Circadian Variation in Mouse Hematopoiesis. I. Sex Difference in Cellularity of Femoral Diaphyseal Marrow[1] (35247)

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Several hematopoietic parameters exhibit a circadian periodicity. One of these is the marrow mitotic index, a measure of the proliferative activity of blood-forming tissue. Thus, the mitotic activity of human marrow (1) is minimal during the daylight hours and maximal at midnight. Recently (2) it has been found that rats and mice apparently are phase shifted when compared to man so that their peak mitotic activity is attained in the morning (*e.g.*, 10:00 a.m. in mice). This preliminary report presents data on yet another parameter apparently showing a circadian variation: the cellularity of mouse femoral bone marrow. The data further suggest changes in marrow cell concentration which may be sex related. Such sex differences have already been shown to exist in the periodic variations of the circulating blood cells of rabbits (3).

Methods. Thirty male and 30 female CF-S1 mice (22–33 g) were used in this study. They were placed into a controlled, ventilated environment maintained at 21 ± 0.1° and were subjected to 12 hours of light and 12 hours of darkness. This illumination schedule was controlled by an electric timer set to turn lights on at 6:00 a.m. and off at 6:00 p.m. The animals were kept on this schedule for a minimum of 8 days prior to experimentation; all animals had free access to food and water which was replenished at 12:00 p.m. each day. On the day of sacrifice, animals were given intraperitoneally 0.5 mg/kg of Vincristine sulfate (Eli Lilly and Co., Indianapolis; a stathmokinetic drug known to block mitosis in metaphase) and killed 4 hr later. Controls received saline. Sacrifice times were 8:00 a.m., 12:00 noon, 4:00 p.m., 8:00 p.m., 12:00 midnight, and 4:00 a.m. Five animals comprised each group: 4 drug-treated and 1 control. At each injection time, 5 mice were arbitrarily chosen from 30 prenumbered acclimatized mice. Mice were sacrificed by ether anesthesia; and weighed samples of femoral diaphyseal marrow were suspended in 0.5 ml of hypotonic fetal calf serum (serum:distilled water, 3:1). Hemocytometer counts were made of the suspension for estimation of total nucleated cell numbers. The total nucleated cell count divided by the weight (mg) of marrow in the sample yielded the total nucleated cells per milligram of bone marrow.

Results. As shown in Fig. 1, both male and female mice show daily fluctuations in the numbers of nucleated cells per milligram of

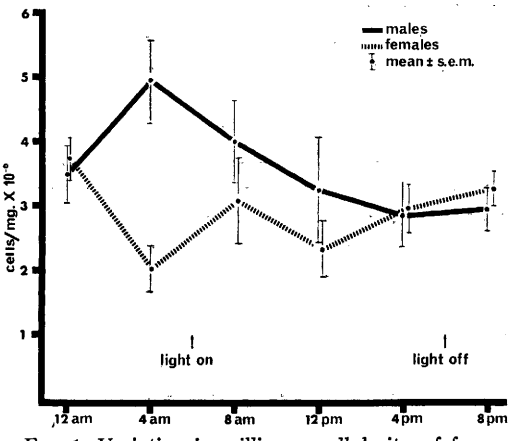

FIG. 1. Variation in milligram cellularity of femoral diaphyseal marrow plotted with respect to time of day: In the males $p<0.05$ for 4:00 a.m. cellularity compared to 4:00 p.m.; in females $p<0.01$ for 12:00 midnight cellularity compared to 4:00 a.m.

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bone marrow. The values that exhibit significant differences ($p<0.05$) are 4:00 a.m. compared to 4:00 p.m. in the males; and 12:00 midnight compared to 4:00 a.m. ($p<0.01$) in the females. Interestingly, the male mice attain peak milligram cellularity at the same time the females show minimal cellularity. At all other times of the day there is little sex difference except that females generally displayed a lower femoral milligram cellularity. The high and low cellularity values in the males followed light changes (on and off) by 10 hr.

Discussion. The alterations in bone marrow cellularity per milligram in the female do not seem to follow any definitive pattern of diurnal variation, although the difference between the midnight and 4:00 a.m. groups is suggestive of a cyclic phenomenon. In the males, however, a clear cut diurnal variation is readily observed, the maximum and minimum cellularity values being 12 hr apart. Similar studies in rabbits (3) have also indicated that males show diurnal variations in several peripheral hematopoietic parameters whereas females do not.

Several factors may account for this diurnal variation in males and the observed sex difference. The fluctuations observed in cellularity per milligram might be related, on a metabolic basis, to the activity of the animal, the mouse being nocturnally active. This may constitute a valid explanation for the data in the male, but does not explain the findings in females which show both maximal and minimal values during the nocturnal, active period. Cyclic variation in other parameters of hematopoiesis have already been reported. Thus, peripheral blood eosinophil counts are known (4) to decrease at night and diurnal variations occur in blood leukocytes in normal and adrenalectomized mice (5). It is well established (5) that for mice the maximal leukocyte numbers in peripheral blood are present at midafternoon. This suggests a possible reciprocal relation between the bone marrow cellularity of our study and the peripheral white blood cell counts.

The levels of endogenous hormone(s) may be responsible for the cyclical variation and the sex difference in cellularity. It has been shown, for example, that adrenal cortical hormones affect the cellularity of femoral marrow (6). Differences in erythrocyte values between the sexes are well known. Also gonadotropic hormone extracts will accentuate the normal differences in red blood cell count between male and female rats (7). Finally humoral influences on hematopoiesis via the sex hormones and erythropoietin and/or the leucocytosis-inducing factor(s) may be implicated in the circadian variation as well as the sex difference. Thus, Gordon *et al.* have shown (8) that testosterone enchances erythropoiesis in mice by increasing the production of erythropoietin. Large amounts of estrogen, on the other hand, exert an inhibitory influence on erythropoietin formation (9).

Summary. The total nucleated cell count in the bone marrow of mice exhibits a sex related daily fluctuation. The male cellularity is highest at 4:00 a.m. and is minimal at 4:00 p.m.; whereas in the female, high cellularity is evident at midnight and assumes its low value at 4:00 a.m.

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