The Generation Time of Marrow Nucleated Erythroid Cells in 6-Day-Old and Adult Rats[1] (34415)

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Available evidence suggests a fundamental difference between the regulation of adult and neonatal erythropoiesis. We proposed a model of adult erythropoiesis in which the most primitive cell is pluripotential and transplantable, *i.e.*, the colony forming unit (CFU) (1). Beyond this is a more differentiated precursor cell which, in the case of the erythroid series, is already committed to erythroid differentiation but requires erythropoietin (EP) to initiate hemoglobin synthesis. The most differentiated marrow compartment contains morphologically recognizable nucleated erythroid cells. The regulation of neonatal erythropoiesis presumably follows the same general model however major differences have been observed within each of the 3 maturation compartments (2, 3). The CFU's derived from fetal liver and neonatal bone marrow promoted an earlier recovery of splenic erythropoiesis in lethally irradiated mice than a comparable number of adult marrow CFU's (2, 3). These results could be attributed to a difference in the generation time (t_G) of the various cellular compartments involved, and they derive further support from studies with tritiated thymidine and hydroxyurea (4, 5). These agents, documented to kill cells in DNA synthesis (S), substantially reduced the surviving fraction of CFU's obtained from fetal liver and neonatal bone marrow. Their effect on adult marrow CFU's was imperceptible. These differences indicate that CFU's derived from fetal and neonatal animals are in active cell cycle with a substantial portion of the cells in DNA S whereas adult CFU's are either in a state of true dormancy (G_0) or have a long t_G with only a small portion of cells in DNA S.

Efforts to assess the intermediate or EP responsive precursor compartment in the intact newborn animal are hampered by the inability to adequately suppress erythropoiesis as well as the rapid growth of the animal which is associated with a continuing change in metabolic requirements and hence in the tissue O_2 supply–demand relationships. Hypertransfusion, starvation, and bilateral nephrectomy which produce red cell aplasia in the adult resulted in only a modest decrease in erythropoiesis in the newborn animal (6). Such differences may relate to extrarenal EP production in the neonate and/or perhaps differences in the relationship of EP effectiveness to the generative cycle of the immediate erythroid precursor cell.

The most differentiated marrow erythroid compartment may be assessed by morphology and kinetics. In the newborn rat for example, red cells are hypochromic and macrocytic whereas in the adult, hypochromia is invariably preceeded by microcytosis (7). Lucarelli *et al.* (8) reported a higher marrow erythroid mitotic index in newborn than adult rats. This observation suggests a difference in the t_G of adult and neonatal differentiated erythroid precursors which in combination with active cell cycle in the pluripotential compartment and the release of hypochromic macrocytes into the peripheral blood would indicate an acceleration of all stages of erythropoiesis. To further evaluate this concept, the t_G of marrow nucleated erythroid precursors was assessed with colchicine in 6-day-old and adult rats. The data reported herein favor comparable generation times.

Materials and Methods. Sprague-Dawley rats injected intraperitoneally with colchicine

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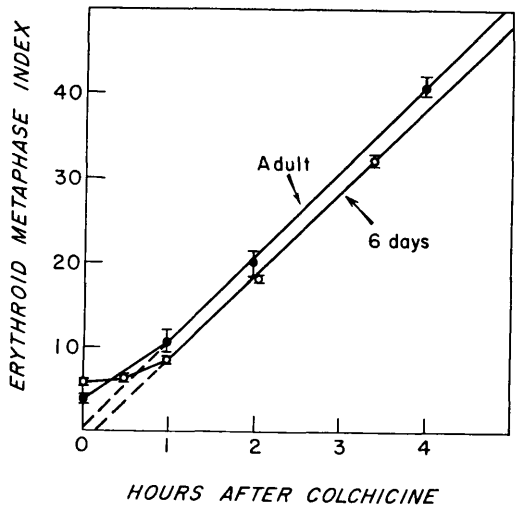

FIG. 1. Percentage of potentially mitotable nucleated erythroid cells arrested at metaphase by colchicine. Adult animals received 1 mg/kg and 6-day-old animals received 0.25 mg/kg.

were sacrificed under ether anesthesia at intervals thereafter. Bone marrow smears were prepared by a brush technic from the split femur. After staining with Wright's and Giemsa, the percentage of erythroid mitoses was determined from counts of 1000 potentially mitotable nucleated erythroid cells (potentially mitotable cells have a nuclear diameter in excess of 5 μ and approximately 50–60% of these cells label initially after an injection of tritiated thymidine). Erythroid mitoses were also classified as to the stage of mitosis per 1000 mitotic figures. For studies in neonatal rats, one animal from each of 5–8 litters was employed for each point on the mitotic accumulation curve and the dose–response curve. One animal from each litter served as a control.

