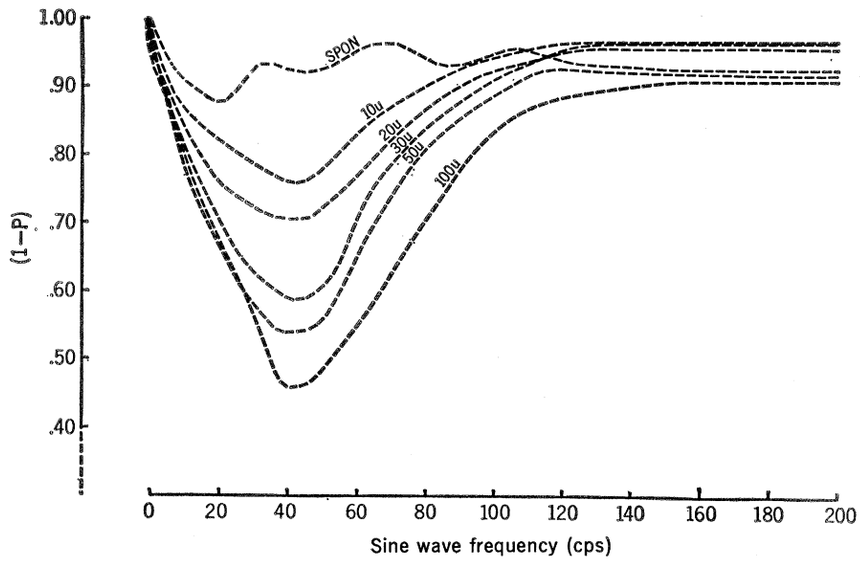


Fig. 3. Tuning curves for neurons in the postcentral gyrus of unanesthetized monkeys activated by sinusoidal mechanical stimulation of the glabrous skin of the contralateral hand. The curves are the averaged results for 13 neurons over a number of stimulus intensities. For the population of time intervals between impulses in the trains of responses to each stimulus (16 trials, each 1 sec in duration) the probability ($P$) was calculated that the stimulus cycle length, ± 20 percent, appeared in the interval distribution. These values were averaged for each point for the 13 neurons and plotted inversely. These cortical neurons are differentially sensitive to low frequencies of stimulation with a best frequency of about 40 hz. The curve for 10 $\mu$m shows that a significant signal of 40 hz appears in the impulse trains evoked by a 40-hz stimulus; 10 $\mu$m is approximately the human threshold for the detection of oscillatory movement of the skin of the hand at 40 hz.

a stimulus amplitude of 10 $\mu$m shows that a significant signal of stimulus frequency appears in the cortical neuronal discharge at 40 hz, at a strength equal to the human threshold for the perception of oscillatory movement at that frequency.

The cortical neurons thought to be related to peripheral Pacinian afferents behave quite differently. They are differentially sensitive to frequencies of 100 to 400 hz and may be driven to maximum and very high rates of discharge by such stimuli having an amplitude of only 1 to 5 $\mu$m, these intensity levels being comparable to human thresholds at these frequencies. Analysis of the temporal ordering of impulse discharges in these responses, however, reveals no significant signal of stimulus cycle length. Cyclic entrainment appears only at stimulus intensities two or more orders of magnitude above human thresholds. It seems possible to conclude that temporal ordering of impulses is not the cortical neuronal code for high-frequency vibration (*3*).

A combination of these results with earlier data on the human sense of flutter-vibration and the responses of peripheral nerve fibers to sinusoidal stimulation of the skin of the hand suggests that flutter-vibration is a dual sensibility, served by two distinct sets of peripheral fibers linked to two equally distinct sets of cortical neurons. Furthermore, low-frequency flutter is a derived form of tactile sensibility, depending for its unique character upon the temporal pattern of the stimuli and coded in the cortex by the temporal order of neuronal discharge. High-frequency vibration has the properties of a true modality, signaled by labeled lines both peripherally and centrally, regardless of the temporal order of discharge.

