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Abstract

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THE MATURATION OF RETICULOCYTES II. LIFE-SPAN OF RED CELLS ORIGINATING FROM STRESS RETICULOCYTES

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Abstract: Life-spans of macroreticulocytes and macrocytic red cells were studied. Rabbits were made anemic by injecting phenylhydrazine. Peripheral blood rich in reticulocytes was drawn, labeled with ^{3}H-amino acids, and injected back into the anemic animal. Autoradiographic observation on circulating red cells revealed that macroreticulocytes matured at nearly the same time as normal-sized reticulocytes but that the macrocytic red cells had a short life-span compared to normal-sized red cells.

Under anemia, extremely large-sized reticulocytes (macroreticulocytes) appear in the circulating blood. These large-sized reticulocytes are probably formed in the anemic bone marrow by early denucleation of younger erythroid cells, and they are supposed to be more fragile than normal reticulocytes (1, 2, 3). In the previous paper the author reported that "stress" reticulocytes introduced into circulating blood disappeared by about 36 hours probably by maturation to red cells and that the large-sized reticulocytes disappeared early from circulating blood, i. e., by 4 to 12 hours after reticulocyte introduction (4). It is still uncertain, however, whether these macroreticulocytes mature to red cells and survive as normal-sized reticulocytes. The early disappearance of macroreticulocytes from circulating blood suggests two possibilities: (a) they disintegrate early in the circulating blood, and are really fragile and short lived or (b) they mature to macrocytic red cells. Even if these cells mature to red cells, they may not survive as long as normal red cells. Many investigators (5, 6, 7, 8) have reported that macrocytes have a short life-span but precise data is not available on whether they disintegrate at the reticulocyte or the macrocytic red cell stage. To examine these points the author investigated the maturation time of reticulocytes that were removed, labeled *in vitro* and then introduced back into the blood of the host animal. In this paper, autoradiographic studies of peripheral red cells are presented. The results indicate that macroreticulocytes decreased in size to some extent by surface fragmentation and matured to red cells or macrocytes in about the same length of time as normal-sized reticulocytes.

After maturation the macrocytes appear to disintegrate earlier than normocytes.

MATERIALS AND METHODS

Two white adult male rabbits weighing about 2 kg each were used. The animals were treated with a daily subcutaneous injection of 2.5% neutralized phenylhydrazine at 0.7 ml/kg for three consecutive days. Three days after the last injection (when the number of peripheral reticulocytes was about 70% of whole red cells and the hematocrit was about 20%) 70 ml of peripheral blood was drawn from the ear vein in a period of more than 30 minutes, using heparinized syringes. Soon after blood depletion, the animals were transfused with about 20 ml of homologous packed red cells suspended in saline to keep the animal alive. The collected blood was centrifuged at about 1,500 rpm for 5 minutes and the packed red cells were collected. The red cells were mixed with 2-^{3}H-glycine (4 μCi/ml), L-leucine-4-5-^{3}H (4 μCi/ml) and L-alanine-2-3-^{3}H (4 μCi/ml) and incubated for 120 minutes at 37°C in a medium free of these three amino acids. After incubation, Eagle's MEM was added to the sample and then centrifuged at 1,500 rpm for 15 minutes. The supernatant was discarded and the cells were washed three times with saline by repeated centrifugation, and the packed cells were suspended in an equal volume of saline and injected back into the ear vein of the host animal. At varying time intervals after reinjection, blood was collected for smears and for autoradiographic preparations. The smears were fixed with methanol, mounted with sensitive emulsions, exposed at 4°C for 20 days, developed and stained with Giemsa. A count of at least 5 grains was required per labeled cell. Labeling indices were obtained on 2,000 red cells from each sample. Price-Jones curves were drawn on 100 labeled cells from smears. Isotopes were obtained from the Radiochemical Center (Amersham) and had a specific activity of 3.8 Ci/nmole in ^{3}H-glycine, 19 Ci/nmole in ^{3}H-L-leucine and 42 Ci/nmole in ^{3}H-L-alanine.

