# Symposium II. Haematopoietic Organs

(Chairman) G. Wakisaka, Univ. of Kyoto

## Erythroblast Kinetics in Bone Marrow

K. NAGAI

*First Department of Internal Medicine, School of Medicine, University of Osaka, Osaka*

Though there are increasing number of ferrokinetic studies on the dynamics of erythron, autoradiographic investigation using $^3$H-thymidine are thought to be most suitable to clarify the dynamics of erythroblasts in bone marrow. But, several problems still exist in the setting of erythroblast compartments, therefore, it seems to be unable to determine the erythroblast kinetics.

In this paper, the determination of erythroblast compartments was firstly attempted on the basis of karyometry, autoradiography with $^3$H-thymidine and the characteristics of each erythroblast. The youngest erythroblast population (K 1) begun to increase 10 hours after bleeding possessed nucleus of which diameter was 10.7 $\mu$ in average. The mean nuclear diameter of the second youngest population (K 1/2) was found to be 9.1 $\mu$ which was equal to that of basophilic macro-erythroblasts. As in the initially non-labeled erythroblasts (K 1/8) the nuclear diameter became smaller accompanying cell maturation, the cells of which nuclei appeared in large side of the peak in distribution curve could be regarded as the newly formed K 1/8. The mean nuclear diameter of these cells was calculated to be 6.3 $\mu$.

From the above, erythroblast were divided into four compartments and their mean nuclear diameters were as follows. (Table 1)

Table 1

| | K1($\mu$) | K$^1/_2$($\mu$) | K$^1/_4$($\mu$) | K$^1/_8$($\mu$) |
|---|---|---|---|---|
| rabbit | 10.7 | 9.1 | 7.7 | 6.3 |
| human | 11.3 | 9.6 | 8.2 | 7.0 |
| rat | 10.6 | 8.6 | 6.9 | 5.6 |

Secondarily, the dynamics of marrow erythroblast was studied under erythroid hyperplastic condition. Our previous report has indicated that reticulocytes are formed when DNA synthesis precedes heme synthesis in the erythroblastic series. On the basis of relative compartment size, our previous results were thought to be corresponded to the increase in K 1 and K 1/2.

Subsequently, changes in time parameters calculated by applying autoradiography in normal and bled rabbits were as follows. (Table 2)

Finally, total erythroblast count in bone marrow was calibrated following Donohue's method.

From these results, the changing rate in the absolute number of each compartment corrected with generation time was illustrated

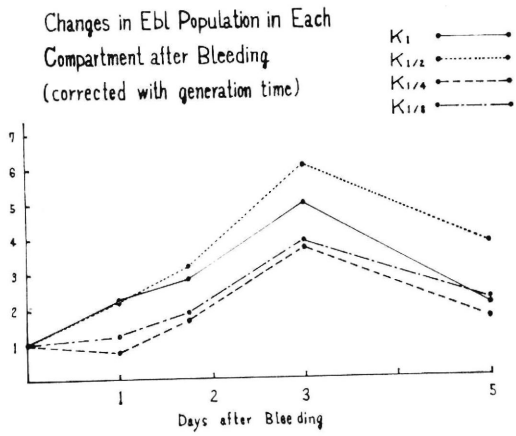


Fig. 1

in Fig. 1. It was also shown that the early denucleation of K 1/2 plays an important role in reticulocyte crisis appearing in the enhanced hematopoiesis.

Table 2

| hours after bleeding | S (hrs.) | GT (hrs.) | | | | total GOT (hrs.) |
|---|---|---|---|---|---|---|
| | | K1 | $K^{1}/_{2}$ | $K^{1}/_{4}$ | $K^{1}/_{8}$* | |
| 24 | 6.8 | 7.2 | 7.3 | 10.7 | 7.5 | 35 |
| 48 | 7.0 | 7.8 | 7.7 | 8.5 | 6.3 | 33 |
| 72 | 7.9 | 8.5 | 8.5 | 9.8 | 8.2 | 37 |
| 120 | 9.1 | 9.8 | 9.8 | 12.0 | 9.3 | 43 |
| normal | 7.9 | 8.9 | 9.5 | 9.5 | 10.0 | 40 |

* represented with compartment transit time.

## Kinetics of Blood and Bone Marrow Cells Studied with Tritium Autoradiography

T. TAKINO and M. TANIGUCHI
*Third Department of Internal Medicine, Kyoto Prefectural University of Medicine, Kyoto*

In normal and blood disorders, the metabolism of DNA, RNA and protein in bone marrow cells as well as peripheral blood was studied by the technique of autoradiography with $^{3}$H-thymidine ($^{3}$H-tdr), $^{3}$H-uridine and $^{3}$H-leucine.

Incorporation of $^{3}$H-tdr revealed that the flash labeling seemed to be established within 30 minutes after $^{3}$H-tdr incubation.

Labeling index of $^{3}$H-uridine was 100% in all nucleated cells in myeloid and erythroid series. The grain count of $^{3}$H-uridine in younger cells increased gradually in time course of incubation.

Incorporation of $^{3}$H-leucine was observed in all nucleated bone marrow cells as well as reticulocytes. Marked protein synthesis was recognized in immature cells. The studies on the ratio of RNA synthesis to protein synthesis in each maturation stages of bone marrow cells revealed greater activity of protein synthesis in mature cells.

Labeling index of $^{3}$H-tdr in vitro was 47~77 % of myeloblast of normal subjects, 1.2~14.2 % of AML, 11~27% of CML, 3.6% of ALL and 0.4~12% of myeloma cells. Decreased proliferative potential was proved in the malignant cells of those blood disorders. RNA synthetic activity indicated the marked reduction in blast cells of AML and ALL, slight reduction in blast cells of CML. On the other hand, RNA synthesis of myeloma cells was almost the same as the level of normal myeloblasts. Protein synthesis was depressed remarrkably in the cells of AML, CML and ALL, but did not decrease in myeloma cells. In general, labeling index of peripheral blast cells was lower than that of bone marrow blasts in leukemia. This finding seemed to support that the leukemic cells were not consisted of homogeneous cell population.

In AML in complete remission, the labeling index of leukemic myeloblasts exhibited nearly normal value of normal myeloblasts after treatment. On the contrary, no improvement of labeling index of $^{3}$H-tdr was observed in all CML investigated.

Generative cycle of normal PHA stimulated lymphocyte and blast cell in CML was investigated by the labeled mitosis curve. In normal PHA stimulated lymphocyte, $G_2$ was 3 hours, S was 10 hours, M+$G_1$ was 6 hours, tG was 19 hours ranging from 18 to 26 hours. In CML, $G_2$ was 6 hours, S was 17 hours. From the result, 2.7~6.4 days of doubling time was estimated in CML cells. Prolongation of the DNA synthesis period and the generation time in leukemic cells was confirmed.