PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 145, 1092–1095 (1974)

No Mitoses in Basophilic Erythroblasts (37959)

HENRY BORSOOK AND SUSAN JIGGINS

Space Sciences Laboratory, University of California, Berkeley, California 94720

Mitoses are rare in basophilic erythroblasts in stained smears of normal rabbit marrow cells, and relatively common among the acidophilic erythroblasts, usually 0.5–1%, even without treatment which accumulates them. The difference cannot be ascribed to slow DNA synthesis in the former. On the contrary, DNA synthesis is more intense in the basophilic (1–9) and the DNA doubling time is about one-third that of the acidophilic erythroblasts (10). The initial purpose of the experiments described here was to observe the effects of colcemid treatment. This substance arrests mitoses in prophase–metaphase and thus causes them to accumulate. One might then see mitoses in basophilic erythroblasts. The long diameters of the erythroblasts were measured and these observations suggested answers to two other questions in this area. One concerns the timing of when the basophilic erythroblasts lose their cytoplasmic basophilia relative to their cell division. The other is the origin of the large acidophilic erythroblasts called Line 2 cells (11, 12). Their average diameter is that of the large basophilic erythroblasts; compared to polychromatic erythroblasts, their cytoplasm stains a clear yellow-orange without the hint of green; they are seen in mitosis more frequently.

Materials and methods. Normal, adult white New Zealand rabbits obtained commercially were used. Colcemid was injected intravenously in doses ranging from 0 to 5 mg/kg. After 6 hr, which is the longest time before the cells escape from the effects of colcemid, the animals were killed, smears made of the marrow cells, fixed in absolute methanol, and stained with benzidine and Giemsa solutions (13). The long diameters of the erythroblasts were measured on the stained smear by means of a micrometer eyepiece. The size recorded was the nearest whole number on the scale; 1 division = 0.794 nm. Eleven animals were used. 21,574 erythroblasts were tallied and classified, 4,142 were measured.

Table II gives the size distribution in five classes of erythroblasts: "large" and "small" basophilic erythroblasts, Line 2 cells, and acidophilic erythroblasts not in mitoses and in mitoses. The partition of the basophilic erythroblasts into "large" and "small" was based on the hypothesis that some of the large gave rise to the Line 2 cells, the "small" to the acidophilic erythroblasts in mitosis, in each case without change in size. The numerical details are as follows. As there were a negligible number of acidophilic erythroblasts in mitosis of size 13 (scale divisions) or larger, the percent of the total Line 2 cells of sizes 13, 14, and 15 was taken to be equal to the percent of "large" basophilic erythroblasts of the same sizes, in this case 48.7%. There were 366 basophilic erythroblasts of these sizes; the total of "large" basophilic erythroblasts was therefore $366/48.7 \times 100 = 751$. The total basophilic erythroblasts counted were 976; the total of "small" basophilic erythroblasts was therefore $976 - 751 = 225$. Their size distribution was taken to be the same as that of the acidophils in mitosis. These, as fractions of 225, were subtracted from the observed number of basophilic erythroblasts. The remainders were the numbers of "large" basophilic erythroblasts of each size. Another route by which the approximate number of the "small" basophils could be obtained was on the assumptions that all those of sizes 8, 9, and 10 belonged to the class

Printed in U.S.A.

of "small" and that the percent of their total was the same as that in these size groups of the acidophils in mitosis. The total number of "small" basophils obtained in this way was 227. The average size given in Table II were

$$\frac{\Sigma(\text{number of each size} \times \text{size})}{\text{Total number}}$$

Results. Table I shows the accumulation of mitoses under the influence of colcemid in the acidophilic erythroblasts and Line 2 cells and their absence even under these conditions in the basophilic erythroblasts. The Line 2 cells had their characteristic very high mitotic index. The mitoses increased with the dose of colcemid both in the Line 2 cells and acidophilic erythroblasts. Evidently the lower the level of colcemid, the greater the number of mitoses which were not arrested and could progress to completion and disappearance.

