24-Hour Rhythms at Several Levels of Integration in Mice on Different Lighting Regimens.* (23915)

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Ordinarily, lighting regimen controls the timing of eosinophil rhythm(1), as well as activity rhythm(2), in mice and other experimental animals(3,4). A shift in timing of daily periods of light and darkness can therefore be used to displace the daily time of peak or trough of the eosinophil rhythm(3), as long as physiologic conditions are maintained [*e.g.*, in fully-fed, healthy animals, but not in mice severely restricted in dietary calories(1)]. It will be shown that daily "high" and "low" of other 24-hour rhythms also may be shifted by manipulation of illumination. An earlier suggestion, that lighting is ordinarily the dominant synchronizer of eosinophil rhythm in mice(5) can now be extended to a number of additional 24-hour periodic phenomena, some of them metabolic and/or cellular functions, others reflecting operation of integrative controls(3,6) of rhythm.

Materials and methods. Mice of inbred strains, maintained in the Division of Cancer Biology at the University of Minnesota, by brother-to-sister mating, for over 50 generations were used: D_8 (Dilute Brown) mice for the study of epidermal mitoses (males, ~ 4 months of age), of liver glycogen (both sexes, ~ 7 months of age) and of blood "corticosterone" (females, ~ 15 months of age); C (Bagg Albino) mice for the study of liver mitoses (males, ~ 5 weeks of age), of liver DNA (both sexes, ~ 5 weeks of age), of liver phospholipid and RNA (males, ~ 6 weeks of age), and of blood sugar (males, ~ 2½ months of age). From the time of weaning and throughout the investigations, Purina Laboratory Chow and tap water were available to the animals *ad libitum*. The mice were first "standardized" for at least one week prior to each study. For this purpose, they were placed in single cages, in rooms maintained at a temperature of 78 ± 2°F and illuminated by artificial light only. The mice were disturbed only once weekly for cage cleaning and food replacement. The cages were last cleaned and fresh food was given 3 days prior to killing. Thereafter, nobody entered the room, except for a daily brief and quiet checking of the water bottles and, in the experiments involving radiophosphorus, for tracer injection, 2 hours before killing, as described(7). Metal cages admitting light solely through the galvanized mesh door were used. Prior to study, photometer readings were made during the light period in selected cages, from different positions on the racks. On the top rows, light intensities varied from the front to the back of a given cage, from ~ 14 to ~ 3 footcandles; on the bottom rows the corresponding variation was from ~ 3 to ~ 1 footcandles. In each room, a clock-controlled switch turned the lights on and off, at 12-hour intervals, according to a set schedule. Two schedules of illumination were used: the "regular" schedule provided for light from 6 a.m. to 6 p.m., while the "reversed" schedule provided for light from 6 p.m. to 6 a.m. All of the mice were first placed on the regular schedule for the week of standardization. Thereafter, in the studies of blood glucose, liver glycogen and phospholipid, the regular schedule was continued for some of the mice, while others were placed on the reversed schedule. Either schedule was then maintained unchanged for 8 days (phospholipid) or for 2 weeks (blood glucose and liver glycogen). Finally, the mice on both schedules were killed on the same day, at the same 2 times which were those of the daily "high" and "low" in a given variable (previously established on a regular schedule of lighting, in experiments based upon data from 7 groups of mice, killed at 4-hour intervals, during each of 2 independent 24-hour periods) (8,9, and unpublished data). In these studies

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the groups on the 2 schedules of lighting, were actually killed in succession, at each of the 2 times of sampling--the hours listed with the results representing the mid-points of the period needed for the killing of both groups (the regular and the reversed). This total period of sampling was of about one-hour duration, mice in a given group being killed at 2-minute intervals. Mitotic activity in pinnal epidermis and hepatic parenchyma, and hepatic RNA and DNA metabolism were studied at 2 time points, with mice on a reversed schedule for 8 days (hepatic mitoses and RNA), 2 weeks (DNA) and 23 days (pinnal mitoses), respectively. Earlier data for comparable mice, kept on the regular schedule will be used for comparison(8,9 and unpublished data). The two series on serum corticosterone each describe 7 groups of mice, killed at 4-hour intervals, throughout a 24-hour period; in this study, the group on the regular schedule was sampled first. For the remainder of the mice, the reversed schedule was then instituted and maintained for 2 weeks prior to killing. Moreover, for the study of "corticosterone," pools of serum were used, by contrast to the rest of the data, all of which describe individual mice. Methods described elsewhere were used for determinations of blood glucose(10), blood "corticosterone" (according to Silber, Bush and Oslapas), liver glycogen(11), and relative specific activity (RSA) of hepatic phospholipid (7), RNA(12), DNA(13). The tissues processed for mitotic studies were fixed in Bouin's solution, embedded in paraffin, cut at 5 μ, stained with Heidenhain's iron hematoxylin and mitotic counts were made as previously described(14), all of the counts in a given study being done by the same individual.

