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Schedule-Shifts, Circadian Rhythms and Lifespan of Freely-Feeding and Meal-Fed Mice[1]

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NELSON, W. AND F. HALBERG. *Schedule-shifts, circadian rhythms and lifespan of freely-feeding and meal-fed mice.* PHYSIOL BEHAV 38(6) 781–788, 1986.—Mice feeding ad lib were subjected to weekly 12-hr shifts of the daily light-dark (LD) schedule beginning at either 7, 20 or 52 weeks of age and continuing until death. Other mice were meal-fed and, from 7 weeks of age until death, experienced weekly 12-hr shifts of the LD schedule alone (with mealtime fixed) or of both the LD schedule and mealtime. Telemetered core temperature data indicated marked differences in response to the different shift conditions and suggested, in the case of meal-fed animals, involvement of a food-anticipatory rhythm. Shifting of the LD schedule had no statistically significant effect on the mean survival time of mice feeding ad lib, regardless of when shifting began. While meal-feeding in itself prolonged life, the added imposition of schedule-shifting had no statistically significant effect on mean survival time, regardless of whether the meal schedule reinforced or opposed shifts of the LD schedule. In the latter case, tenth-decile survival time may have been increased.

Circadian rhythms Schedule-shifting Lifespan

CIRCADIAN rhythms adjust only gradually to a sudden change in timing of daily environmental schedules. At least several days elapse before circadian maxima occur at times appropriate for the new routine [1,10]. In addition, the rate and even the direction of adjustment may differ for various rhythms [1, 10, 14]. The resulting alteration of relations among rhythms lasts until all achieve resynchronization with the new schedule.

An isolated schedule shift may have only slight and/or short-lived consequences for health. An example in man is the familiar jet lag following transmeridian flight. A study of body temperature and drug susceptibility rhythms in mice subjected to a shift of their daily lighting regimen indicated alternating spans of relative advantage and disadvantage, in comparison with unshifted controls, for several days after the shift [19].

Effects of repeated schedule-shifting over an extended span have been sought in the case of shift-workers, with mixed results [7, 15, 25]. Shifting of the daily lighting regimen repeatedly throughout life may either shorten or lengthen the lifespan of insects, depending on factors such as the interval between shifts [2,13]. Studies on mice in this laboratory indicated that weekly 12-hour shifts of the daily lighting schedule, begun when the animals were about one year old and continuing until death, shortened lifespan. Subsequent investigations in which shifting at either weekly or twice-weekly intervals was instituted early in life found no such effect [11].

As a follow-up on these latter separate observations, the study described herein examined the lifespan of freely-feeding mice exposed to repeated shifts of their daily lighting schedule beginning at different ages. An organism's response to shifting may also depend on the relation between different environmental schedules. With this in mind, the study included mice restricted to a single daily meal and subjected them to weekly 12-hr shifts of either the lighting regimen alone (with mealtime fixed) or of both the lighting regimen and mealtime. Body core temperature was monitored to determine the effects of the various shift schedules on a representative circadian rhythm.

METHOD

Female CD2F$_1$ mice produced in our colony were weaned at about 4 weeks of age and housed 3 per plastic cage (25×15×13 cm), with San-I-Cel bedding (Paxton Processing Co., Paxton, IL), in a room maintained at about 24°C and 50% relative humidity, and on a daily schedule of fluorescent lighting (L) from 0600–1800 hours and darkness (D) from 1800–0600 hours. Food (Purina Laboratory Chow, Ralston Purina Co., St. Louis, MO) and water were continuously available. After 8–9 days under these conditions, the cages

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were distributed among seven groups such that body weight means and variances were similar. The groups were then assigned to seven different treatments: group 1, ad lib feeding (AL), no schedule shifts; group 2, AL, weekly 12-hr shifts of LD schedule (LD shift) beginning at about 7 weeks of age; group 3, AL, weekly LD shifts beginning at about 20 weeks of age; group 4, AL, weekly LD shifts beginning at about 1 year of age; group 5, meal-fed (MF), with half of the animals fed only at the onset of darkness and the other half fed only at the onset of light each day, no shifts; group 6, MF, weekly LD shifts beginning at about 7 weeks of age, mealtime fixed at 1800 hours; group 7, MF, weekly LD shifts and alternation of mealtime between 1800 hours (when lighting was from 0600 to 1800) and 0600 hours (when lighting was from 1800 to 0600), beginning at about 7 weeks of age. Groups on fixed LD and feeding routines remained in the original room while groups subjected to shifts were housed in an adjoining similar room maintained under the same conditions of temperature and humidity.

