap's method[7]. $\times 1,000$

Fig. 6 Section of spleen of the same animal as in Fig. 3, showing the islets in the sinusoidal cavities. Note that these islets are smaller than in bone marrow and erythroblasts surrounding reticulum cells are of more mature type. $\times 530$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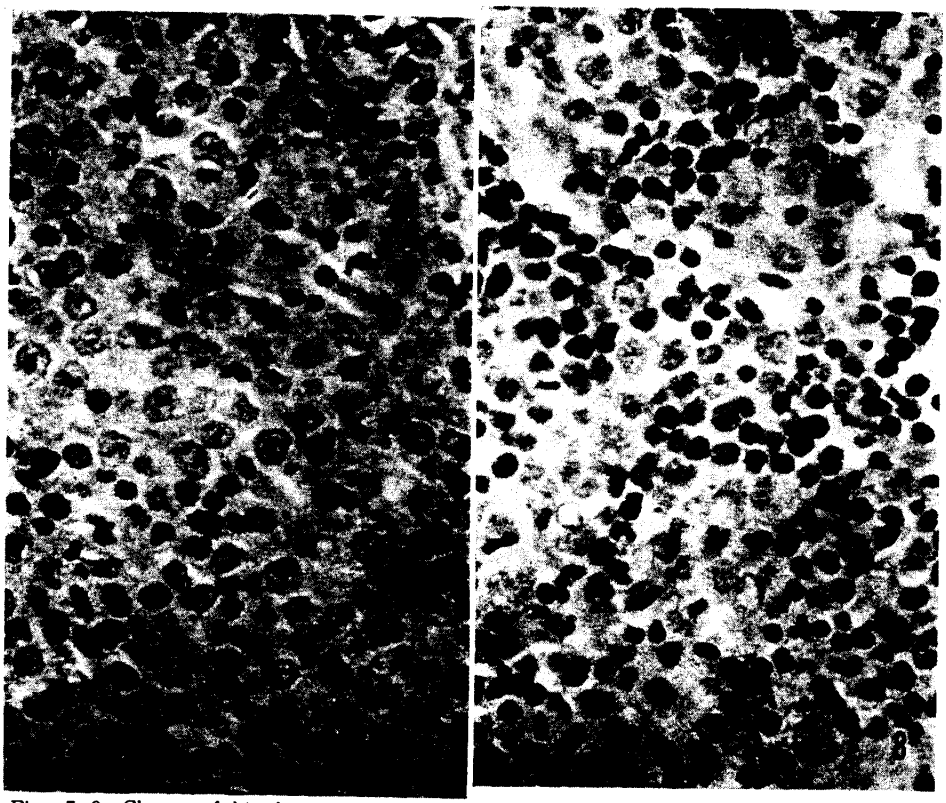

Figs. 7, 8 Change of histologic picture by blood transfusion on a phenylhydrazine injected rabbit. Fig. 7 is the picture before transfusion, predominantly showing large undifferentiated erythroblasts. Compared with this, Fig. 8 after transfusion shows an increase of small more differentiated erythroblasts. ×530

induced, the Price-Jones curve showing three peaks as indicated by the solid thick-line in Fig. 1.

The left peak with the base of 6 to 9.5 μ and with the summit at 7.5 μ corresponds to the Price-Jones curve of normal red cells and mainly composed of introduced red cells; the middle one, with the base of 7.5 to 13.5 μ and with the summit at 10 μ, to that found before the red cell transfusion or that of macrocytes developed by phenylhydrazine injection; and the right one with the summit at 11.5—12 μ is a new one appearing after transfusion. No peak appeared on the right side of the last one. Bone-marrow tissues taken 24 hours after the introduction of red cell suspension showed a picture still retaining active hemopoiesis. A large number of erythroblasts constituting erythroid islets was observed microscopically. However, in contrast to the tissue at critical stage of anemia, erythroblasts of a relatively mature type were predominant, while large

immature ones were relatively less in number (Figs. 7, 8).

DISCUSSION

According to the extensive studies of WEICKER[8], the nuclear volume of erythroblast reduces by one half at each cell division, and that of orthochromatic erythroblast is only 1/16 of that of the tetraploid proerythroblast. SENO and associates[4] have demonstrated that the cell volume of erythroblasts is also reduced by one half at each cell division and the cell size becomes smaller and smaller with the advance of maturation, their cytoplasmic volume being reduced proportionately to the reduction rate of nuclear volume. This means that the red cell size indicates directly the denucleation stage, as no cell division occurs after denucleation[9]. Erythroid cell production may require a certain high level of oxygen. OHEDA[10] stated that in the bone marrow of young rabbit arterial branches reach directly the epiphyseal area where the arterioles are densely distributed and active hematopoiesis can be seen. Under such a highly oxygenated environment, undifferentiated cells seem to be located in the area of relatively low oxygen, such as the central part of erythroid islet. It is the general concept in biology that undifferentiated cells live in relatively anaerobic environments while the differentiated ones in the aerobic environment. According to WARBURG[11], the cells proliferating actively as tumor cells consume less oxygen than general somatic cells; the former get much energy from anaerobic glycolysis, while the latter rely largely on the energy produced by oxidative phosphorylation. This seems to suggest that the cell cannot differentiate without getting the energy liberated by oxidative phosphorylation, even though it develops m-RNA for its differentiation. Erythroid cells are not an exception. The cellular configuration of erythroblastic islet, in which undifferentiated young cells are located in the central part of the islet and differentiated ones in the outer layer, is consistent with this view, because it is thought that oxygen tension is rather low in the central area than in the outer layer, which directly faces the sinusoidal lumen.

As just mentioned, extramedullary hematopoietic foci found in spleen develop in the sinusoidal cavities having a sufficient blood supply, and these islets are mainly composed of rather mature erythroblasts.

In any area where the blood supply is supposed to be well maintained, there should be erythroblasts of more differentiated type and in hypoxic area undifferentiated ones, indicating that erythroid cell proliferation and differentiation are controlled by oxygen tension, probably enhancing the proliferation of undifferentiated cells with suppressed somatic protein synthesis in hypoxia and *vice versa*.

SUMMARY

For the purpose to clarify the control mechanism of erythroid cell differentiation, the author observed morphologic changes in bone-marrow cells and circulating red cells in phenylhydrazine anemia of rabbits by introducing a mass of red cells into vein at one time and reached the following conclusions.

1. After red cell transfusion in a mass to animal showing a marked hematopoietic activity, anisocytosis or macrocytosis becomes distinct with the appearance of big reticulocytes and red cells as large as four times the normal in volume. This suggests, judging from their volume, the accelerated denucleation of erythroblast as early as at the late basophilic stage.

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3. From these observations and other results obtained from the studies on reticulocyte maturation and RNA synthesis of erythroblast, the author stresses that erythroid cells can differentiate as long as it is provided with a certain level of oxygen, even though it may develop m-RNA for differentiation. In other words, there should be two steps in the differentiation of erythroblast, the first is m-RNA synthesis induced by the information and the second is the somatic protein synthesis with oxygen supply. This seems to be directly connected to the control mechanism of hematopoiesis by oxygen.

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