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## Erythropoiesis in the Rat: Differential Rates of DNA Synthesis and Cell Proliferation

Abstract. *Direct in vivo estimates of DNA synthesis time in early and late erythroblasts were obtained by using the $H^3$- and $C^{14}$-thymidine double-labeling technique. A double-emulsion autoradiographic procedure was used to resolve the two isotopes. Early erythroblasts were found to proliferate at a rate about five times that of late cells. This results primarily from a shorter mean DNA synthesis time in early cells (2.5 hours) than in late cells (6.5 hours).*

The mean duration of DNA synthesis time ($T_s$) is a basic component of any model of cell proliferation kinetics (*1*). Since little evidence is available to the contrary (*2*), the assumption is usually made that average $T_s$ exhibits little variation within homogeneous cell populations. However, differences in the length of $T_s$ have been suggested (*3*, *4*) for cell populations consisting of both less mature and more mature elements. Thus, the validity of assuming constancy of $T_s$ throughout all maturational stages of a given cell lineage must be reexamined. This report presents results of a study of the proliferative characteristics of less mature and more mature rat erythroblasts, made by using double labeling–double emulsion autoradiography.

A more complete explanation of the techniques employed appears elsewhere (*5*, *6*). The double-labeling technique consists of administering in vivo two DNA pulse labels. The first label, $H^3$-thymidine (*7*), is followed 1 hour later by $C^{14}$-thymidine (*8*). Fifteen minutes after administering the second label the animals are killed. During the 1-hour time interval ($T_a$) between the administration of labels, a segment of cells in the DNA synthesis phase (S phase) leave this phase of the cell cycle labeled only with $H^3$ and are thus unable to incorporate the second label. Cells entering and remaining in the S phase incorporate the second label ($C^{14}$-thymidine) and are thereby distinguished from the first segment of $H^3$-labeled cells. The ratio of the number of cells labeled with $H^3$ alone ($N_{H^3}$) to all other labeled cells ($N_{C^{14}+H^3}$) yields an average estimate of $T_s$, since, $N_{H^3}/N_{C^{14}} = T_a/T_s$ (*9*). The labeling index can also be obtained from these preparations by determining the ratio of $C^{14}$-labeled cells to all cells in the population.

Thirty-two male Long-Evans rats were divided into three age groups (Fig. 1). The labeled thymidines were administered intraperitoneally according to the above-described schedule. Fifteen minutes after the second label ($C^{14}$) the rats were killed with ether (*10*). Methanol-fixed femoral diaphyseal marrow smears were prestained by a modification (*11*) of Ralph's (*12*) benzidine technique to differentiate nucleated erythroid cells. To aid in distinguishing between $H^3$- and $C^{14}$-containing cells, the double emulsion autoradiographic technique of Baserga and Nemeroff (*13*) was employed as follows: benzidine-prestained slides were dipped in Kodak NTB-2 liquid emulsion and exposed at 5°C for 3 weeks, developed in Kodak D-19 and counterstained with Giemsa (*14*), coated with a 2 percent nitrocellulose solution and air-dried, dipped again in NTB-2 and exposed for 6 weeks, and developed. The interposition of a celloidin layer between the two emulsion layers prevents the weak $H^3$ beta rays from entering the second emulsion (*5, 13*), whereas the more energetic $C^{14}$ beta rays readily penetrate into the second emulsion layer. The processed autoradiograms were scored for cells labeled with $H^3$ alone (grains in first emulsion only) and cells labeled with $C^{14}$ (grains in both emulsions). Benzidine-positive erythroblasts were divided, by morphologic and karyometric criteria, into two subpopulations: "early" [10 $\mu$ in diameter or larger, showing definite morphologic features of immaturity—high N/C ratio; extensive cytoplasmic basophilia—but containing benzidine-positive areas (*15*)] and "late" (5 to 9 $\mu$ in diameter; very coarse nuclear chromatin, intensely yellow-green cytoplasm).

A total of 5000 erythroblasts were scored for each animal, exclusive of postmitotic orthochromatic normoblasts (*3*). Since background fogging was negligible in both emulsion layers, erythroblasts having three or more grains located directly over the cell nucleus were considered labeled.

The results (Fig. 1, bottom) indicate a striking difference in average S-phase duration of early and late erythroblasts. No significant differences in $T_s$ are found when the three groups (ranging in age from 3 weeks to 8 months) are compared. Average $T_s$ in late erythroid cells remains consistently 2.5 times longer than in the less differentiated cells. The differences in labeling indices between early and late cells likewise indicate a higher rate of cell turnover in the less mature cells (Fig. 1, top), with $C^{14}$-labeling indices averaging about 1.8 times greater in the early cells. The results support the contention (*3*) that less differentiated erythroid cells proliferate at a higher rate than more mature erythroblasts.