Results. The results of this study are shown in the figures, which reveal considerable variability in individual values (height of columns) and/or the standard error of each mean (see shaded areas around means, indicating $\pm$ 1 standard error). The limitations of our illumination which varied from the top to the bottom of the racks as well as from the front to the back of a given cage (see methods), may have contributed substantially to this variability. It is further recognized that with the "spot-checks" carried out herein at only 2 time points on the reversed schedule, the completeness of inversion of rhythm cannot be ascertained, a consideration which may be particularly pertinent to the data on hepatic mitoses (Fig. 1f). We can conclude, however, for all of the functions studied that an important shift of rhythm had taken place following the shift of lighting schedule. It also is pertinent in this connection that except for blood "corticosterone," the day-night differences on both schedules were tested by Student's t and were found to be significant. Blood "corticosterone" is the only variable for which a statistical test was not carried out, since each point, in each series, is based upon only one determination, made on a pool of serum. It is pertinent, though, that the peak in blood "corticosterone" occurred at the time previously predicted on the basis of the daily periodic eosinophil behavior of mice recorded by others as well as ourselves on a regular (15-18) or on a reversed schedule(1). While the occurrence of the daily "corticosterone" peak at the time of periodic eosinopenia suggests that it is real, it is noted that the degree of specificity of the fluorometric procedure used for corticosterone determination has not been ascertained in this study. Therefore, instead of plotting absolute amounts, the relative levels of blood "corticosterone" are given in Fig. 1 d, *i.e.* the changes are expressed as per cent of their 24-hour mean.

With particular reference to Fig. 1 f, it may be noted that the day-night differences in mitotic count of liver parenchyma had changed sign and were statistically significant, at 8 days after inversion of lighting, while at the same time point, mitoses in pinnal epidermis were as yet not reversed (data not graphed). But at 23 days after lighting inversion, the shifted timing of mitoses in pinnal epidermis was clearly apparent (Fig. 1 f). That a shift of rhythm occurs only gradually (rather than abruptly) following the sudden change in lighting regimen has previously been shown elsewhere for rhythms at the level of the body as a whole(2,4) and in our laboratory, with special reference to cellular rhythms(1,19). This study, in turn, demonstrates that such shifts of rhythm (by shifts of lighting schedule) are the rule rather than the exception, as