RESULTS

The labeling index of red cells of both animals was about 45‰ immediately after introduction of labeled reticulocytes into the vein (Fig. 1). This value decreased with increased time and reached zero at 9 weeks after reticulocyte introduction. The index remained unchanged during the first 48 hours after reticulocyte introduction, but at 3 days the value fell by about 10‰ reaching a new stable level that continued for about 2 weeks. Thereafter, the labeling index began to decrease gradually, reaching a plateau in 2 to 3 weeks and fell to zero in 2 weeks (at the end of 9th week after the introduction).

Cells heavily labeled with more than 45 grains disappeared by 4 hours. Cells with 30 to 45 grains survived 2 days and then disappeared. From 3 days to 9 weeks after reticulocyte introduction, grain counts per cell were at

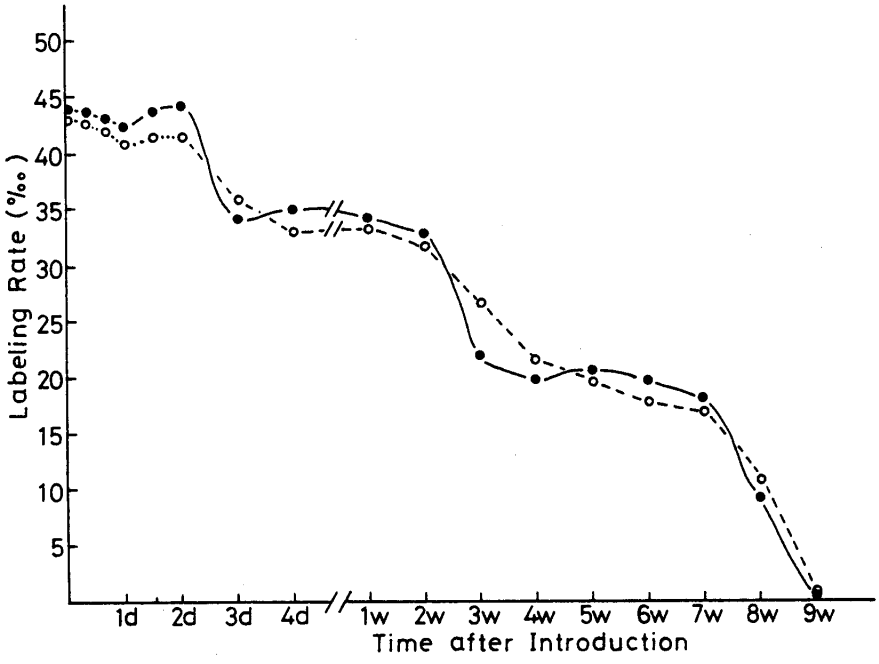

Fig. 1 Labeling percentage of red cells after the intravenous introduction of labeled reticulocytes.