Shifting of the LD schedule always involved a single 24-hr span of darkness. In the case of group 7, the shift of mealtime was always accomplished by a single shortening of the daily span of food deprivation so that feeding occurred at the onset of darkness both before and after the shift. Thus, for group 7, the effects of shifting mealtime and LD were assumed to be mutually reinforcing. In the case of group 6 mealtime was fixed in terms of clock-hour but, because of the LD shift, alternated between early light and early darkness from week to week, so that shifts of the two schedules were considered to be acting in opposition. The respective shift-schedules continued until all animals were dead.

Meal-feeding for groups 5–7 began when the mice were about 6 weeks old and was implemented with an automatic feeding apparatus [18]. This apparatus permitted control of both the timing and duration of food accessibility. For each of these three groups, the mealspan was adjusted, on the basis of weekly measurements of food consumption, to achieve restriction to about 75% of the food intake by the unshifted AL group. The resulting mealspans varied between 2.75 and 4 hours, depending on the schedule and the stage of the study. Control of food restriction is important because of its known effect on lifespan [17]. Because of the way its mealtime was shifted, group 7 had more meals than did group 6 (15 meals in a 2-week span for group 7 as compared to 14 meals for group 6); to compensate for this, the daily span of food accessibility was shorter for group 7.

The above procedure was performed on four separate cohorts (A–D) of mice produced from BALB/cANN females and DBA/2N males (Charles River Laboratories, Wilmington, MA) at about 3-month intervals. This method of accumulating subjects was adopted because: (1) it allowed the production of a desired large number of mice with a relatively small breeding colony; (2) it could provide evidence that infectious disease was not a factor in determining lifespan, if there were no sudden mortality increases in all cohorts simultaneously; and (3) systematic differences among cohorts in the effects of shifting may indicate a seasonal influence; i.e., mice entering the study at different times of the year might respond differently to the imposed schedules.

The total number of mice involved in the study was 764, with 229, 209, 208 and 118 in cohorts A, B, C and D, respectively. The smaller number in cohort D was due to low production by the breeders; because of this, two of the shifted AL groups (groups 3 and 4 above) were not included in cohort D.

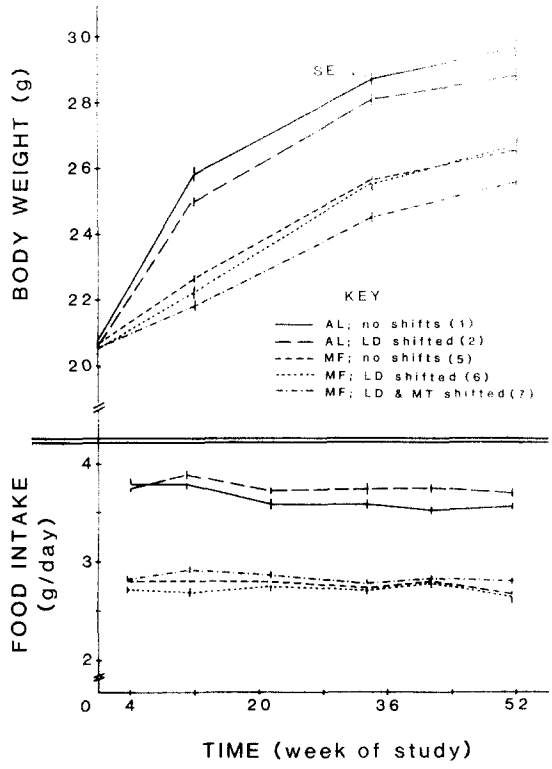

FIG. 1. Food intake and body weight of freely-feeding mice and meal-fed mice with or without weekly schedule-shifts. Data pooled across cohorts, with body weights on 70, 85, 138, 63 and 66 mice and food weights on 26, 33, 52, 26 and 26 cages in groups 1, 2, 5, 6 and 7, respectively. Values for food intake, expressed per mouse, cover a 2-week span and so 2 successive schedule shifts. Abbreviations in key: AL=ad lib feeding; MF=meal-fed; LD=light-dark schedule; MT=mealtime. Group number in parentheses.