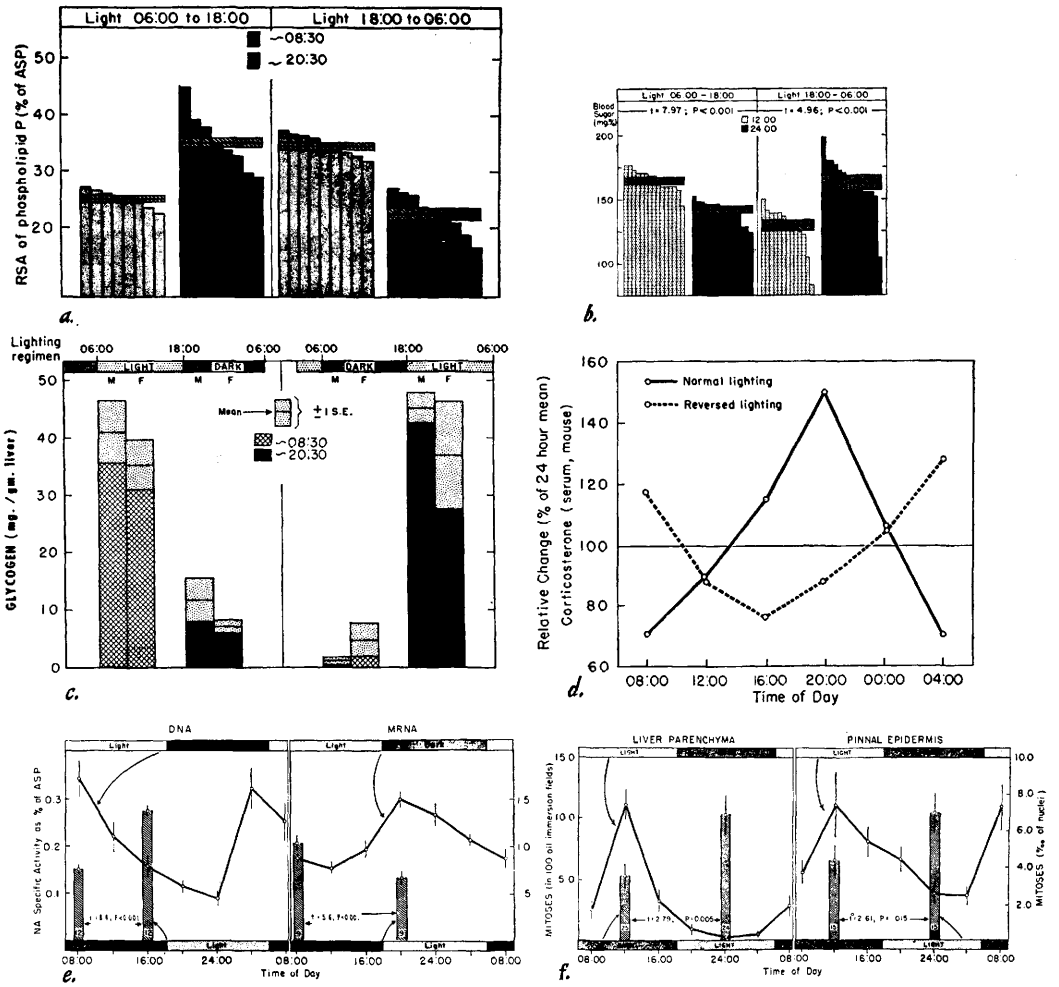

FIG. 1. Physiologic 24-hr rhythms at various levels of organization. Mouse. Data on schedule of light from 6 p.m. to 6 a.m., alternating with 12 hr of darkness, are compared with data on light from 6 a.m. to 6 p.m. Previously published data are used for lines in Fig. 1e(8). t value for day-night difference in epidermal mitoses based upon $\log_{10}$ transformation of counts.

regards 24-hour periodic phenomena at different levels of organization(20-33).

Summary and conclusions. The following variables were studied in the mouse under conditions standardized for evaluation of 24-hour periodicity: glucose and "corticosterone" in blood; mitoses in pinnal epidermis and hepatic parenchyma; liver glycogen and the hepatic metabolism of cytoplasmic phospholipid and ribonucleic acid and of nuclear deoxyribonucleic acid. For each variable, data obtained with light from 6 p.m. to 6 a.m. alternating with 12 hours of darkness, were compared with data obtained with light from 6 a.m. to 6 p.m. A shift in timing of 24-hour rhythms, following the inversion of lighting regimen came clearly and consistently to the fore. Ordinarily, environmental lighting is the dominant synchronizer of various 24-hour rhythmic functions, at different levels of organization of mice: by instituting an appropriate schedule of lighting and by allowing thereafter for the necessary shift time, one may set the peak or trough of rhythm to any chosen clock hour. Moreover, by controlling the timing of various periodic functions with respect to the environment, defined phase relations are brought about among the various physiologic rhythms themselves. The possibility to control timing of these rhythms by an

easily manipulated environmental factor is of obvious practical interest, but it also constitutes a requisite for a basic analysis of functions, within the range in which they vary under ordinary circumstances.

