Circadian Timing of Single Daily "Meal" Affects Survival of Mice[1] (37678)

W. NELSON, L. CADOTTE, AND F. HALBERG

Chronobiology Laboratories, Department of Laboratory Medicine and Pathology, University of Minnesota, Minneapolis, Minnesota 55455

The susceptibility of mice and other organisms, including man, to a number of chemical and physical agents varies according to circadian rhythms (1). Reports of time-dependent responses to nutrient intake also accumulate (2–8). The studies described in this report were part of efforts to restrict feeding by mice to a single relatively short span of time each day and to vary the timing of this "meal." The effects of "meal-feeding" have been extensively studied in rats (9–14), but we are aware of only one investigation involving mice (15). The consequences of systematically changing the timing of single meals also appear to be unexplored, apart from the aforementioned study on mice (15), an investigation of differences in anticipatory behavior of rats (16), and early work concerning the hepatic glycogen rhythm (17).

Materials and Methods. Inbred Bagg albino (BALB/c) mice were weaned at 4 weeks of age, housed 6–8 per cage, and placed in light (L) from 0600 to 1800 hr alternating with darkness from 1800 to 0600 hr (an L (0600–1800 hr) regimen) at a room temperature of 24 ± 2°, and with food (Purina Rat Chow) and water continuously available. In a first experiment, 93 females were housed singly at age 5–7 weeks, and were randomly distributed (with stratification by weight) among 4 groups—A, B, C, or D. Each cage had a floor area 6 × 10 in., a height of 5 in., and a wire-grid top holding food and a water bottle, and providing about 30 square in. of open area for ventilation. Each cage was placed into a separate sound-deadened compartment of an environmental housing unit ("hive") so that one mouse could be handled with minimal disturbance of others. Each hive had centrally-controlled temperature (24 ± 1.0°) and lighting. Humidity was not controlled or monitored.

The temporal placement of the 12-hr light span differed among groups so that food could be removed and replaced at different stages of the lighting cycle yet at the same clock hours. Thus, group A and about one-third of group D controls were put on an L (0800–2000 hr) regimen in one hive, group B and another third of group D were placed on an L (2000–0800 hr) regimen in a second hive, while group C and the remaining third of group D were on an L (1200–2400 hr) regimen in a third hive. Following the institution of these different lighting regimens, all animals continued on food and water *ad libitum* for one week. Thereafter, the accessibility of food to mice in groups A, B, and C was restricted to the 0800–1200-hr interval each day for the remainder of the study, while animals in group D remained on *ad libitum* feeding as controls. Thus, mice in group A received food only during the early part of the light span of their daily lighting regimen, while animals in groups B and C received food only during the early and late parts of the daily dark span, respectively (see Fig. 1, first study). Water was freely available to all mice at all times. The condition of the animals was checked twice daily at times of food replacement and removal.

Although groups A, B, and C of this first study were housed in hives controlled at similar temperatures (24 ± 1° at the control point) it seemed possible that, under the burden of food restriction, even small differences

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in environmental temperature within the control limits, or in humidity and ventilation, could be important. Furthermore, although it is known that the lighting regimen is a strong synchronizer of circadian rhythms in mice (18), one week was probably not sufficient to ensure an equivalent resynchronization of all rhythms on each of the three different regimens involved in this study (19). Different results might therefore be obtained on a fixed lighting regimen with different clock-hour spans of food accessibility. To investigate this possibility and other factors which might have contributed to the results of the first experiment, a second study was performed on mice all housed on open shelves in the same room maintained at 24 ± 0.5° and 45 ± 5% relative humidity. Cages from different study groups were intermingled to reduce the possibility of slight environmental differences among groups.

This second experiment involved 92 male mice, 6–10 weeks old, randomly distributed (with stratification by weight) among three groups of 20 each (groups A, B and E) and two groups of 16 (groups C and D). Mice in groups A, B, and E were housed singly in plastic cages with wire covers, identical to those used in the first experiment. Mice in groups C and D were housed in subgroups of 4 in plastic cages with a floor area 6 × 6 in., a height of 5 in., and a plastic top with slots providing about 12 in.^{2} of open area for ventilation. Food and water were supplied from the side and were freely available to all groups during a 3-day span of adjustment, with light from 0600–1800 hr.

