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Acta Medica Okayama

Volume 29, *Issue* 4 1975 *Article* 4

AUGUST 1975

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Abstract

Investigations were conducted on the life-span of "stress" reticulocytes and the fate of the early denucleated large-sized reticulocytes in circulating blood. Reticulocyte disappearance was examined after reticulocyte introduction into the vein and into the peritoneal cavity of polycythemic and normocythemic animals. The results indicated that these introduced reticulocytes matured to red cells by about 36 hours after injection under both the polycythemic and normocytehmic conditions. The large-sized reticulocytes disappeared by about 4 to 12 hours after introduction. The maturation of reticulocytes was largely arrested when the cells were introduced into the peritoneal cavity.

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Acta Med. Okayama 29, 273—282 (1975)

THE MATURATION OF RETICULOCYTES I. FOLLOWING INTRODUCTION OF RETICULOCYTES INTO POLYCYTHEMIC AND NORMOCYTHEMIC ANIMALS

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Received for publication, March 5, 1975

Abstract: Investigations were conducted on the life-span of "stress" reticulocytes and the fate of the early denucleated large-sized reticulocytes in circulating blood. Reticulocyte disappearance was examined after reticulocyte introduction into the vein and into the peritoneal cavity of polycythemic and normocythemic animals. The results indicated that these introduced reticulocytes matured to red cells by about 36 hours after injection under both the polycythemic and normocythemic conditions. The large-sized reticulocytes disappeared by about 4 to 12 hours after introduction. The maturation of reticulocytes was largely arrested when the cells were introduced into the peritoneal cavity.

The maturation time of reticulocytes to red corpuscles has been reported to be from 24 to 29 hours in the peripheral blood of man (1). A few reticulocytes may be seen in oxalated blood kept *in vitro* as long as 24 hours (2). Seno and his collaborators (3) reported that reticulocytes from anemic rabbits matured to red corpuscles by about 24 hours *in vitro* and under the oxygenated condition, the maturation time was considerably shortened. This suggests the reticulocyte life-span may be different under differing environmental conditions. The present author examined the life-span of reticulocytes following their introduction into the circulating blood and peritoneal cavity of polycythemic and normocythemic mice and rabbits.

One problem is the reticulocyte itself. Large-sized reticulocytes denucleated at the early differentiation stage, and the macrocytic mature red cells were reported to have a short life-span and destined to disintegrated earlier than normal reticulocytes (3, 4, 5). But Strickmans *et al.* (6) reported that macroreticulocytes have a long life-span compared to normal-sized reticulocytes. It is thus uncertain whether the macroreticulocytes have a short life-span and if so, whether they disintegrated at the reticulocyte stage or at the mature red cell stage. To examine this problem, the author has morphologically observed the macroreticulocytes at varying time intervals after the

Produced by The Berkeley Electronic Press, 1975

intravenous introduction of reticulocytes to polycythemic and normocythemic animals that were previously treated to stop reticulocyte production.

In this study, the life-span of reticulocyte was found to be about 36 hours in circulating blood in both the polycythemic and normocythemic animals but cell maturation was severely arrested when the reticulocytes were introduced into the peritoneal cavity with macroreticulocytes disappearing from circulating blood by 4 to 12 hours after reticulocyte introduction.

MATERIALS AND METHODS

Preparation of reticulocytes. Reticulocytes used were obtained from inbred adult ddN mice of both sexes weighing about 20 g each and from female white rabbits weighing about 2 kg. Anemia was induced in the animals by phenylhydrazine injectons or blood depletion. In the former case, animals were treated with a daily subcutaneous injection of 2.5% neutralized phenylhydrazine (0.25 ml/kg for mice and 0.7 ml/kg for rabbits) for three consecutive days. Three days after the last injection (when the reticulocyte number in the peripheral blood reached about 70% of whole red blood cells) blood was drawn from heart (mice) or ear vein (rabbits) with a heparinized syringe. Red blood cells rich in reticulocytes were sedimented by centrifugation, washed with saline by repeated centrifugation, and suspended in an equal volume of saline. In the second method of inducing anemia, blood was withdrawn daily from the retrorbital sinus in mice (0.3 ml) and from an ear vein in rabbits (30 ml) for four consecutive days. Two days after the last blood withdrawal (when the reticulocyte number in peripheral blood reached about 40% of whole red blood cells) blood was collected and red cell suspensions were prepared as described earlier. The red cell suspensions obtained from two animals with the same type of anemia were mixed and introduced into one recipient animal.