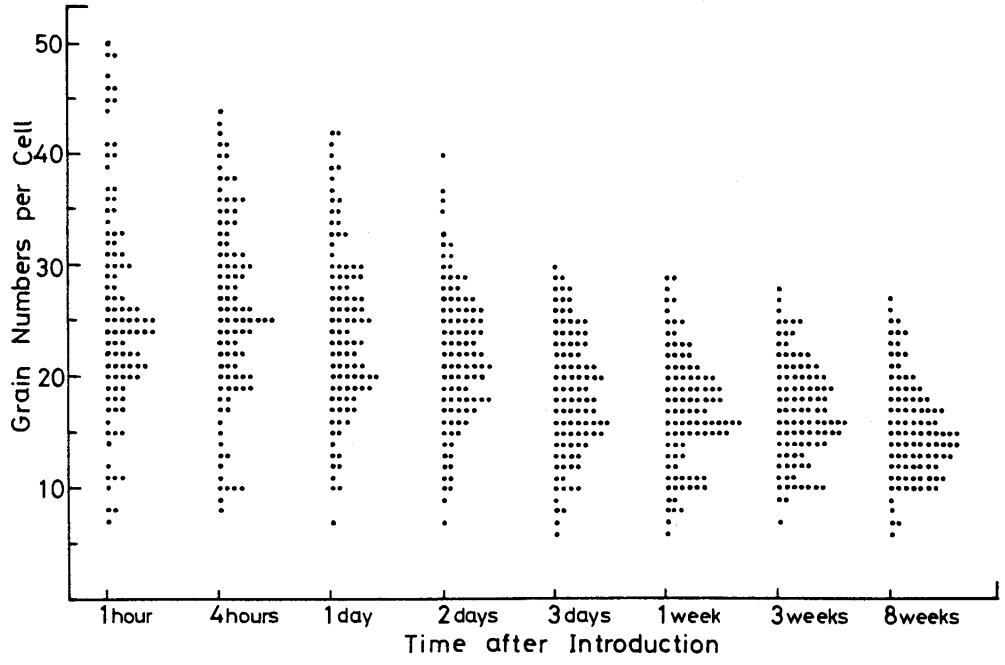

Fig. 2 Grain counts of labeled red cells at varying time intervals after the intravenous introduction of labeled reticulocytes.

nearly the same level (Fig. 2). The estimation of labeled cell size showed that the more heavily labeled cells had a larger diameter (Fig. 3). The Price-Jones curve of labeled cells immediately after cell introduction into the vein showed a peak at 7.0 μ. During the first 4 to 36 hours, when almost all labeled reticulocytes matured, the peak of the P-J curve shifted to the left, from 7.0 to 6.0 μ. In addition, the extremely large-sized cells with a diameter more than 8.0 μ disappeared during the first 4 hours and cells those diameter was 7.5 μ disappeared 36 hours after reticulocyte introduction (Fig. 4). This data is consistent with the wet cell measurements reported in the previous paper in which the peak of the P-J curve of reticulocytes moved from 7.5 to 6.5 μ in the fiirst 4 to 12 hours with the disappearance of reticulocytes larger than 8.5 μ in diameter (4). The peak of the P-J curve shifted from 6.5 to 5.5 μ on the third day, and thereafter the peak was fixed at 5.5 μ until the labeled cells completely disappeared from circulating blood. The number of relatively large-sized cells (7.0 μ in diameter) decreased on the third day and disappeared completely 3 weeks after being introduced. P-J curve on the 8th week showed nearly the same pattern as normal red blood cells from healthy controls (Fig. 4).

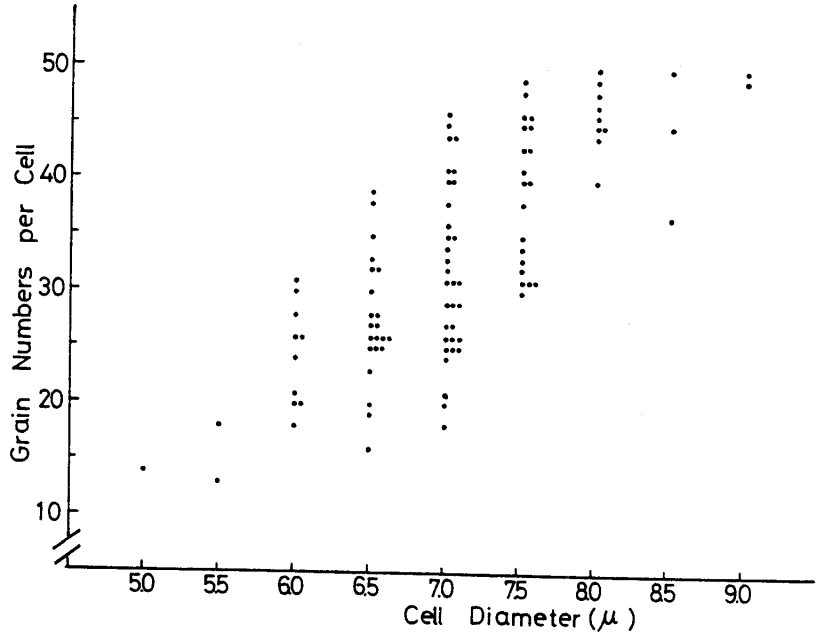

Fig. 3 Relationship between grain counts of labeled red cells and cell diameter immediately after intravenous introduction of labeled reticulocytes.