1. Halberg, F., Visscher, M. B., Bittner, J. J., *Am. J. Physiol.*, 1953, v174, 313.
2. Johnson, M. S., *J. Exp. Zool.*, 1939, v82, 315.
3. Halberg, F., *Z. f. Vitamin-, Hormon- und Fermentforschung*, in press.
4. Calhoun, J. B., *J. Tenn. Acad. Sci.*, 1945, v20, 228, 291, 373; 1946, v21, 208, 281.
5. Halberg, F., Visscher, M. B., Bittner, J. J., *Am. J. Physiol.*, 1954, v179, 229.
6. Halberg, F., *Acta Med. Scand.*, 1955, Suppl., 307, 117.
7. Halberg, F., Vermund, H., Halberg, E., Barnum, C. P., *Endocrin.*, 1956, v59, 364.
8. Barnum, C. P., Jardetzky, C. D., Halberg, F., *Texas Rep. Biol. and Med.*, 1957, v15, 134.
9. Jardetzky, C. D., Barnum, C. P., Halberg, F., *Am. J. Physiol.*, 1956, v187, 608.
10. Nelson, J., *J. Biol. Chem.*, 1944, v153, 375.
11. Stadie, W. C., Haugaard, H., Marsh, J. B., *J. Biol. Chem.*, 1951, v188, 167.
12. Barnum, C. P., Huseby, R. A., *Arch. Biochem.*, 1950, v29, 7.
13. Barnum, C. P., Nash, C. W., Jennings, E., Nygaard, O., Vermund, H., *ibid.*, 1950, v25, 376.
14. Chaudry, A. P., Halberg, F., Bittner, J. J., PROC. SOC. EXP. BIOL. AND MED., 1956, v91, 951.
15. Halberg, F., Visscher, M. B., *ibid.*, 1950, v75, 846.
16. ———. *Endocrin.*, 1952, v51, 329.
17. Louch, G., Meyer, R. K., Emlen, J. T., PROC. SOC. EXP. BIOL. AND MED., 1953, v82, 668.
18. Brown, H. E., Dougherty, T. F., *Endocrin.*, 1956, v58, 365.
19. Halberg, F., Bittner, J. J., Smith, D., *Z. f. Vitamin-, Hormon- und Fermentforschung*, 1957, v9, 69.
20. Bykow, K. M., Grosshirnrinde und Innere Organe. *VEB Verlag, Volk und Gesundheit*, Berlin, 1947, pp320.
21. Galbraith, J. J., Simpson, S., *J. Physiol.*, 1903, v30, XIX.
22. Pitts, G. C., *Am. J. Physiol.*, 1943, v139, 109.
23. Elfvin, L. G., Petren, T., Sollberger, A., *Acta Anat.*, 1955, v25, 286.
24. Kleitman, N., *Physiol. Rev.*, 1949, v29, 1.
25. Blumenfeld, C. M., *Arch. Path.*, 1944, v38, 321.
26. Riley, G. M., *Anat. Rec.*, 1937, v67, 327.
27. Hastings, J. W., Wiesner, M., Owens, J., Sweeney, B., *ibid.*, 1956, v125, 611.
28. Brown, F. A., Jr., Webb, H. M., *Physiol. Zool.*, 1949, v22, 136.
29. Panzenhagen, H., Speirs, R., *Blood*, 1953, v8, 536.
30. Litman, T., Halberg, F., Ellis, S., Bittner, J. J., *Endocrin.*, 1958, v62, 361.
31. Halberg, F., Stephens, A. N., *Fed. Proc.*, 1958, v17, 439.
32. Halberg, F., Bittner, J. J., Gully, R. J., Albrecht, P., Brackney, E. L., PROC. SOC. EXP. BIOL. AND MED., 1955, v88, 169.
33. Halberg, F., Barnum, C. P., Ellis, S., Bittner, J. J., *J. Clin. Endocrin. and Metab.*, in press.

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Staphylococcal Enterotoxin: Increased Vomiting Incidence in Monkeys Following Subemetic Doses of Dihydroergotamine.* (23916)

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Very little definitive information is available on mode and site of action of staphylococcal enterotoxin. It has been suggested that action of enterotoxin is peripheral rather than central and that it affects either the sensory nerve endings or the smooth muscle of small intestine(1). Experiments with isolated intestinal strips show no direct stimulatory action of enterotoxin (unpublished, 2). The action of enterotoxin upon some specific area of the central nervous system has also been suggested(3). The results with animals subjected to various denervation and surgical procedures have been interpreted as indicating the site of action in the forebrain(4), but enterotoxin induced vomiting in chronic, totally decorticate cats with the third ventricle and surrounding structures intact(5). The

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