Food accessibility was then abruptly restricted to the early light span (0600–1000 hr) for groups A and C, and to the early dark span (1800–2200 hr) for groups B and D (see Fig. 1, second study). The change from *ad libitum* to time-restricted feeding began with a 20-hr span of food deprivation for all 4 groups. Group E remained on *ad libitum* feeding as a control. Water was freely available to all groups at all times. Daily food consumption was determined by weighing food containers before the 4-hr span of food accessibility. Through oversight, food weighing for the grouped mice (C, D) did not begin until the second day of restriction. Survival was checked 4 times a day—at 0600, 1000, 1800 and 2200 hr.

Results. Figure 2 presents survival data for the 4 groups in the first study and shows that, while none of the group D control animals died, mice restricted to 4 hr of food accessibility in the early light span (group A) began dying within 3 days, with 50% mortality by the fifth day. On the other hand, mice in groups B and C, receiving food only during the early or late dark span, respectively, began dying on days 5 or 6, with 35% mortality by day 13, at which time the study was discontinued. A chi-square test comparing group A with groups B and C combined, on day 13, indicates a statistically significant difference in survival ($\chi^2 = 18.6$, $p < 0.005$).

Results of the second study are presented

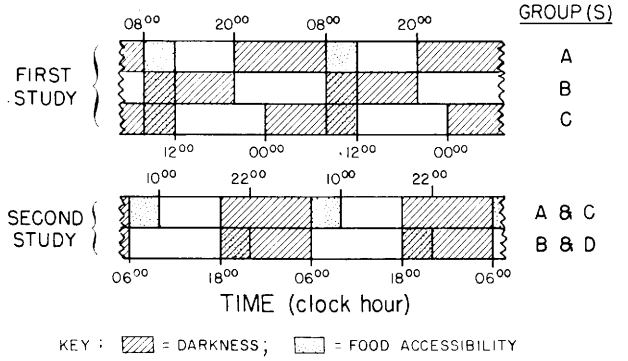

FIG. 1. Relations between feeding and lighting regimens. Two 24-hr cycles shown. In first study, clock hours of food accessibility were identical for all restricted groups but times of lighting changes differed. In second study, clock hours of food accessibility differed among groups while lighting changes were at the same times for all.

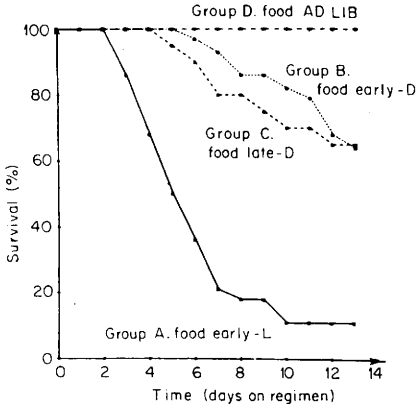

FIG. 2. Survival of singly-housed young female mice after restriction to 4-hr span of daily food accessibility at one of three different phases of lighting regimen [LD (12:12)]. Initial group sizes: A = 28, B = 28, C = 20, D = 17.

in Fig. 3. One of the group A mice escaped on the third day of restriction and had access to nearby food containers prior to recapture; this mouse was eliminated from the study. Statistically significant differences in survival of singly-housed mice, dependent on the timing of food accessibility, appeared on the fifth day of restriction ($\chi^2 = 4.32$, $p < 0.05$). By the seventh day only 2 mice survived from the group of 19 allowed food only during the early-light span (group A) while there were 13 survivors out of the 20 group B mice allowed food only during the early dark span ($\chi^2 = 10.02$, $p < 0.005$). There were no deaths in the *ad libitum* control group (E) nor in groups C and D, despite the fact that the latter, like the singly-housed mice in groups A and B, were abruptly restricted to 4 hr of food accessibility per day.

A comparison of the amounts of food taken daily by mice of groups A–D in the second study is presented in Fig. 4.

