Circadian Variations in Mouse Hematopoiesis
II. Sex Differences in Mitotic Indices of Femoral Diaphyseal Marrow Cells[1] (35926)

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Mitotic activity of several mammalian tissues are known to exhibit circadian fluctuations. Thus, within intestinal mucosa (1) and other epithelial tissues (2, 3), marked variations in daily cell division cycles have been discerned. In the bone marrow (4, 5) there is some indication that alterations in overall mitotic activity exist, but the studies performed to date have not been specific as to the hemic cell types involved. In a study of humans (4), such nonspecific mitotic activity was found to be minimal during the daylight hours and maximal at midnight. Recently (5), it has been shown that female rats and mice exhibit fluctuations in mitotic activity with peak mitotic count occurring during daylight hours; and it has been suggested that life style may be responsible for the observed differences between rodents and man. Fox and Laird (6) have recently observed that only male rabbits manifested diurnal variation in peripheral hematologic parameters and suggested that failure to detect similar diurnal rhythms in females might be due to the hormonal changes associated with the estrous cycle and its effects on hemopoiesis and the peripheral hemogram. The present investigation was undertaken in mice to answer two obvious questions raised by these earlier studies, namely: (i) Does diurnal variation occur in the mitotic activity of specific blood cell precursors? and (ii) Does a sex difference exist? Results obtained indicated an affirmative answer to both questions.

Methods. Adult male and female CFS-1 mice were used in this study.

Cardinali *et al.* (8) found that 4 hr was the optimal treatment time when Vincristine sulfate (0.5 mg/kg body wt) was used to arrest and permit the accumulation of marrow cell mitoses in the DBA/2 mouse. It remained necessary, however, to determine this experimentally for the CFS-1 mouse. Therefore nine male CFS-1 mice were used in a preliminary investigation which confirmed the optimum duration of treatment with Vincristine as 4 hr. Eight days prior to experimentation animals were acclimated to a controlled, ventilated environment maintained at 21 $\pm$ 0.1° and were subjected to 12 hr of light and 12 hr of darkness. Five male and 5 female CFS-1 mice subjected to prior 4 hr Vincristine treatment (0.5 mg/kg of body wt) were killed every 4 hr over a 24 hr period. At sacrifice, one femur was rapidly dissected out of each animal. The marrow was suspended in hypotonic fetal calf serum (serum:distilled water, 3:1), and smears were made for determination of the mitotic index for the specific cell lines. Specific mitotic indices were ascertained by total counts of: 200 dividing granulocyte precursors from smears stained with Wright–Giemsa; 200 lymphocytes from smears stained with LoBue and co-workers' modification (9) of Ralph's (10) hemoglobin stain (counterstained with Giemsa); and 2000 dividing erythroid cell precursors (cells larger than 5 μ and which are known to initially incorporate tritiated thymidine). All counts were done blindly with coded numbers.

Results. There appeared to be a bimodal

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TABLE I. Marrow Mitotic Indices (MI) (mean % ± SEM).

Time	Granulocyte precursor elements		Lymphocyte elements		Erythroid precursor elements	
	Male MI	Female MI	Male MI	Female MI	Male MI	Female MI
12:00 noon	2.4 ± 0.55	2.5 ± 0.68	2.25 ± 0.60[a]	2.8 ± 0.78	0.68 ± 0.14	0.76 ± 0.21
4:00 p.m.	3.3 ± 0.88[a]	3.1 ± 0.75	1.63 ± 0.88	2.0 ± 0.65	1.40 ± 0.27[a]	0.58 ± 0.08
8:00 p.m.	0.63 ± 0.13	1.4 ± 0.32	0.63 ± 0.13	1.6 ± 0.52	0.99 ± 0.07	0.33 ± 0.08
12:00 mid.	1.6 ± 0.55	2.3 ± 0.48	1.38 ± 0.55	4.1 ± 2.07	0.68 ± 0.11	0.88 ± 0.28
4:00 a.m.	2.6 ± 1.13	3.0 ± 0.79	1.75 ± 0.52	2.4 ± 1.30	0.26 ± 0.04	0.45 ± 0.12
8:00 a.m.	1.13 ± 0.24	3.3 ± 0.75	0.63 ± 0.13	2.5 ± 0.74	0.14 ± 0.01	0.36 ± 0.08