TABLE I. Erythroid Metaphase Index in Adult and 6-Day-Old Rats at Intervals after Colchicine.[a]

Time (min)	Adult[b]	6 day old[c]
Control	3.8 ± 0.3	5.8 ± 0.5
30		6.3 ± 0.3
60	10.7 ± 1.5	8.4 ± 0.5
120	19.8 ± 1.5	18.0 ± 0.6
210		32.3 ± 0.7
240	41.0 ± 1.1	

[a] Values represent mean erythroid index ± SE, 5–8 animals/point.

[b] 1.0 mg/kg of colchicine.

[c] 0.25 mg/kg of colchicine.

Results. Adult rats injected with 1 mg/kg of colchicine showed a 4-hr linear accumulation of erythroid mitoses in the bone marrow (Fig. 1, Table I). Extrapolation of this curve to 100% provided an estimate of 10.5 hr for the t_G of nucleated erythroid precursors. In 6-day-old rats 1 mg/kg of colchicine was uniformly fatal within 1–3 hr after injection. Therefore, a dose response to colchicine was performed in 6-day-old rats. That dose of colchicine which resulted in a maximal accumulation of erythroid mitoses at 2 hr was 0.25 mg/kg (Fig. 2, Table II). Larger doses resulted in lower accumulations of arrested erythroid mitoses. Accordingly the estimate of t_G in the 6-day-old rat using 0.25 mg/kg of colchicine was 10.5 hr (Fig. 1). The slope of the accumulation of arrested mitotic figures with time was parallel to the adult; however there was a slight difference in the

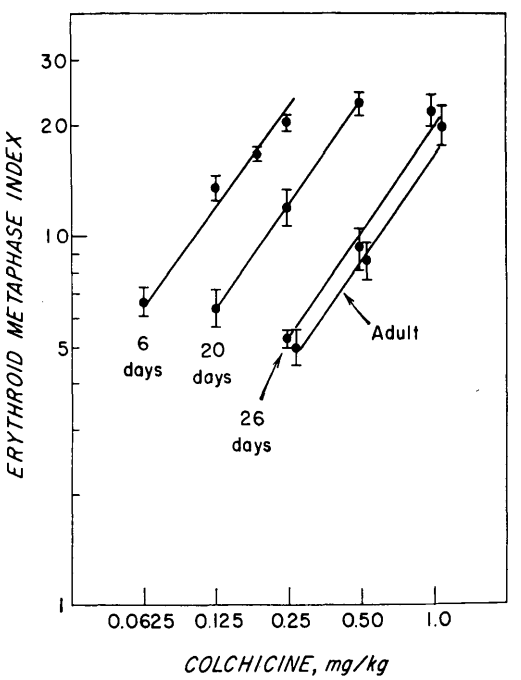

FIG. 2. Percentage of potentially mitotable nucleated erythroid cells arrested in metaphase by a 2-hr *in vivo* exposure to colchicine. Data were plotted according to age and dose.

TABLE II. Erythroid Metaphase Index after Colchicine in Animals of Various Ages.[a]

Colchicine dose (mg/kg)	6 Day	20 Day	26 Day	Adult[b]
Control	5.9 ± 0.5	5.9 ± 0.2	5.5 ± 0.6	3.8 ± 0.3
0.03	6.2 ± 0.7			
0.0625	8.1 ± 0.9			
0.125	14.0 ± 0.9	7.5 ± 0.6		
0.1875	16.7 ± 0.7			
0.25	20.4 ± 1.0	12.7 ± 1.3	6.3 ± 0.5	5.0 ± 0.6
0.375	19.4 ± 1.1			
0.5	17.0 ± 0.7	23.0 ± 1.6	10.2 ± 0.8	8.6 ± 1.0
0.66	14.0 ± 0.5			
1.0	11.8 ± 0.7	23.6 ± 1.0	22.3 ± 1.4	20.0 ± 1.5
2.0		18.3 ± 1.2	20.9 ± 0.7	17.7 ± 1.6

[a] Animals of various ages were sacrificed 2 hr after an intraperitoneal injection of colchicine. Values represent mean erythroid metaphase index ± SE, 5–9 animals/point.

[b] 150–170 g female Sprague-Dawley rat.

T_0 intercepts. Control erythroid mitotic index was 5.8% in the 6-day-old rat and 3.8% in the adult. The mitotic period (t_M) derived from t_G and mitotic index was 60 min for the 6-day-old and 40 min for the adult. In control marrows, 88% of erythroid mitotic figures were classified as metaphase in the 6 day old whereas the distribution was 78% in the adult. The metaphase period ($t_{Metaphase}$) calculated from t_M and % metaphase was 53 min in the 6 day old compared to 31 min in the adult.

The dose response to colchicine showed a gradual transition from day 6 to 26. The response observed in 26-day-old rats was similar to the adult response (Figure. 2, Table II). Acute survival data were also related to age (Table III).