Discussion. Rats are reportedly able to survive in good health when food is available for as little as 1–2 hr per day (9, 20). Our results indicate, however, that abrupt restriction of young, singly-housed BALB/c mice to 4 hr of food accessibility daily results in deaths even when feeding is permitted at presumably optimal times (during darkness). Assuming that the size of the mammalian stomach, like that of other organs, is proportional to about the 0.9 power of body weight (21), while metabolic rate is proportional to the 0.75 power of body weight (22), one can perhaps explain this species difference by the approximately 10-fold difference in body weight; *i.e.*, stomach engorgement will not sustain a mouse as long as it does a rat.

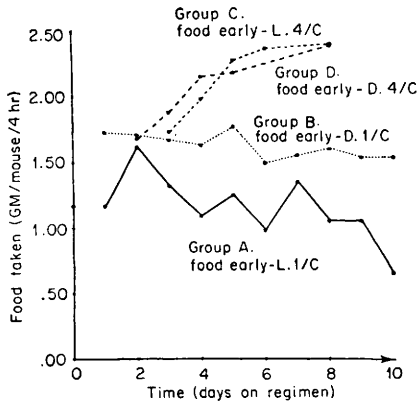

FIG. 4. Food consumption by young male mice as a function of housing conditions and timing of 4-hr span of daily food accessibility.

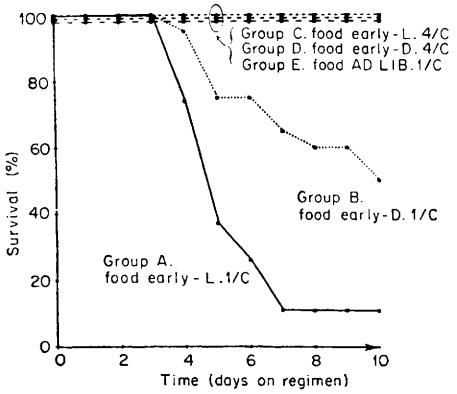

FIG. 3. Survival of young male mice dependent on housing conditions and timing—in relation to lighting regimen [LD (12:12)]—of 4-hr span of daily food accessibility. Initial group sizes: A = 19, B = 20, C = 16, D = 16, E = 20. 1/C = 1 per cage; 4/C = 4 per cage.

On the other hand, our results (Figs. 2 and 3) also indicate that the *timing* of food accessibility can play a critical role. In an earlier study, unknown to us when this investigation was performed, 7 out of 10 C3H/HeJ mice, housed 2 per cage and allowed to feed only between 0600 and 1400 hr (during daylight) each day, died "during the first 3

weeks of the experiment." In contrast, 10 mice similarly housed and permitted daily access to food between 1800 and 0200 hr (in darkness) all survived and "grew at a rate similar to that of controls" (15). The importance of timing probably depends on the extent of departure from habitual feeding times, the speed of a learning process, and the influence of environmental factors on appetite and/or nutritional requirements. When food is available *ad libitum*, mice eat and drink mostly during the dark span (23). The required change in feeding habits is therefore greatest for the animals allowed food only during the early light span. Because changes in food habits require time (24), this group of mice is presumably at a disadvantage in satisfying its needs. Thus, our results on food-taking in the second study (Fig. 4) indicate that in the case of singly-housed mice, the group permitted to feed only in the early light span (group A) took less food on the average than the group given food in early darkness (group B) ($p = 0.01$; sign test of 10 pairs of mean values).

In contrast, mice grouped 4 per cage and restricted to 4 hr of food accessibility in the early light span (group C) or early darkness (group D) appear to increase their food consumption gradually, exceeding the average amounts taken by singly-housed animals, at corresponding times, from the third day onward.

The fact that most singly-housed mice failed to adjust to an abruptly restricted span of food accessibility while all grouped mice responded favorably, regardless of timing, suggests that factors other than a change in feeding habits also play a role. The grouped mice were frequently seen to be huddled, thus reducing heat loss and caloric requirement. The cage microenvironment, influenced by cage design as well as by the presence of other animals, could be an important factor (25).[2]

Summary. The survival of young BALB/c mice after abrupt restriction to a single 4-hr span of daily food accessibility can depend on the temporal placement of this feeding span in relation to the lighting regimen. Housing conditions are an important codeterminant of this response.

[2] Follow-up work shows that raising room temperature to 28° prevents deaths among singly housed mice restricted to food in early-L.

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