[a] $p < 0.05$ when compared with the 8:00 a.m. group.

circadian variation for the mitotic index of granulocyte precursors in male mice with peak activity at 4:00 p.m. and 4:00 a.m. (Table I). Both peaks were significantly different from the 8:00 p.m. minimum value; whereas only the 4:00 p.m. group exceeded the second minimum value at 8:00 a.m. at a confidence level of $p \leq 0.05$. The data for the females showed no significant daily fluctuations, although the 8:00 p.m. values appeared lowest. For lymphocytes, a circadian rhythm was again apparent in males with only one peak at 12:00 noon but with the same minima as for granulocytes. The females exhibited erratic values which prevented drawing any conclusions concerning circadian variation in this cell line. Males showed a clear cut diurnal variation in erythroid mitotic index with the peak activity at 4:00 p.m. being significantly different ($p \leq 0.05$) from the 4:00 and 8:00 a.m. groups. The females exhibited peak activity at midnight and minimal values at 8:00 p.m. but these differences were not significant.

Discussion. The alteration in bone marrow mitotic index for specific cell types in the female did not follow any definitive pattern of diurnal variation although some trends indicative of cyclic behavior were suggested. In the male, however, several significant rhythms were found. There appeared to be a bimodal variation in granulocyte mitotic index with both peaks and minima following light changes by 10 hr. There was a circadian variation in lymphocyte mitoses with minima at the same time as the dividing granulocytes and manifesting a single diurnal variation.

The presence of a circadian variation in males and the apparent absence of such fluctuations in the females is of interest. We have already demonstrated that males exhibit a circadian variation in total marrow mg cellularity whereas females do not (7). It is known from the studies of Fox and Laird that only male rabbits show daily fluctuations in peripheral red blood cell parameters. Metabolic activity has been suggested as a possible factor in control of cellular proliferation and mitotic rate (11). Since mice are nocturnal this should result in peak mitotic activity during the evening hours, given that general metabolism was the only factor to be considered. However, peak cell mitotic activity varied depending upon the specific cell type and sex. In a cell renewal system such as the bone marrow daily rhythms of mitotic division are probably affected, in part, by the manifold physiologic alterations attending homeostatic mechanisms generally. On the biochemical level it has been suggested (12, 13) that sequential activation and repression of specific genes lead to a critical state during which there is a qualitative shift in macromolecular synthesis which is in turn essential for DNA replication, the critical prerequisite for normal mitotic activity. Thus the passage of cells from one subcycle phase to another may be dependent on interaction and activation of gene complexes which may in turn be humorally induced. Regulation of the duration of any phase of the cell cycle (*i.e.*, mitotic time) may be due to such gene action. Clearly, since the mitotic index is a function of the ratio: mitotic time/generation time; factor

changing this ratio could manifest itself as an alteration in mitotic index. In harmony with this notion, Brown and Berry (3) have recently reported that diurnal mitotic rhythms result from partial synchronization of DNA synthesis; the mitotic variations being a reflection of the temporal pattern of the S-phase cohorts of the cell cycle. They suggest that hormones which inhibit mitosis may trigger this partial synchrony which is then expressed as a mitotic rhythm. Adrenal cortical hormones are known to affect numbers of hemic cells (14); these hormones also exert lympholytic effects in addition to inhibiting the mitotic phase of the cell cycle (15). Humoral influences on hematopoiesis via the sex steroids may be implicated in the sex differences; the enhancing effect of testosterone, and the inhibiting effect of estrogen on erythropoiesis are well documented (16, 17). It may be that these hormones act either in concert with or in opposition to specific hematopoietic factor (erythropoietin and possibly "leukopoietin") as well as interaction with specific genes in enzyme activation to produce a partial synchrony which might then result in a diurnal rhythm in mouse bone marrow.

Summary. Circadian fluctuations in the mitotic index of 3 specific cell lines of the bone marrow were studied. The males showed bimodal fluctuations for the dividing granulocytes and one peak with two minima for lymphocytes. Erythroid mitotic indices exhibited a clear cut diurnal variation with peak activity in late afternoon and lowest in early morning. No circadian fluctuations could be ascertained for mitotic indices of bone marrow cells from female mice.

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