Table 1. Mean values for labeling indices and DNA synthesis time of early and late erythroblasts in rats of three age groups. $C^{14}$ = a 15-minute $C^{14}$-thymidine labeling index; $H^3$ = a 1-hour $H^3$-thymidine labeling index. $T_s$ = mean estimate for DNA synthesis time (hours).

| Age group | Early | | | Late | | |
|---|---|---|---|---|---|---|
| | $C^{14}$ * | $H^3$ | $C^{14} + H^3$ | $C^{14}$ † | $H^3$ | $C^{14} + H^3$ |
| I | 64.2 | 28.4 | 92.7 | 36.5 | 5.6 | 42.1 |
| II | 60.1 | 23.5 | 84.3 | 34.6 | 5.3 | 39.9 |
| III | 62.8 | 27.2 | 90.0 | 32.1 | 5.5 | 37.6 |
| Means | 62.4 | 26.4 | 89.0 | 34.4 | 5.5 | 40.0 |

*$T_s$: I, 2.4; II, 2.8; III, 2.4; mean, 2.5. † $T_s$: I, 7.0; II, 6.7; III, 5.9; mean, 6.5.

A major advantage of the double-labeling technique is that it yields both kinetic measures from a single bone marrow sample (*6*): a ratio of differentially labeled cells gives a direct estimate of $T_s$, whereas the ratio of labeled to nonlabeled cells yields the labeling index. More importantly, $T_s$ measures are thus independent of the labeling index.

The occurrence of similarly high initial labeling indices in early erythroid precursors has been reported previously (*3, 4, 16, 17*). Considering only cells in cycle, an increase in the labeling index could be due to (i) a concomitant diminution in the non-S phase durations (that is, $T_{G2}$, $T_M$, and/or $T_{G1}$) without a decrease in S phase; (ii) an increase in S phase accompanied by reductions in other subcycle times; or (iii) reduction in all subcycle phases including S, but proportionately greater in $G_1$ and $G_2$. In all three cases the result would be the same: a decreased mean cell cycle time and hence a greater cell turnover rate. Our data indicate that the less mature erythroblasts have a very short $T_s$ in relation to a high labeling index, a situation which could only be consistent with (iii). From a number of observations on the variability of the remaining phases of the cell cycle (*6, 18*) it would seem that $T_{G1}$ is by far the most variable and in the present case may be largely reduced or eliminated altogether.

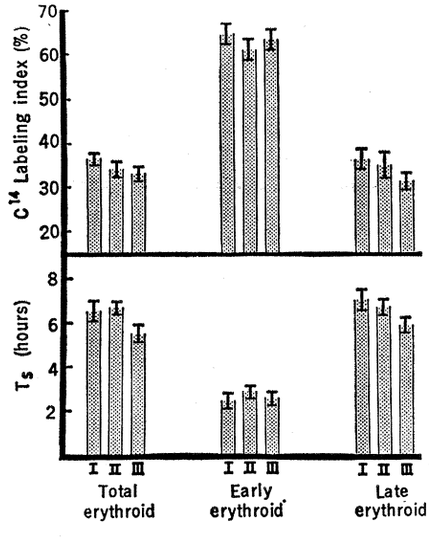


Fig. 1. (Bottom) Mean duration of DNA synthesis time ($T_s$, in hours) in early and late erythroid precursors of 32 rats divided into three age groups: group I: 12 rats, 3 weeks old (50 to 58 g); group II: ten rats, 3 months old (250 to 290 g); and group III: ten rats, 8 months old (385 to 440 g). Vertical bars, ± one standard error of the mean. (Top) Fifteen-minute pulse labeling indices with $C^{14}$-thymidine.

The mean grain count data on early and late human erythroblasts from two earlier reports (*3, 4*) could indicate that the rate of DNA synthesis is twice as fast in younger cells. Although other interpretations were made at that time (*4, 19*), these results and those of our study suggest differential rates of DNA synthesis somehow related to a cell's maturational level. The presence or absence of nondividing erythroid cells in no way affects the $T_s$ estimates, since all labeled cells, upon which the estimates are based, were in S phase at some time during the experiment. By combining all labeled cells into one category, we obtain a total labeling index, representing a 1¼-hour pulse labeling with both $C^{14}$ and $H^3$, which indicates the relative proportion of cycling cells in S phase over the 75-minute period. When early and late cells are compared (Table 1), these total labeling indices are 89 and 40 percent, respectively, suggesting that no more than 11 percent of the early cells could be noncycling or nondividing cells. It would be reasonable to assume that if the pulse period were lengthened (for example, to 2 hours or longer) nearly 100 percent labeling of the early erythroid cells would be achieved (*4*). If this is the case, there is reason to believe, as others have suggested, that

most (if not all) early erythroblasts are dividing cells (*4*). A total labeling index of only 40 percent for the late erythroid cells indicates that the presence of nondividing components or slower rates of production, or both, may be a large factor in determining the extent of labeling in these cells.