Conditioning of recipient animals. To examine the maturation time of reticulocytes *in vivo*, the recipient animals were treated to stop the production of reticulocytes by red cell transfusion or by repeated injections of actinomycin D (AMD). Blood transfusion was carried out by injecting homologous, packed red cells (0.5 ml in mouse and 30 ml in rabbit daily) for four consecutive days for a total volume of 2 ml for each mouse and 120 ml for each rabbit. Two days after the last transfusion of red cells, reticulocytes disappeared completely from circulating blood whose mean hematocrit value was about 65% in mice and 50% in rabbits. AMD was administered by subcutaneous injection (0.002% solution in mice and 0.025% solution in rabbits, 120 μg/kg to mice and 100μg/kg to rabbits) daily for three consecutive days. Two days after the last injection, reticulocytes disappeared from the circulating blood.

Observation of reticulocyte maturation. Following reticulocyte introduction into reticulocyte depleted animals, the reticulocytes in circulating blood were counted by supravital staining method with Nile-Blue at varying time intervals. Counts were taken in each sample twice and the mean value was obtained. The cell diameters of reticulocytes and normal mature red cells were

recorded in wet samples. Price-Jones curves were drawn on 100 to 500 cells. The maturation of reticulocytes was investigated according to Heilmeyer's classification (7).

RESULTS

After intravenous transfusion of reticulocytes to reticulocyte depleted animals, the reticulocytes appeared initially in circulating blood as expected and their numbers diminished hourly and disappeared completely by 32 to 36 hours after introduction. The reticulocyte number decreased rapidly during the first two hours after introduction. Such a decrease in the circulating blood was observed in reticulocytes collected from both phenylhydrazine anemia and blood depletion anemia animals and in both polycythemic and normocythemic condition of both animal species (Fig. 1 to Fig. 4).

After reticulocyte introduction into the peritoneal cavity, the reticulocyte number in circulating blood increased gradually and reached a maximum value 16 hours after introduction and then decreased, disappearing 52 hours after introduction. The time required for reticulocyte maturation in circulating blood was about 36 hours calculated from the time of maximal value.

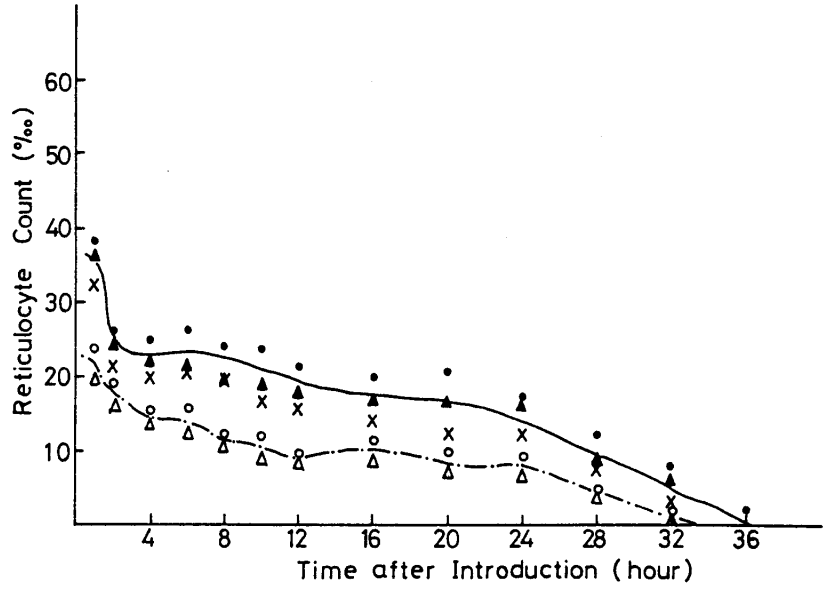

Fig. 1. Mouse reticulocyte percentages after introducing reticulocytes into circulating blood of polycythemic mice.
Dark circles, triangles and crosses with solid line are reticulocyte values of phenylhydrazine anemia mice; circles and triangles with broken line are reticulocyte values of blood depleted anemia mice.

