percent fertile and contained the chromosomal fragments and rearrangements shown in Fig. 1, C and F. The other two F_2 males (25.5 and 35.1 percent fertile) had chromosomal fragments or rearrangements that were less drastic but caused a reduction in their fertility. Also, the fertility of some of the eight daughters was affected (range 0.8 to 72.5 percent fertile), and presumably they represent the range of the various types of viable gametes produced by the F_1 male parent (Fig. 1, B and E). Similar types of chromosome aberrations and a consequent reduction in fertility were found in some of the F_3 males.

The persistence of the chromosomal fragments through the multitude of mitotic and meiotic cell divisions that occur in three insect generations is strong evidence for the holokinetic nature of the chromosomes of this species. Furthermore, the transmission of fragments through meiotic divisions demonstrates that the kinetochore activity is not restricted to a limited region of the chromosomes during meiosis as previously suggested by Heizer (*4*).

The drastic reduction of fertility of the progeny bearing fragmented and translocated chromosomal complements adds further evidence that inherited partial sterility observed in the Lepidoptera (*5*), which also have holokinetic chromosomes, is based on the continued transmission of aberrant chromosomal complements. These data provide cytogenetic support for the potential application of inherited partial sterility as an innovation in insect control (*6*).

LEO E. LACHANCE
MAURICE DEGRUGILLIER
Metabolism and Radiation Research Laboratory, Agricultural Research Service, U.S. Department of Agriculture, Fargo, North Dakota

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Circadian Periodicity of Bone Marrow Mitotic Activity and Reticulocyte Counts in Rats and Mice

Abstract. *Mitotic proliferation in the bone marrow of female rats and mice kept under standardized conditions with light from 6:00 a.m. to 6:00 p.m. exhibited a significant circadian periodicity with the greatest activity occurring from 6:00 a.m. to 12:00 noon. The reticulocyte levels in peripheral blood were highest at 8:00 a.m.*

Circadian periodicity occurs in a number of hematological parameters such as the mitotic activity in human bone marrow (*1*), circulating lymphocyte and eosinophil counts in man (*2*) and mice (*3*), and plasma iron levels (*4*). Spontaneous variations of this type in intact normal animals have been found to be of large magnitude and must be considered in assessing experimental results (*5*). In this study, a circadian periodicity was demonstrated in the mitotic proliferation of the bone marrow and in the numbers of circulating reticulocyte levels in rats and mice.

Female Sprague-Dawley rats (190 to 210 g) and female white Swiss mice (20 to 25 g) were kept for 7 to 10 days under standardized conditions consisting of exposure to light from 6:00 a.m. to 6:00 p.m., isolation in individual cages, maintenance of room temperature at 70°F (21°C), and protection from disturbances except for daily feeding and watering. All animals had free access to Purina Laboratory Chow.

To demonstrate the circadian variation in the mitotic activity in bone marrow, groups of ten rats and ten mice were given colchicine intraperitoneally (1.0 and 1.2 mg/kg of body weight, respectively) 4 hours before bone marrow samples were taken at intervals throughout the day and night. This was done in order to arrest mitoses in the metaphase during these 4-hour periods. Each animal was anesthetized with ether and rapidly exsanguinated. A femur was split and bone marrow was removed. Smears were prepared, fixed in methyl alcohol, and stained with Giemsa's stain. Two to three thousand cells were counted per animal, and the

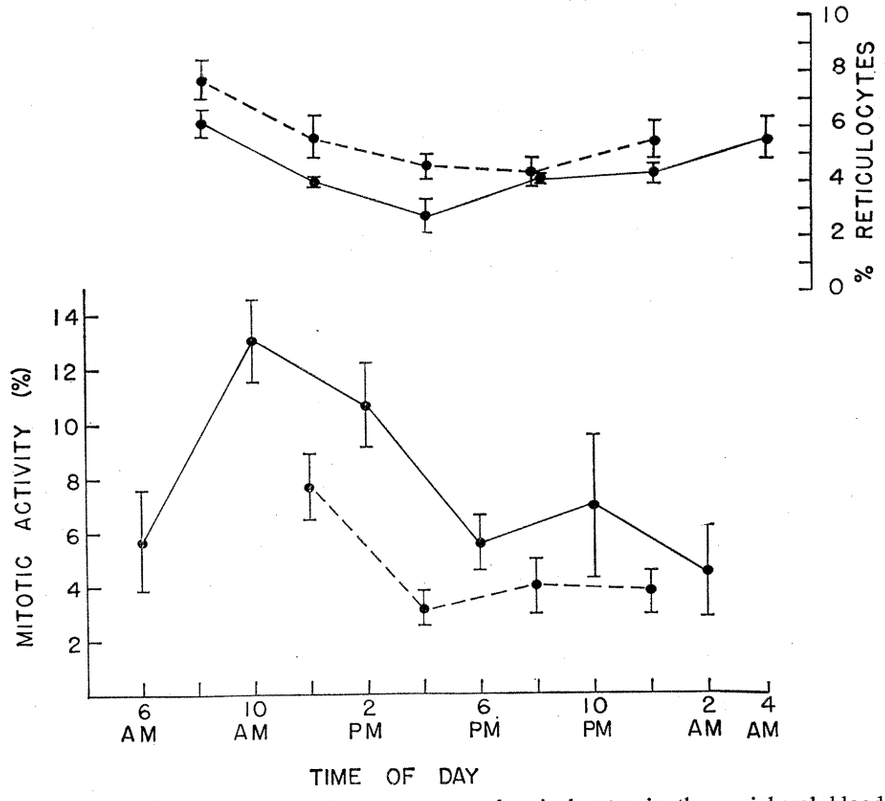

Fig. 1. Circadian variation in the percentage of reticulocytes in the peripheral blood (upper portion) and in the frequency of mitoses (lower portion) of rats (solid lines) and mice (dashed lines). Animals were maintained in light from 6 a.m. to 6 p.m., alternating with darkness. Vertical lines represent standard errors of the means.

percentage of cells in division was calculated. The overall mitotic activity was determined, and no attempt was made to separate mitotic figures in the myeloid or erythroid series. All counts were performed blindly. Four hours after colchicine injection has been found to be an optimal time for study of mitotic activity in bone marrow of mice (*6*).