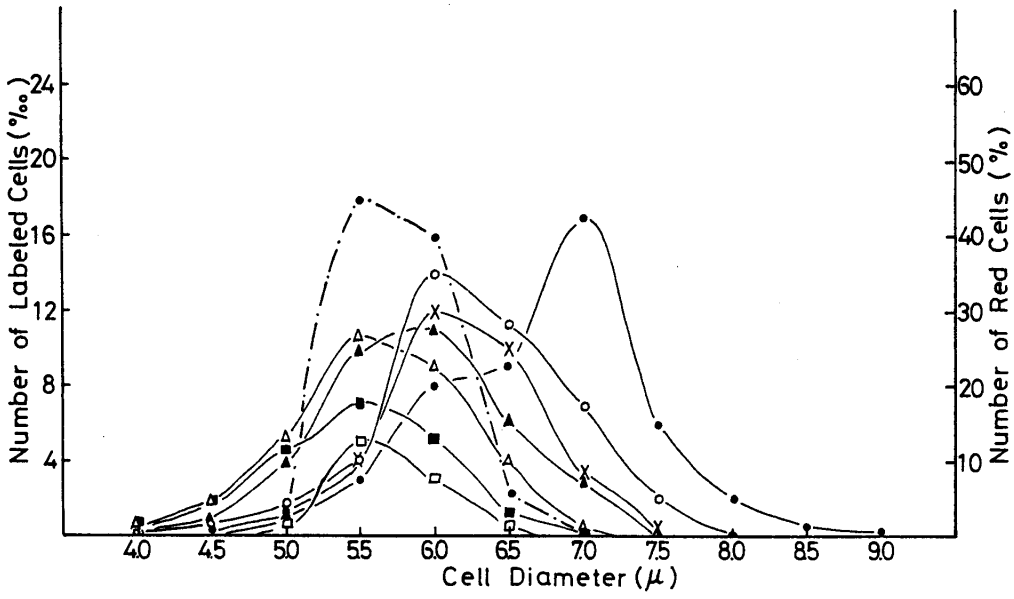

Fig. 4 Relative changes of Price-Jones curves of labeled cells observed at varying time intervals after the intravenous introduction of labeled reticulocytes.
Dark circles with solid line, immediately after introduction; open circles with solid line, 4 hours after introduction; crosses with solid line, 24 hours after introduction; dark triangles with solid line, 36 hours after introduction; open triangles with solid line, 3 days after introduction; dark squares with solid line, 3 weeks after introduction; open squares with solid line, 8 weeks after introduction; dark circles with broken line, P-J curve of mature red cells.

DISCUSSION

The data presented here showed that macroreticulocytes larger than 8.0 μ disappeared from circulating blood during the first 4 hours after being introduced into the vein. The data is consistent with the previous report in which similar changes were observed in wet cells larger than 8.5 μ (4). In contrast, the labeling indices remained unchanged during this period (about 45‰ in the present observation). It is, therefore, unlikely that the rapid disappearance of large-sized reticulocytes from circulating blood resulted from their disintegration at the early stage or by division into small-sized cells. On the other hand, heavily labeled cells having more than 45 grains disappeared completely from circulating blood by 4 hours. In addition, the peak of the P-J curve of labeled cells moved from 7.0 to 6.5 μ during the first 4 hours after cell introduction. The data suggest that the large-sized and heavily labeled reticulocytes decrease in size to some extent by surface fragmentation early in circulating blood and mature to red cells, although they may still be macrocytic. These data are consistent with recent report by Come *et al.* (9).