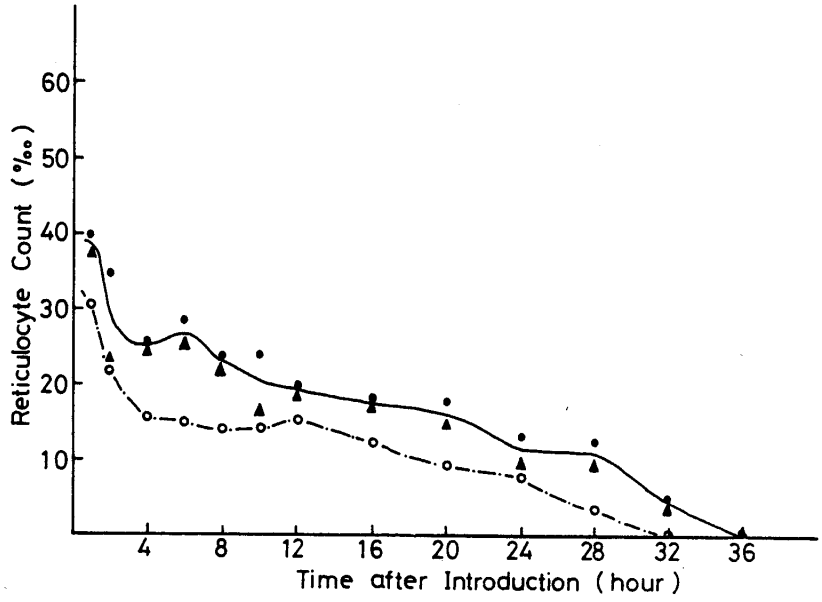

Fig. 2. Mouse reticulocyte percentages after introducing reticulocytes into circulating blood of normocythemic mice.
The graphic symbols are the same as those shown in Fig. 1.

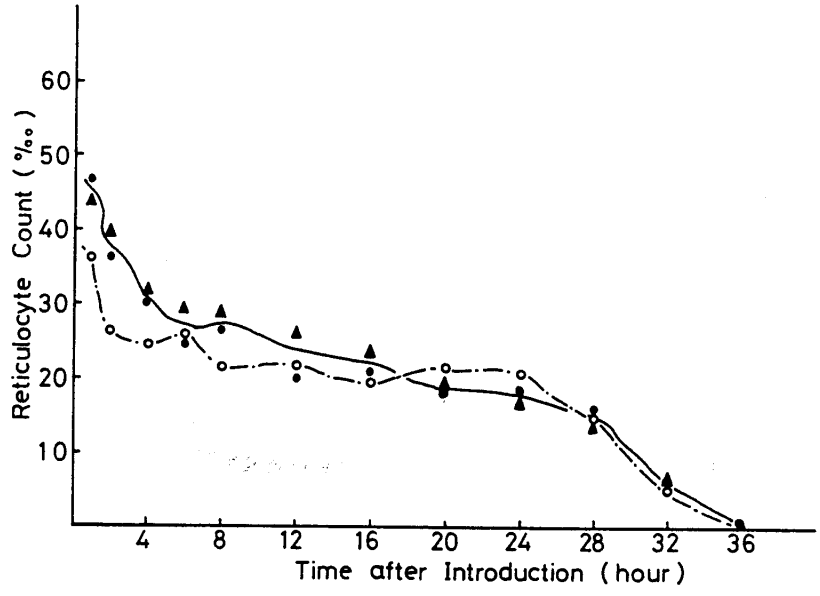

Fig. 3. Rabbit reticulocyte percentges after introducing reticulocytes into circulating blood of polycythemic rabbits.
The graphic symbols are the same as those shown in Fig. 1.

