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Kinetics of Erythroid Cell Precursors in the Newborn Rat[1] (39494)

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The cell-cycle time of erythroid cells in normal animals has been estimated by a number of investigators (1-3) but there have been few studies of the cell-cycle time of the recognizable erythroid precursors in neonatal rats.

In the newborn rat during the first week of life there is a striking increase of the erythropoiesis in the bone marrow that shows at this time significant numbers of undifferentiated cells similar to those seen in the liver during the hepatic phase of erythropoiesis. These cells have a mean diameter of about 30-40 μm, a pale basophilic cytoplasma, a leptochromatic nucleus with large nucleoli, and frequently occur in syncytia.

As the animal matures, these undifferentiated cells gradually decline in frequency and there is also a gradual change in the physiologic response of the bone marrow to various red cell perturbations, known to effectively abolish erythropoiesis in the adult rat, such as bilateral nephrectomy, hypertransfusion induced plethora, starvation, and posthypoxic polycythemia (4).

It has been reported that CFU's derived from fetal liver promote an earlier recovery of splenic erythropoiesis in lethally irradiated mice than a comparable number of adult marrow CFU's, suggesting a difference in the generation time of fetal liver erythroid precursor cells and, by inference, of bone marrow erythroid precursors during he first 2 weeks of life, when erythropoiesis s proceeding at the fastest rate (5).

In the work to be reported in this paper, he cell-cycle time of the recognizable erythoid precursors in 5-, 10-, and 20-day-old nd adult rats was measured by the technique of labeled mitoses.

Materials and Methods. Newborn rats of the Sprague-Dawley strain, aged 5, 10, and 20 days, and 10- to 14-week-old female rats of the same strain weighing 180-200 g were given a single intraperitoneal injection of [methyl-^{3}H] thymidine (^{3}H-Tdr) (1.0 μCi per g of body weight, sp act 10 Ci/mmole).

One to three rats were sacrificed from each age group at intervals thereafter. Three to seven animals were used for each experimental point for a total of 235 newborn and 48 adult rats in three separate studies.

The smears were prepared from bone marrow, air-dried, and fixed in methyl alcohol for 3 min.

Autoradiographs were prepared by the dipping technique, using Kodak Nuclear Track Emulsion, type NTB 3, diluted 1:1 with distilled water.

After a suitable exposure time (21 days), the slides were developed with Kodak D19 developer at 19°C for 2 min, rinsed in 0.5% acetic acid, stop bath, fixed for 2 min in Kodak's Unifix, rinsed for 2 hr in running water, and then soaked for 1 hr in a buffer solution at pH 6.3.

The slides were stained with Wright's and Giemsa solutions.

The percentage of erythroid mitoses was determined by counting at least 100 mitoses on each preparation.

A mitosis was considered as labeled if more than three grains could be attributed to the chromosomes.

Approximate values for the cell-cycle parameters were obtained from the labeled mitoses curve (FLM) by a graphic method (6).

The experimental data were analyzed using the method of Steel and Hanes (7) for computer simulation of FLM curves.

Results. The cell-cycle is typically subdivided into the following phases: M, mitotic phase; S, DNA synthetic phase; G_1, postmitotic presynthetic phase; and G_2, postsynthetic premitotic phase. The method of la-

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beled mitoses is one of the most important methods for studying and estimating the intermitotic intervals and the length and position of the S period. It consists of pulse-labeling the cells with tritiated thymidine and observing the proportion of mitoses which are labeled (FLM) at a series of subsequent times after injection of the label (6).

The FLM method is based on the assumption that all cells in the S phase at the time of the injection of the tracer will be labeled, and that no others will take up label.

A theoretical curve for a synchronous population in which all cells have no variation of the time spent in the different parts of the cycle would have a rectangular shape, and the duration of the nuclear cycle and its phases could be estimated from such data.

However, it is clear from the available experimental data obtained by the technique of the FLM that all cell populations have a considerable variability in the duration of the phases of the cycle, therefore, the curves show a greater or lesser degree of damping, in which case, approximate estimates for the mean values of the cell-cycle parameters can be obtained.

Figures 1 and 2 show the FLM curves for pro- and basophilic erythroblasts in 5-, 10-, and 20-day-old and adult rats.

The points represent the mean of three to seven experimental points and the solid lines are the curves calculated by the computer.

The usual method of estimating the replication time from a FLM curve is to measure the interval between the first and the second ascending limb of the curve (6).