It is possible from our data to calculate hourly cell production rates for both early and late erythroblasts. This is obtained from the ratio (*20*): labeling index (%)/$T_S$ (hours), giving values of 25 and 5.3 percent per hour for early and late erythroblasts, respectively. For the less mature cells this represents a turnover time of about 4 hours (*21*), and for the more mature cells, of about 20 hours. An hourly production rate of 25 percent in the early erythroblast compartment is far greater than that given by others for the rat (for example, 7 percent per hour) (*17*). The discrepancy lies in the vastly different $T_S$ durations used in calculating production rates, figures between 6 and 8 hours usually being employed (*17*). From our data it now appears that this contention of a constancy of $T_S$ in early and late erythroblasts is no longer valid and that gross underestimations of early cell proliferation kinetics result if it is accepted. Some workers (*16*, *17*) have suggested that $T_S$ could be shorter in earlier stages of differentiation but they made no direct observations to confirm this. Since $T_S$ is basic to models of cell proliferation it will be necessary to determine the extent to which $T_S$ differs among members of a given maturational sequence before such models are constructed.

The greater proliferative "efficiency" of the earlier stages in cell lineages has been noted before (*3*, *4*) and may prove to be a general phenomenon. It is now clear that a pool of dividing cells, belonging to the same cell line, can vary greatly in its kinetic constitution. The presence of nondividing cells and two or more subpopulations in cycle contained within the confines of a single cell lineage decrease the value of average measurements as parameters of kinetic analysis (*1*, *3*).

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## Flagellar Adenosine Triphosphatase from Sea Urchin Sperm: Properties and Relation to Motility

Abstract. *Adenosine triphosphatase activated by divalent cations is apparently a component of the motile apparatus in flagella of* Arbacia *sperm, as judged by the activity of this enzyme in intact flagella, glycerol-extracted flagella, and soluble extracts prepared from flagella. However, the variation in the physical properties and in the amount of enzyme obtained after a variety of treatments suggests that additional components are involved in the motile mechanism. These features distinguish the soluble flagellar enzyme from adenosine triphosphatases of other motile cells.*

Adenosine triphosphatase components generally analogous to those of muscle cells are believed to participate in the motility of spermatozoa. Extraction of this enzyme in a soluble form (*1*) has made possible a study of its enzymatic and physical properties and its relationship to flagellar activity.

Male *Arbacia punctulata* were injected with 0.5*M* KCl solution (0.5 ml), and semen was collected in dry, chilled Stender dishes (*2*). All subsequent manipulations were carried out in the cold to minimize metabolic activity. After low-speed centrifugation to remove gross contamination, semen was diluted with ten volumes of cold CMFSW (the calcium- and magnesium-free synthetic seawater prepared by the Marine Biological Laboratory). Sperm were sedimented at 1000*g* for 10 minutes, washed by suspension in CMFSW and sedimented again, resuspended in 20 volumes of CMFSW, and homogenized 3 minutes in a glass homogenizer with Teflon pestle. Nearly quantitative separation of heads and flagella was achieved with very little debris. Heads and intact sperm were sedimented by two or three successive centrifugations at 1000*g* for 10 minutes, until the supernatant was essentially free of heads. The flagellar fraction was sedimented at 10,000*g* for 15 minutes. As indicated in Table 1 (preparation 1), such isolated flagella displayed substantial adenosine triphosphatase activity as well as motility. No measurable activity was extracted by Weber-Edsall solution (*3*) without prior mechanical or chemical disruption of the flagella, indicating the presence of structural barriers to extraction. The membrane nature of these barriers was shown by the facilitation of extraction by freezing and thawing (Table 1, preparation 2) or digitonin (preparation 3); flagella were exposed to digitonin-tris-$Mg^{++}$ solution (*4*) [tris is tris(hydroxymethyl)aminomethane] for two successive 10-minute periods, each terminated by centrifugation at 10,000*g* for 10 minutes. The removal of membranes and cytoplasmic components from the motile apparatus by