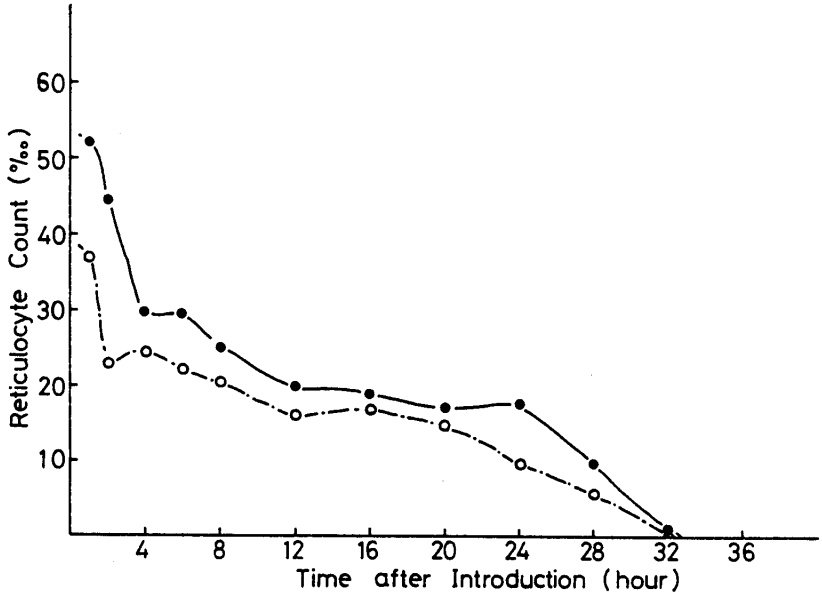

Fig. 4. Rabbit reticulocyte percentages after introducing reticulocytes into circulating blood of normocythemic rabbits.
The graphic symbols are the same as those shown in Fig. 1

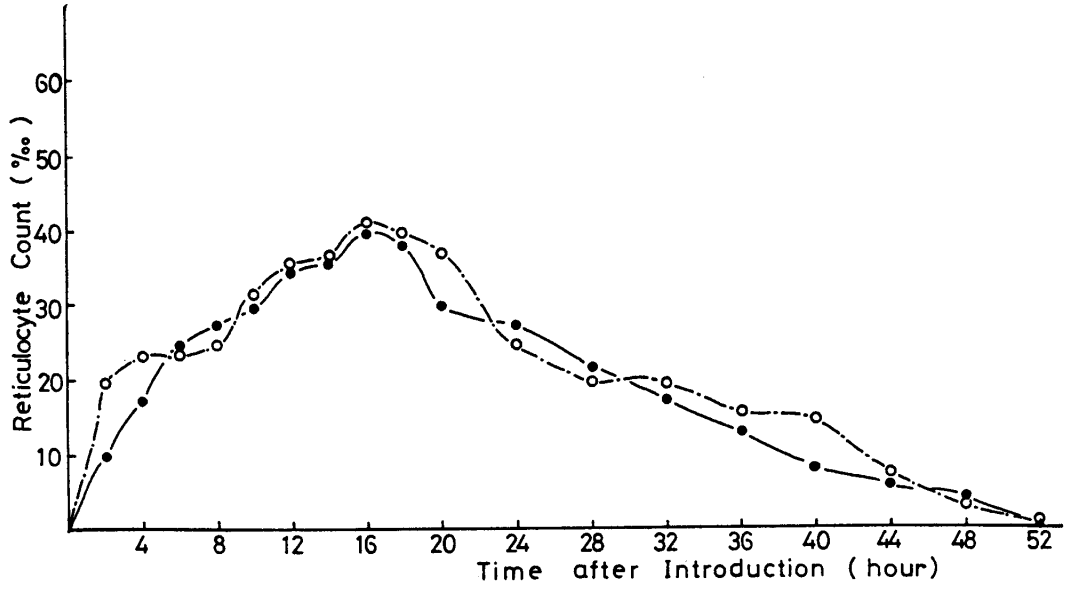

Fig. 5. Reticulocyte (phenylhydrazine anemia) percentages after introducing reticulocytes into the peritoneal cavity of polycythemic and normocythemic mice.
Dark circles with solid line, polycythemic mice; open circles with broken line, normocythemic mice

The same results were present in both polycythemic and normocythemic mice (Fig. 5).