Inspection of Figs. 1 and 2 reveals that

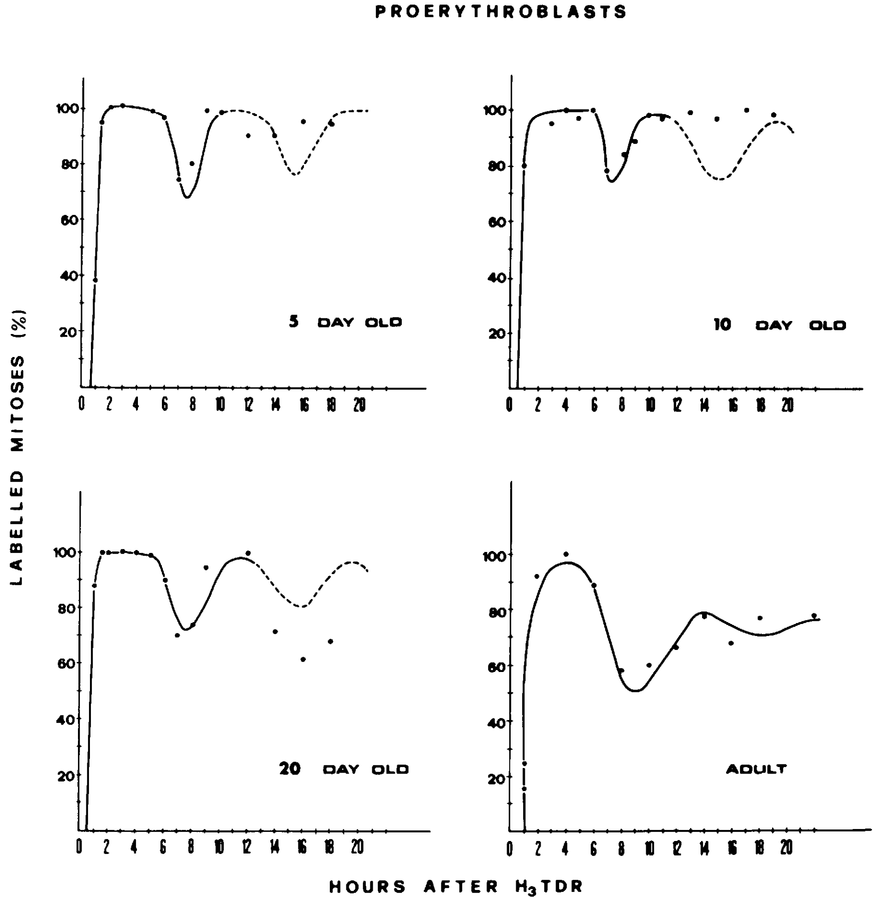

FIG. 1. Labeled mitoses curves for proerythroblasts in 5-, 10-, and 20-day-old and adult rats, at various times after injection of ^{3}H-thymidine. Each point represents the mean of three to seven animals. The curves drawn were calculated by computer analysis of the data.