Discussion. Despite the accelerated rate of erythropoiesis in the 6-day-old rat, the t_G of differentiated marrow nucleated erythroid precursors estimated with colchicine was identical to the estimate obtained in the adult rat, ~ 10.5 hr. It is of interest that Lucarelli and co-workers derived a t_G of 7–8 hr for neonatal erythroid elements from evaluation of labeled mitoses in newborn animals of the same strain (9). Data derived from thymidine labeling probably represents a minimum estimate of t_G, and further, the shape of the curve of labeled mitoses may be adversely affected by asynchrony as well as the entry of labeled cells from the precursor compartment during the course of the study. The extrapolation of metaphase accumulation after colchicine probably provides an average estimate of t_G and the possibility exists that at least some of the undifferentiated precursor cells might be blocked at metaphase before they are transformed into definitive erythroid elements. The erythroid mitotic index in untreated control animals compared favorably with the report of Lucarelli *et al.* (8): 5.8% in the 6 day old and 3.8% in the adult. This difference, in view of comparable estimates of t_G, appears best explained by the t_M. Since direct estimates of t_M are not feasible *in vivo*, the product of t_G and mitotic index provides an estimate t_M which is necessarily low because the *sine qua non* of prophase, dissolution of the nuclear membrane, is frequently beyond the resolution of light microscopy. As a result the mitotic in-

TABLE III. The Effect of Colchicine on Survival.[a]

	Colchicine dose (mg/kg)				
Age	0.125	0.25	0.5	1.0	2.0
6 Day	0	100			
15 Day	0	20	100		
20 Day	0	0	0	100	
26 Day	0	0	0	0	100
Adult[b]	0	0	0	0	80

[a] Animals dying (%) within the first 24 hr after administration of intraperitoneal colchicine.

[b] 150–170 g Sprague-Dawley rat.

dex will be underestimated but nevertheless, the difference in t_M and $t_{Metaphase}$ appear substantial: 60 and 53 min, respectively, for the 6 day old and 40 and 31 min for the adult. The duration of metaphase thus appears to account in large part for the difference in mitotic index. In the 6-day-old rat, most organ systems are in a phase of rapid growth and conceivably differing demands for energy sources may in part explain the longer mitotic interval. In support of this concept we reported that starvation in newborn rats was associated with metaphase arrest in marrow nucleated erythroid elements (7). In the adult rat, starvation leads to a suppression of erythropoiesis but mitotic arrest is not observed.

The data at hand do not provide an explanation for the difference in dose response to colchicine between 6-day-old and adult rats. In the 6-day-old animal, the effective dose for arresting mitosis (0.25 mg/kg) was uniformly lethal within the first 24 hr. In contrast, the effective dose in the adult was unrelated to survival. In part these differences may relate to hepatic detoxification of colchicine (10). The liver of the neonatal rat is rather immature and therefore one might anticipate a reduced capacity for detoxification and biliary excretion.

In tissue culture, it has been reported that exposure of the cells to colchicine for increasing periods of time led to increasing periods of further accumulation of mitoses after colchicine was removed from the medium (11). Equilibration studies with tritiated colchicine revealed rapid penetration into the cells and equilibration with extracellular colchicine. When the cells were washed, the bound colchicine was slowly lost. These results were consistent with a mechanism involving reversible binding of colchicine to a critical fraction of sites within the cell, presumably those sites involved in spindle formation. If this be the case for marrow nucleated erythroid cells *in vivo*, then perhaps the difference in response between 6-day-old and adult rats may also relate to the number of critical binding sites as well as the dissociation constant that defines the relationship between bound:unbound colchicine.

For all age groups in the present study, doses of colchicine in excess of the effective dose resulted in lower than expected accumulations of arrested erythroid mitoses. This finding was most marked in the 6-day-old animal. These observations suggest that colchicine in high doses may inhibit cells from entering mitosis and supports the earlier contention of Eigisti and Dustin (12) in this regard. With increasing age there was a gradual transition of effective dose of colchicine toward the adult response. The rate of this transition compares favorably with reported time relationships for the transition of neonatal to adult erythropoiesis in the rodent. Although estimates of t_G reported herein were comparable, differences of time parameters for the various phases of the generative cycle might be anticipated in view of the calculated difference of t_M.

Summary. The generation time of marrow nucleated erythroid cells was estimated with a colchicine technic in adult and 6-day-old Sprague-Dawley rats. Extrapolation of metaphase accumulation to 100% provided comparable estimates for these age groups. Cell cycle parameters were: generation time (t_G) ~ 10.5 hr: mitotic interval (t_M) 40 min adult vs 60 min 6 day old; metaphase interval ($t_{Metaphase}$) 31 min adult vs 53 min 6 day old. The higher erythroid mitotic index (MI) in the neonatal animal appears best explained by a longer duration of mitosis. Neonatal animals were more sensitive to the stathmokinetic effects of colchicine. The change in sensitivity to colchicine appeared to parallel the transition of neonatal to adult erythropoiesis.

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