Price-Jones curves showed high peaks as follows: normal mature red blood cells, 6.0 to 6.5 μ; normal reticulocytes, 7.0 μ; and stress reticulocytes, 7.5 μ (Fig. 6). After the introduction of stress reticulocytes into the circulating blood of polycythemic animals having no reticulocytes, the reticulocyte peak on the P-J curve appeared at 7.5 μ soon after cell introduction; at 4 to 12 hours, the peak shifted to 6.5 μ; at 24 hours, the 6.5 μ diameter cells diminished markedly but some 7.5 μ cells remained; and at 36 hours, 7.5 μ diameter cells disappeared leaving a small number of 6.5 μ cells (Fig. 7). The results indicated that large-sized reticulocytes of 8.5 μ to 9.5 μ disappeared from the circulating blood 4 to 12 hours after the introduction of reticulocytes.

The observation on cell size and cell maturity according to Heilmeyer's classification showed that large-sized reticulocytes belong to the younger group (Fig. 8). After the introduction of reticulocytes into circulating blood, the cells disappeared in the sequence from immature to mature cells (Fig. 9).

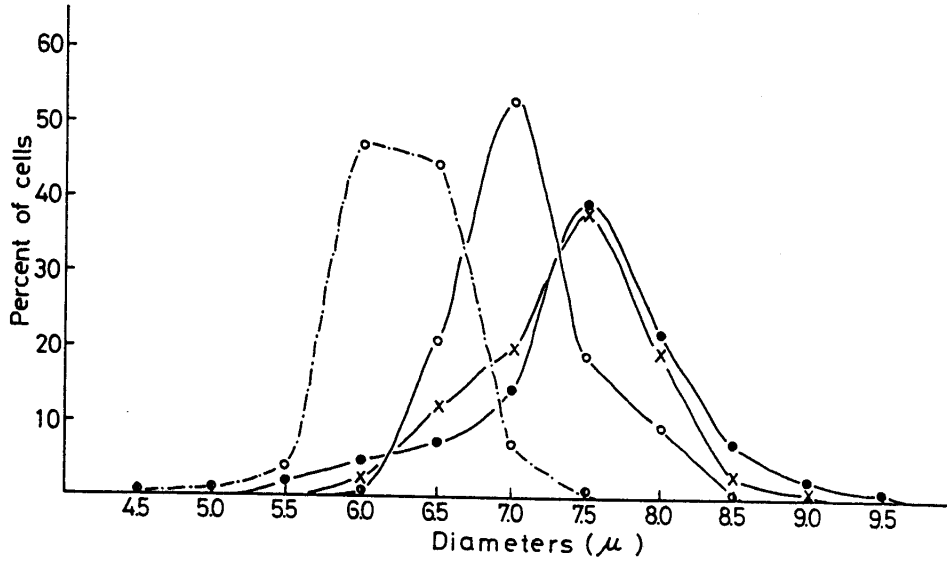

Fig. 6. Price-Jones curves of mature red cells and reticulocytes of normal and of anemic rabbits.
Open circles with broken line, mature red cells of a normal rabbit; open circles with solid line, reticulocytes of a normal rabbit; dark circles with solid line, reticulocytes of an anemic rabbit (phenylhydrazine anemia); crosses with solid line, reticulocytes of an anemic rabbit (blood depletion anemia).