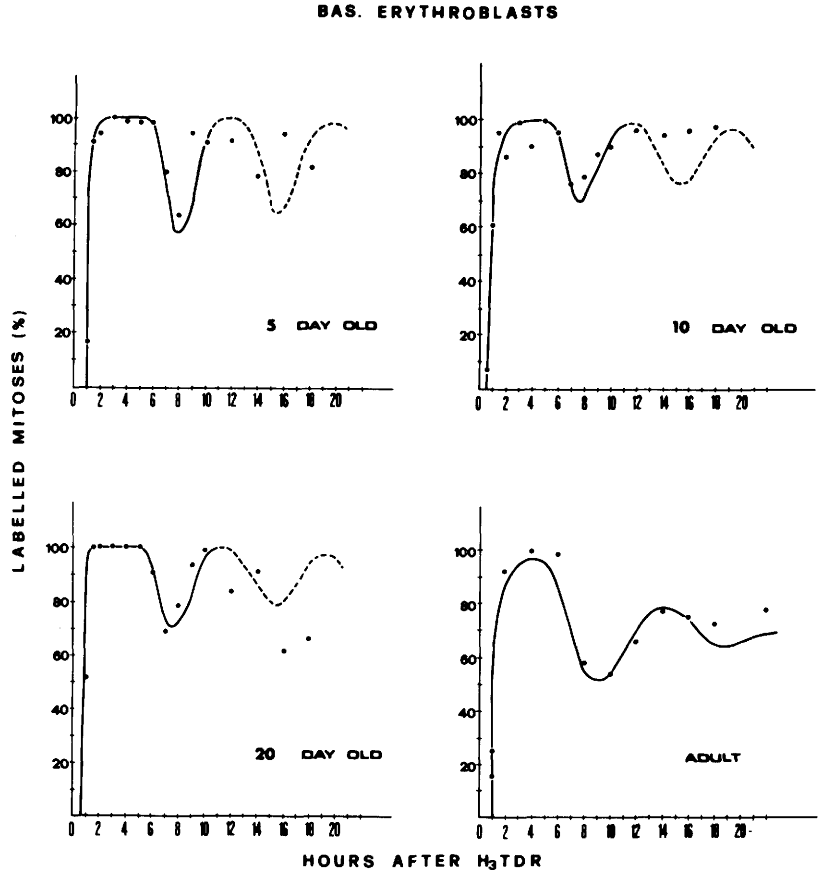

FIG. 2. Labeled mitoses curves for basophilic erythroblasts in 5-, 10-, and 20-day-old and adult rats, at various times after injection of ^{3}H-thymidine. Each point represents the mean of three to seven animals. The curves drawn were calculated by computer analysis of the data.

the mean generation time in the adult rat is about 10 hr, and in the neonatal rats the cycle time is about 7.5 hr.

The first labeled mitotic figures appear between 30 and 45 min after tritiated thymidine administration, thus, the premitotic rest period (G_2) is about 0.75 hr. The duration of DNA synthesis is 5.50 hr and this leaves $7.5 - (5.5 + 0.75) = 1.25$ for $G_1 + M$.

Although it is easy to get approximate values for the cell-cycle parameters from the FLM curve, there is no graphical method which has any degree of precision.

A more detailed analysis of the labeled mitoses curves has been obtained using the method of Steel and Hanes (7), a development of the method of Barrett (8) in which theoretical curves are computed on the basis of a model which assumes a log-normal distribution of the duration of G_1, S, and G_2 phases; the analysis consists in finding a labeled mitoses curve which is the best fit to the experimental data. For the purpose of this analysis, the duration of mitosis (M) is divided equally between G_1 and G_2.

The curves fitted to the experimental points in Figs. 1 and 2 are computed curves, obtained by Steel's method.

The computed curves do not fit the experimental data adequately over a whole cell cycle (broken curves), especially for the newborn rats, but the computer was able to generate curves to fit the first wave of labeled mitoses in each case.

The analysis should therefore be restricted to the first cycle, which is sufficient to give reasonable estimates of the cell-cycle parameters; the means, standard deviations, and medians of these parameters are shown in Table I.

The values obtained for the cycle times by

Steel's method are in good agreement with those estimated by simple inspection of the curves.

The durations of both G_1 and G_2 for the earlier erythroblasts of the newborn rats are always shorter than those of the adult, but S is not appreciably different from the adult.

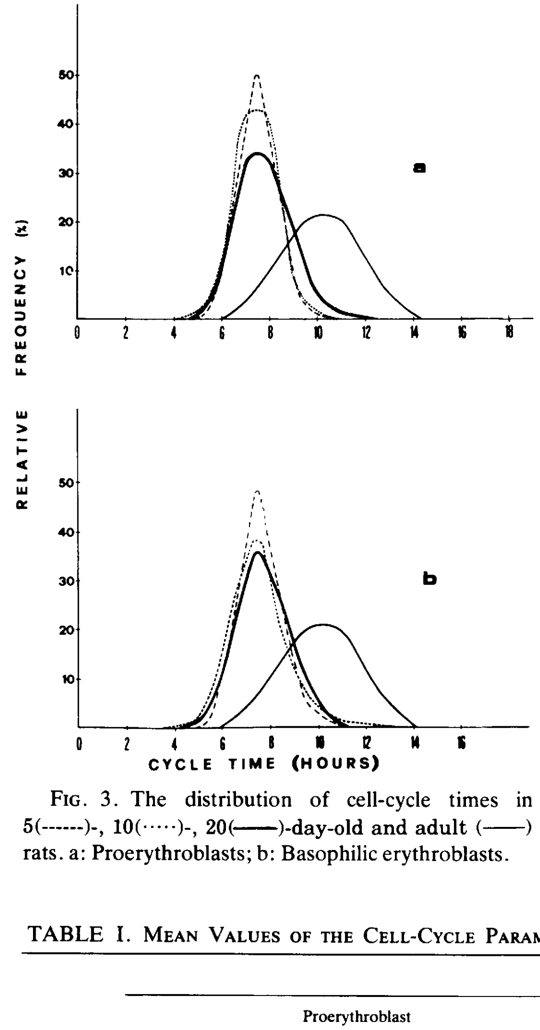

FIG. 3. The distribution of cell-cycle times in 5(------)-, 10(·····)-, 20(——)-day-old and adult (——) rats. a: Proerythroblasts; b: Basophilic erythroblasts.