They observed a markedly disproportionate loss of membrane against hemoglobin in stress reticulocytes introduced intravenously after labeling the membrane phosphatidylethanolamine with ^{32}P and the hemoglobin with ^{14}C during the first 24 hours. They suggested that stress reticulocytes go through a process of surface remodelling durling maturation. In the present observation, however, macrocytes showed decreased labeling and disappeared 3 days after being introduced into the vein, suggesting that macrocytes formed by maturation of macroreticulocytes were short-lived compared to normal-sized red cells whose life-span is calculated to be about 9 weeks (Fig. 5).

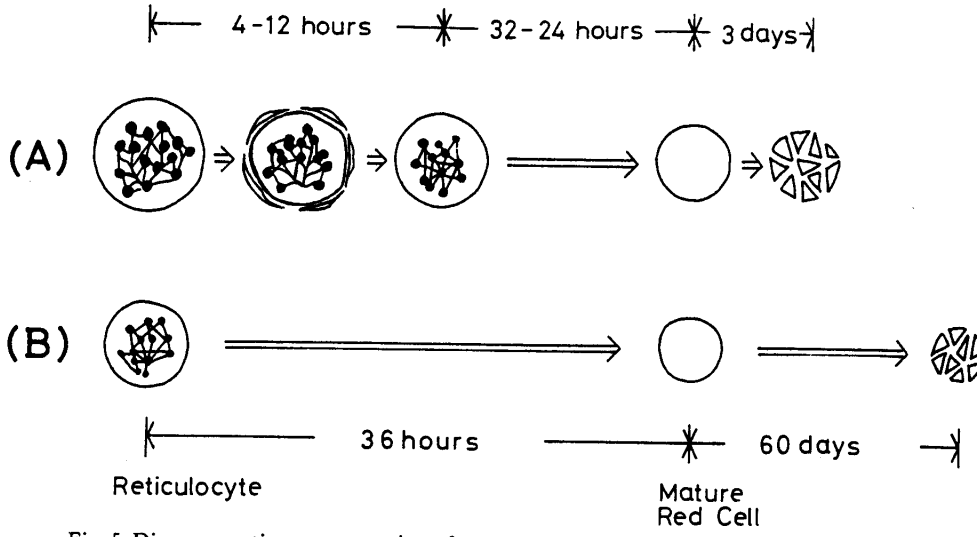

Fig. 5 Diagrammatic representation of maturation of macroreticulocyte (A) and normal-sized reticulocyte (B) and their life-spans.

It is not clear why these large-sized reticulocytes diminished in size by surface fragmentation and are destined to be destroyed within a short period of about 3 days. It is known that reticulocytes are more adhesive than mature red cells and circulate at a much slower rate than adult red cells (10). The stress reticulocytes are excessively large sized (1). These characteristics make them more susceptible to physical damage as they pass through capillary space, e. g., in the spleen where macrophages may shed off the red cell surface. The loss of membrane label being minimized in splenectomized animals (9) suggests the action of macrophages on surface fragmentation of red cells. Koyama and his collaborators (11) presented an electronmicrophotograph of the pitting function of spleen macrophage that removed Heinz bodies from the red cells of phenylhydrazine anemia. This occurs when red

cells pass through the narrow arterial capillary and are trapped there by the stromata on sinus wall and areas having Heinz bodies are selectively engulfed by macrophages. Macroreticulocytes may be trapped easier than normal red cells and these macroreticulocytes may disintegrate easier than normal red cells due to damage while passing through the narrow capillary lumen. The macrocytes may be easily destroyed because of their discoidal shape compared to reticulocytes which are round and may escape complete destruction by surface fragmentation.

The life-span of rabbit erythrocyte has been reported to be 50 to 70 days by many investigators using different methods (5, 12, 13, 14). The present data also shows the life-span to be about 60 days.

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