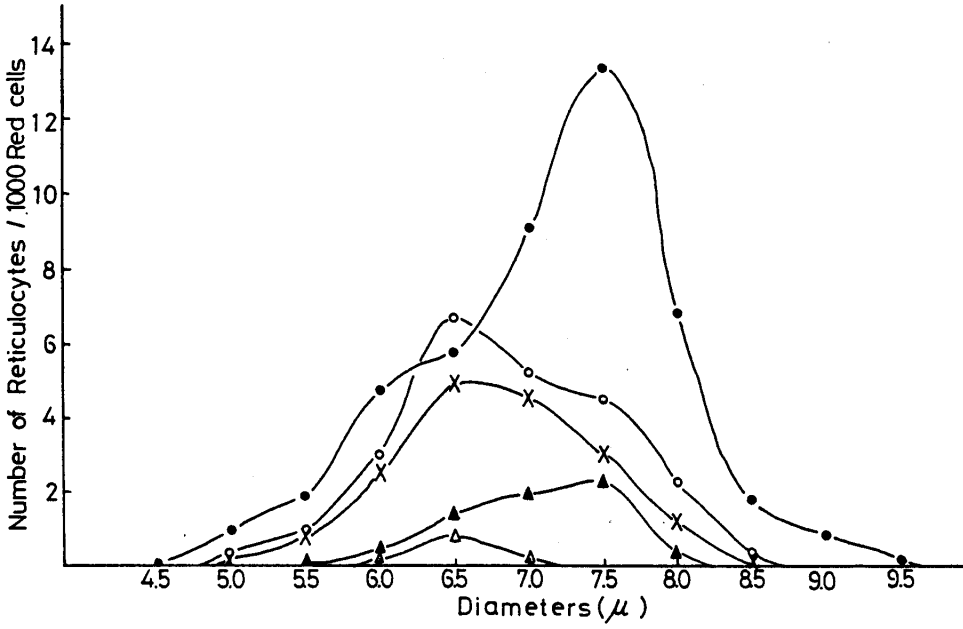

Fig. 7. Price-Jones curves of stress reticulocytes (phenylhydrazine anemia) after reticulocyte introduction into the circulating blood of polycythemic rabbits.
Dark circles, soon after introduction; open circles, 4 hours after introduction; crosses, 12 hours after introduction; dark triangles, 24 hours after introduction; open triangles, 36 hours after introduction.

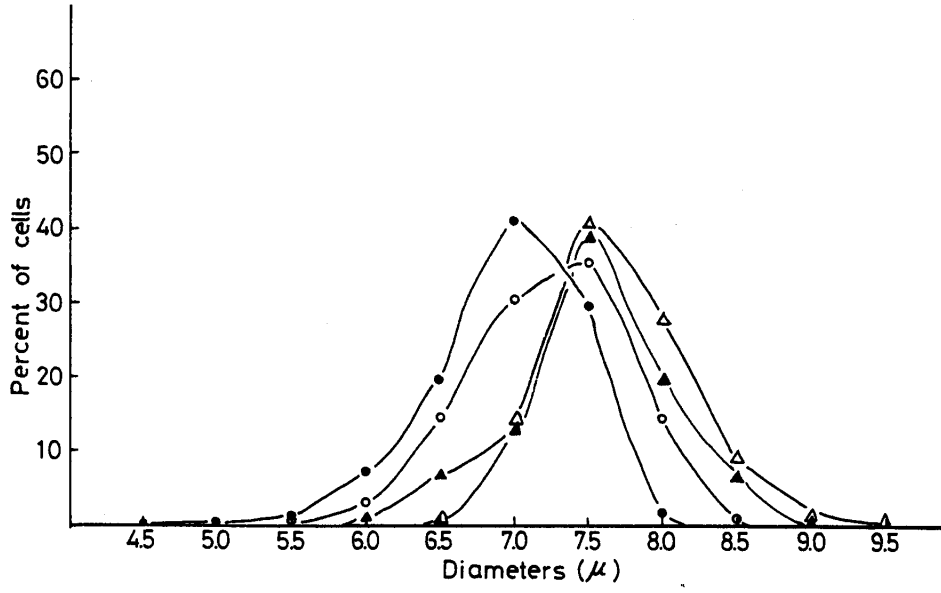

Fig. 8. Price-Jones curves of reticulocytes using the Heilmeyer classification.
Open triangles, Group I; dark triangles, Group II; open circles, Group III; dark circles, Group IV.