The connection between cytoplasmic basophilia in erythroblasts and the low incidence (or absence) of mitosis is not known at present. But it appears worth noting that the basophilia in rabbit erythroblasts is due to RNA with acid groups free to combine with basic dyes. This was found by treating the smears fixed by methanol with RNAse or DNAse. The former enzyme dispelled the cytoplasmic but not the nuclear basophilia, the latter enzyme the reverse.

Table II gives the size distributions as a percent of the total cells counted in each class. The numbers pertaining to the Line 2 cells and the two classes of acidophilic erythroblasts are free of assumptions. The partition of the basophilic erythroblasts into two classes—"large" and "small"—is an hypothesis. Of the total basophilic erythroblasts, the same partition of 76.5% "large" and 23.5% "small" was obtained by the two independent assumptions described above.

Discussion. The median long diameter of the basophilic erythroblasts was 13, average 12.4, scale divisions; of the acidophilic erythroblasts in mitoses, the median long diameter was 10, average 10.6, scale divisions; of those not in mitoses 8 and 8.6, respectively. These size differences connote cell division. No mitoses were observed among the basophilic, many among the acidophilic erythroblasts. Hence, mitoses occurred after the cytoplasmic basophilia had disappeared. However, the necessary doubling of the DNA probably occurred in the basophilic stage. The reasons for this surmise are that DNA synthesis is more intense (9) and the DNA doubling time about one-third (10) in the basophilic than in the acidophilic erythroblasts, even though mitoses are rarely to be seen in the former.

A related question concerns the origin of the "small" fraction of basophilic erythroblasts, which are about 25% of the total and have the size distribution of the acidophilic erythroblasts in mitoses. Two pieces of evidence support the interpretation that the "small" arise by division of the "large," which, it may be said, is the current consensus. One is that the same proportion of "large" to "small" is obtained by either method of computation described above.

TABLE I. Distribution of Erythroblasts and Mitoses. Effect of Dose of Colcemid.

	Basophilic erythroblasts		Line 2 cells Per cent		Acidophilic erythroblasts	
Dose of colcemid (mg/kg)	of total erythroblasts	mitoses in the class	of total erythroblasts	mitoses in the class	of total erythroblasts	mitoses in the class
0	11.3	0	0.8	7.1	87.9	2.1
0.5	9.1	0	0.35	12.5	90.5	3.6
1.0	8.6	0	2.1	17.1	89.3	9.8
2.0	7.4	0	2.4	25.0	90.3	13.1
5.0	6.4	0	4.7	57.6	88.9	17.1

TABLE II. Size Distribution: Percent.

Size: scale divisions	Basophilic erythroblasts		Line 2 cells	Acidophilic erythroblasts	
	Large	Small		not in mitosis	in mitosis
6	0			1.9	
7	0			18.3	
8	0	1.9	0.1	29.6	1.9
9	3.0	8.3	0.3	24.4	8.3
10	5.4	37.3	5.3	17.3	37.3
11	13.7	37.0	15.5	6.5	37.0
12	20.4	13.8	24.2	1.6	13.8
13	20.7	1.2	23.7	0.4	1.2
14	15.3	0.3	15.0		0.3
15	12.6		10.0		
16	4.3		4.0		
17	3.8		1.6		
18	0.8		0.3		
19	0.1				
Average long diameter: in scale divisions	12.98	10.59	12.82	8.64	10.51
Average long diameter: in microns	10.3	8.4	10.2	6.9	8.3

Then the size distribution of the "large" corresponds to that of the Line 2 cells; $P > 0.9$ by chi square, if one ignores the sizes where in one or both groups the incidence was less than 1%.

The other piece of evidence is obtained after translating the long diameters into volumes. For this purpose, one approximation is to assume that all the cells were spheroids and when they are flattened on the smear, the effect on their long diameters as measured was relatively the same. With these assumptions, when the volume is halved the ratio of the long diameters is 0.794. The values in Table III are near to this value.