For the basophilic erythroblasts, the reduction of G_1 and G_2 is associated with a slight reduction of S (from 7.5 to 6.6-6.9, respectively).

The analysis of the corresponding distributions of cell-cycle times shown in Fig. 3 demonstrates a considerable decrease of spread in the cell-cycle in the newborn rats.

Discussion. The labeling mitoses curves represent the resultants of the time intervals for the replicating cell populations.

In the present study, the FLM curves for the newborn rats show that the descending limb falls only to 60-70% and then rises again to 95-98%.

This may be due to the overlapping of groups of rapidly dividing cells so that the "in phase" pattern is lost, the lengthening of the second cell-cycle as a result of the radiation effect of ^{3}H-Tdr (9), and the entry into erythroid compartment of labeled cells from an actively proliferating precursor compartment.

Reutilization of tritium may also be playing some part because erythropoiesis is very active and more erythroid cells are losing their initially labeled nuclei during maturation, possibly making more DNA available for reutilization (10).

However, had any of the above mentioned events occurred then the FLM curves would have given an overestimate of the generation time.

The estimates obtained by Steel's method show that in the newborn rats considerable

TABLE I. MEAN VALUES OF THE CELL-CYCLE PARAMETERS IN 5-, 10-, AND 20-DAY-OLD AND ADULT RATS[a]

	Cell type							
	Proerythroblast				Basophilic erythroblast			
Rats (days old)	G_1 0.5 M (hr)	S (hr)	G_2 + 0.5 M (hr)	Median t_c (hr)	G_1 + 0.5 M (hr)	S (hr)	G_2 + 0.5 M (hr)	Median t_c (hr)
5	0.6 ± 0.3 (0.5)	6.9 ± 0.7 (6.9)	0.1 ± 0.1 (0.05)	(7.5)	1.0 ± 0.4 (0.9)	6.6 ± 0.6 (6.6)	0.1 ± 0.3 (0.03)	(7.6)
10	0.4 ± 0.6 (0.2)	6.8 ± 0.7 (6.7)	0.2 ± 0.1 (0.07)	(7.3)	0.8 ± 0.6 (0.6)	6.7 ± 0.9 (6.7)	0.1 ± 0.1 (0.09)	(7.5)
20	0.5 ± 0.2 (0.5)	7.1 ± 1.1 (7.0)	0.3 ± 0.1 (0.2)	(7.8)	0.5 ± 0.2 (0.5)	7.0 ± 1.0 (6.9)	0.3 ± 0.2 (0.2)	(7.7)
Adults	1.9 ± 1.4 (1.5)	7.0 ± 1.8 (6.7)	1.2 ± 1.6 (0.7)	(9.8)	1.4 ± 1.5 (1.1)	7.9 ± 2.4 (7.5)	1.8 ± 1.0 (1.6)	(10.7)

[a] Mean values were derived by Steel's method. The median values are in parentheses; for a log-normal distribution, median = $\left[\text{Mean}/\left\{1+\left(\frac{\text{Standard deviation}}{\text{Mean}}\right)^2\right\}\right]^1$.

reductions occur in the cell-cycle time of the recognizable erythroid precursors, and that these reductions would seem mainly due to decreases in the duration of G_1 and G_2 phases.

The results reported here are in fair agreement with those estimated by Roylance (11) in young rats and with those of Hanna (12), who observed distinct shortening of the cell-cycle time of erythroid precursors in response to anemia in bled and phenylhydrazine-treated adult rats.

It has been extensively documented that in the rat, at birth, there is hypocromic anemia (4) and from this one may infer that the changes in the generation time of erythroid elements in the newborn rats are due to overproduction of erythropoietin associated with hypochromic anemia.

However, the erythropoietic activity recovered from the plasma of newborn rats exposed to hypoxia was significantly less than that observed in adults that were similarly exposed, indicating a low rate of erythropoietin synthesis in early neonatal life (13).