To determine whether a circadian variation in reticulocytes in peripheral blood occurred, tail vein blood was obtained from groups of five rats every 4 hours beginning at 8:00 a.m., each animal being used only once. Samples were obtained from 10 to 12 mice by exsanguination at the same times (except 4:00 a.m.). Reticulocyte counts were performed by utilizing supravital fluorescent staining (*7*). The results with this technique compare well with the results obtained with standard wet preparation Cresyl Blue staining, except that the values have been found to be somewhat higher and more easily reproducible. Blood was drawn to the 1 mark of a white blood cell pipette and diluted with a 0.1 percent solution of acridine orange, a fluorochrome which combines with the RNA of reticulum to produce an orange fluorescence under ultraviolet light. This was placed beneath a cover slip and sealed to prevent drying. Counts were first performed by using dark-field light to determine the number of erythrocytes in each field, then the same area was viewed under ultraviolet light to determine the percentage of reticulocytes. Student's *t*-test was used to determine the significance of experimental differences.

A marked circadian periodicity occurred in the frequency of mitoses of the bone marrow of both rats and mice (Fig. 1). The proliferation of the marrow was highest during the period from 6:00 a.m. to 2:00 p.m. and then dropped and remained low throughout the rest of the day. In the rat, the peak activity at 10:00 a.m. was significantly higher than those at other observed times except at 2:00 p.m. and 10:00 p.m. In the mouse, the peak activity at 12:00 noon was significantly higher than that at any other observed time. The total 24-hour mitotic rate in the rat was 46.5 percent (calculated from the sum of the percentages at each of the six times of day, thus representing an entire 24-hour period). If this same figure were calculated only on the basis of the value at 10:00 a.m., it would appear to be 78.6 percent over a 24-hour period, or if calculated from the 2:00 a.m. figure it would be only 27.0 percent. Thus, overall daily mitotic activity cannot be extrapolated from the mitotic activity determined at any one time.

This variation of cell division in the bone marrow of rats and mice, with the peak mitotic activity occurring during the morning hours, is very similar to mitotic rhythms found in other rodent tissues such as gastric mucosa (*5*), epidermis (*8*), and liver (*9*). In a study of human bone marrow, the largest number of mitoses was seen in the evening and the smallest in the morning (*1*). Opposite patterns are expected in rodents and humans, of course, as rodents are nocturnally active while humans have primary daytime activity.

A circadian variation was present in reticulocytes in both rats and mice, with the highest percentages occurring at 8:00 a.m. and the lowest from 4:00 p.m. to 8:00 p.m. (Fig. 1). The 8:00 a.m. percentage in the rat was statistically significantly higher than that at any other time of day except 4:00 a.m., and in the mouse the percentage at 8 a.m. was significantly higher than that at any other time except 12:00 noon.

Thus, there is a circadian periodicity present in both the bone marrow mitotic activity and peripheral blood reticulocyte levels in both rats and mice. Experimental results involving measures of hematologic function may be difficult or impossible to assess without considering these circadian variations. Studies done at one standard time of day may not be sufficient to eliminate these problems. The result of drug administration, for example, may be dependent upon the time of day if the drug's activity is primarily directed toward one portion of the cell cycle.

RAY H. CLARK
DONALD R. KORST
St. Joseph Mercy Hospital, Ann Arbor, Michigan

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Lysergic Acid Diethylamide: Role in Conversion of Plasma Tryptophan to Brain Serotonin (5-Hydroxytryptamine)

Abstract. *Injections of* D-*lysergic acid diethylamide decrease the turnover rate of 5-hydroxytryptamine of rat brain, as measured from the conversion of* ^{14}C*–tryptophan into* ^{14}C*–5-hydroxytryptamine. The 2-bromolysergic acid diethylamide given in doses fivefold greater than those of lysergic acid diethylamide fails to change the rate of* ^{14}C*–tryptophan conversion into* ^{14}C*–5-hydroxytryptamine. The effect of* D-*lysergic acid diethylamide is discussed with regard to its action on brain serotonergic neurons and its psychotomimetic effects.*

From indirect information available, we now can infer that D-lysergic acid diethylamide (LSD) may reduce the turnover rate of brain 5-hydroxytryptamine (HT) in rats (*1*, *2*). The validity of this inference is somewhat doubtful because it is based on results obtained with nonisotopic methods (*3*) derived from the application of steady-state kinetics to the dynamic equilibrium between the rates of HT formation and its metabolism and efflux from the brain. When a drug like LSD changes the "steady-state" concentrations of brain HT and 5-hydroxyindoleacetic acid (HIAA) in opposite directions (*1*, *4*), it is difficult to assume that the dynamic relation between the rates of formation of HT and HIAA remain comparable to that at the steady-state. In fact, the data on the turnover rate of brain HT in rats injected with LSD (*1*, *2*) might also be interpreted to indicate that this drug changes the route of metabolism of brain HT. One might infer that, as a result of LSD action, HT may be diverted toward a novel metabolic pathway which normally plays an insignificant role in the metabolic degradation of this brain amine. For instance, LSD