From the data and the above approximations and assumptions, one obtains the following picture of basophilic erythroblast maturation. The "large" have two pathways to acidophilia. One is via Line 2 cells of the same size. The high incidence of mitoses in these cells (Table I) indicates that they divide rapidly. The progeny have the size of the acidophils seen in mitosis (Table III). The other route is first division of the "large" into the "small." This cell division is amitotic. Amitotic division among erythroblasts has been proposed before (14, 15). The "small" basophils lose their cytoplasmic basophilia without change in volume, the resulting acidophils divide mitotically. Either route leads to acidophils of the same size. We cannot assess the "traffic" along the two routes.

The average long diameter of rabbit erythrocytes as measured here is about 6.5 nm; that of the acidophils not in mitoses was 6.9 nm; some of this difference is due to the nucleus. Evidently most of these erythro-

TABLE III. Ratios of Average Sizes (from Table II).

$\frac{\text{Small basophils}}{\text{Large basophils}}$	0.82
$\frac{\text{Acidophils not in mitosis}}{\text{Acidophils in mitosis}}$	0.82
$\frac{\text{Acidophils in mitosis}}{\text{Line 2 cells}}$	0.82

blasts (orthochromatic) have undergone their terminal division, which is in accord with autoradiographic observations that about 95% do not synthesize DNA.

Summary. In the rabbit, under conditions in which mitoses accumulated in the acidophilic erythroblasts, none were observed in the basophilic erythroblasts. On the basis of certain assumptions, the basophilic erythroblasts were divided into two size classes: of average long diameters (as measured here) 10.3 and 8.4 nm. The larger have two pathways of development. One is to lose their cytoplasmic basophilia to form acidophils of the same size (Line 2 cells); the other is to divide amitotically into small basophils which eventually lose their cytoplasmic basophilia to become acidophils of the same size. Both groups of acidophils undergo mitotic division. Those from the large basophils divide once more; for those from the small basophils, it is a terminal division.

This investigation was supported by NIH HL-12236-05 and a Grant NGR-05-003-460 from NASA to the University of California, Berkeley.

1. Bond, V. P., Fliedner, T. M., Cronkite, E. P., Rubini, J. R., and Robertson, J. S., *in* "The Kinetics of Cellular Proliferation" (F. Stohlman, Jr., ed.), p. 188. Grune and Stratton, New York, NY (1959).

2. Cronkite, E. P., Bond, V. P., Fliedner, T. M., and Rubini, J. R., Lab. Invest. **8**, 263 (1959).

3. Cronkite, E. P., Fliedner, T. M., Bond, V. P., and Rubini, J. R., Ann. N. Y. Acad. Sci. **77**, 803 (1959).

4. Bond, V. P., Odartchenko, N., Cottier, H., Feinendegen, L. E., and Cronkite, E. P., *in* "Erythropoiesis" (L. Jacobson and M. Doyle, eds.), p. 162. Grune and Stratton, New York, NY (1962).

5. Lajtha, L. G., Nature **173**, 587; **174**, 1013 (1954).

6. Lajtha, L. G., Oliver, R., and Ellis, F., Brit. J. Cancer **8**, 367 (1954).

7. Lajtha, L. G., Physiol. Rev. **37**, 50 (1957); *in* "The Kinetics of Cellular Proliferation" (F. Stohlman, Jr., ed.), p. 173. Grune and Stratton, NY (1959).

8. Borsook, H., and Weil, A., Proc. 9th Congr. Eur. Soc. Haematol., p. 865. S. Karger, Basel/New York (1963).

9. Borsook, H., Ann. N. Y. Acad. Sci. **119**, 523 (1964).

10. Monette, F. C., LoBue, J., Gordon, A. S., Alexander, P., Jr., and Po-Chuen, C., Science **162**, 1132 (1968).

11. Borsook, H., Ratner, K., Tattrie, B., and Lajtha, L. G., Nature **217**, 1024 (1968).

12. Lord, B. I., Nature **217**, 1026 (1968).

13. Borsook, H., Ratner, K., and Tattrie, B., Blood **34**, 32 (1969).

14. Burkl, W., A. Z. f. Zellforsch. Mikroscop. Anat. **34**, 584 (1949).

15. Bucher, O., Protoplasmatologia **6**, 1 (1959).

Received June 18, 1973. P.S.E.B.M., 1974, Vol. 145.