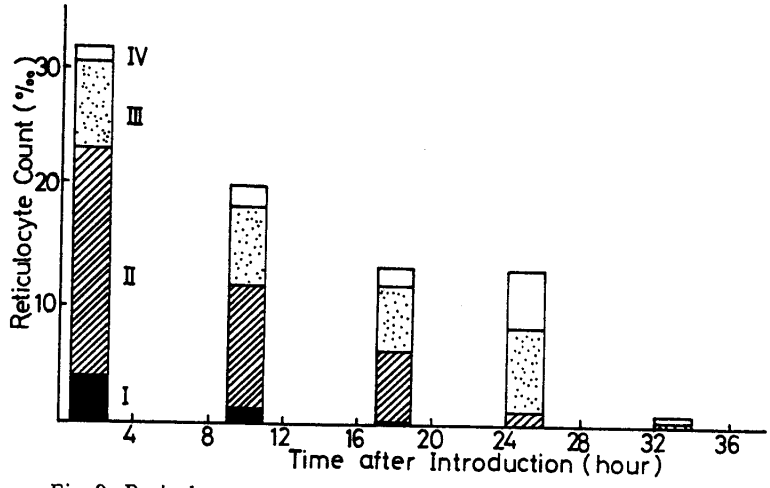

Fig. 9. Reticulocyte counts at various time periods using the Heilmeyer classification.
Dark area, Group I; hatched area, Group II; dotted area, Group III; open area, Group IV.

DISCUSSION

Mice reticulocytes introduced into the circulating blood of a mouse of the same strain disappeared from circulating blood by 36 hours. The time of disappearance was nearly the same in "stress" reticulocytes from animals of both the phenylhydrazine anemia and blood depletion anemia. Reticulocytes introduced into polycythemic or normocythemic animals took nearly the same time (32 to 36 hours) for complete disappearance. Similar experiments repeated in rabbits produced the same results. A study with labeled reticulocytes showed that reticulocyte disappearance from circulating blood was due to cell maturation (8). This means that reticulocytes obtained by different methods have the same maturation characteristics and the maturation time seems to be constant irrespective of the polycythemic or normocythemic condition. Furthermore, the author tried to examine reticulocyte maturation time under anemia, but failed in arresting reticulocyte production in recipient anemic animals. Reticulocytes introduced into the peritoneal cavity showed a severe arrest in maturation. The cells took 52 hours to disappear from circulating blood compared to 36 hours when reticulocytes were introduced directly into circulating blood. Soon after introduction into the peritoneal cavity, reticulocytes appeared in circulating blood and increased with time reaching a maximum level 16 to 18 hours after reticulocyte introduction. Later their numbers decreased gradually and disappeared completely by 36 hours. Thus, it seems that maturation of reticulocytes was arrested in the

peritoneal cavity and the cells matured as usual after being discharged into circulating blood. The result is consistent with the report by Seno *et al.* (3) who observed a considerable delay in the maturation of reticulocytes *in vitro* in the deoxygenated environment. That is, the arrest in reticulocyte maturation in the peritoneal cavity may be due to a low oxygen tension compared to circulating blood. Thus, the reticulocyte behaviour under phenylhydrazine anemia was the same as that under blood depletion. Reticulocytes from both anemias showed the same maturation time and the same maturation arrest in the peritoneal cavity.

Observations on reticulocyte size revealed that macroreticulocytes were immature and disappeared early from circulating blood. The reticulocytes may disintegrate early or divide into smaller cells or become small-sized reticulocytes by surface fragmentation. The hypothesis of reticulocyte division proposed by Weicker in 1953 (9) is unlikely (10). Many investigators have suggested the possibility of an early disintegration of large-sized cells (3, 4, 5, 11, 12), but it is uncertain whether these cells disintegrate while in the reticulocyte stage. A likely explanation is that these macroreticulocytes are reduced to smaller cells by surface fragmentation (13, 14). Further experimental data on the latter possibility will be reported in a subsequent paper (8).

Acknowledgment: The author expresses her thanks to Prof. S. Seno and Dr. S. Okada for their valuable discussion and suggestions on this study.

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