A number of differences has been reported between fetal and adult murine CFU's.

Using the thymidine suicide technique, Becker *et al.* (14) found no depression of CFU's in normal adult marrow, but found extensive killing of CFU's with cells from fetal liver. This suggested that few CFU's in adult marrow are in cell-cycle, while a high proportion of CFU's in fetal liver may be in active cell-cycle. Kubanek *et al.* (15) tested the proliferation of adult marrow and fetal liver CFU's, using secondary transplants (16). After transplantation of adult marow, they noted a 3-4 day lag phase followed by an exponential growth of splenic CFU's. In recipients of fetal liver tissue this lag period was significantly diminished and the exponential growth had a faster doubling time, indicating a shorter generation time of fetal liver CFU's.

A shorter doubling time of fetal liver CFU's was also found by Schofield (17).

Since the shift from hepatic to myeloid erythropoiesis begins in rodents in late fetal life, the neonatal rat is in transition from fetal to adult erythropoiesis (4).

From our results it would appear that the generation time of erythroid elements in early neonatal life is shorter than that observed for adult erythroid elements.

Whether the explanation of such a difference resides in a different stem cell, similar to that present in the fetal liver, is moot, but it is tempting to suggest that this is the case.

Summary. The kinetics of erythroid proliferation have been studied in the newborn rat of different ages by the method of ^{3}H-Tdr-labeled mitoses. The experimental results have been analyzed by the method of Steel and Hanes (7).

A generation time of 10 hr has been obtained in the adult rats for the proerythroblasts and basophilic erythroblasts, while in the newborn rat the generation time for the same cells was found to be of the order of 7.5 hr. It has been shown that these reductions are mainly due to decrease in the duration of G_1 and G_2 phases.

The possibility is suggested that the shorter cell-cycle of the erythroid cells could, at least in part, account for the erythroid hyperplasia observed in the rat during the first 2 weeks of life.

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1. Bond, V. P., Odartchenko, N., Cottier, M., Feinendengen, L., and Cronkite, E. P., *in* "Erythropoiesis" (L. O. Jacobson and M. Doyle, eds.) p. 173. Grune and Stratton, New York (1962).
2. Lala, P. K., Patt, H. M., and Maloney, M. A., *Acta haemat.* **35,** 311 (1966).
3. Frindel, E., Tubiana, M., and Vassort, F., *Nature (London)* **213,** 1017 (1967).
4. Lucarelli, G., Porcellini, A., Carnevali, C., Carmena, A., and Stohlman, F., Jr., *Ann. NY Acad. Sci.* **149,** 544 (1968).
5. Kubanek, B., Rencricca, N., Porcellini, A., and Stohlman, F., Jr., *in* "Hemopoietic Cellular Proliferation" (F. Stohlman Jr., ed.), p. 205. Grune and Stratton, New York (1970).
6. Quastler, M., and Sherman, F. G., *Exp. Cell Res.* **17,** 420 (1959).
7. Steel, G. G., and Hanes, S., *Cell Tissue Kinet.* **4,** 93 (1971).
8. Barret, J. C., *J. Nat. Cancer Inst.* **37,** 177 (1966).
9. Post, J., and Hoffman, J., *Exp. Cell Res.* **36,** 111 (1964).
10. Odartchenko, N., Bond, V. P., Feinendengen, L. E., and Cottier, H., *in* "Cell Proliferation" (L. F. Lamerton and R. J. M. Fry, eds.), p. 172. F. A. Davis, Philadelphia (1963).
11. Roylance, P. J., *J. Anat.* (Abstr.) **102,** 137 (1967).

12. Hanna, I. R. A., Tarbutt, R. G., and Lamerton, L. F., *Brit. J. Haemat.* **16,** 381 (1969).
13. Carmena, A. O., Howard, D., and Stohlman, F., Jr., *Blood* **32,** 376 (1968).
14. Becker, A. J., McCulloch, E. A., Siminovitch, L., and Till, J. E., *Blood* **26,** 296 (1965).
15. Kubanek, B., Rencricca, N., Porcellini, A., Howard, D., and Stohlman, F., Jr., *Blood* **35,** 64 (1970).
16. Siminovitch, L., Till, J. E., and McCulloch, E. A., *J. Cell. Physiol.* **62,** 327 (1963).
17. Schofield, R., *Cell Tissue Kinet.* **3,** 119 (1970).

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