Several mice in each of groups 1, 2, 4, 5, 6 and 7 from cohorts A and B were anesthetized with pentobarbital (75 mg/kg) and implanted intraperitoneally with temperature transensors (Model M Mini-Mitter, Mini-Mitter Co., Sunriver, OR). These transensors provided temperature data at 10-minute intervals over a span of several months. The resulting large amount of data was reduced to hourly averages for storage and subsequent examination. At the end of temperature monitoring, the transensors were removed, again under pentobarbital anesthesia.

Mortality, environmental conditions and automatic feeder operation were checked daily. Clean cages, bedding and water bottles were provided at about-weekly intervals, on days when the light-span of the shifted room was from 0600–1800 hours (and so encompassed the caretaker's usual working hours). Food was also replenished at that time. For each cohort, body weight and food intake were determined for groups 1, 2, 5, 6 and 7, initially at about-weekly intervals, and less frequently as the study progressed. Food intake was measured by the change in weight of the food hoppers. There was no evidence of food waste on any of the schedules. Body weights were determined about 8.5 hr after the last food access for the MF groups and about 8.5 hr after the end of darkness (presumably the end of major feeding activity) for the AL groups. This timing was standardized because of the great effect of feeding on the body weight of meal-fed mice

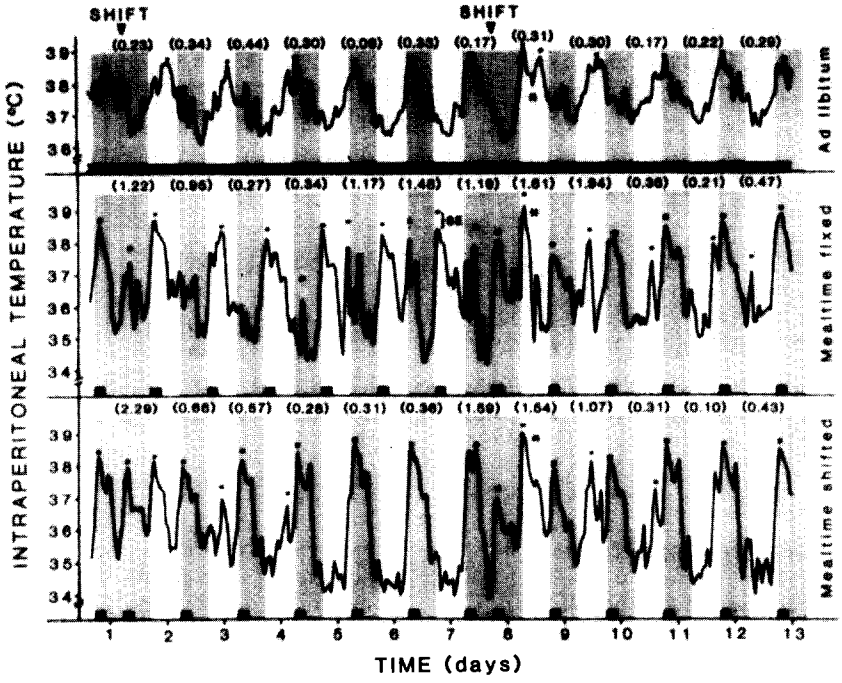

FIG. 2. Effects of schedule-shifting on circadian temperature rhythms. Results from groups 2, 6 and 7 shown in top, middle and bottom rows, respectively. Times of two successive shifts indicated at top of figure. Scale marks on abscissa are at 0000 hours of indicated day. Spans of darkness portrayed by stippling. Food accessibility indicated by bar(s) at bottom of each row. Curves connect hourly means of data telemetered from 4, 6 and 6 mice in groups 2, 6 and 7, respectively. Dots above peaks indicate standard errors of highest hourly means. Asterisks placed near peaks in light span of day 8 indicate probable alteration by disturbance of mice (cage-changing). There was no such disturbance after shift on day 1. Numbers in parentheses across tops of rows are ratios between amplitudes of 12-hr and 24-hr components of mathematical model fitted to each day's data (see text).

[20]. The mice on shifting schedules were weighed at the end of weeks with lighting from 0600–1800 hours (and so with mealtimes in early darkness for group 6 as well as group 7). As mice died, the number of animals/cage was maintained at two or three, by moving survivors among cages of the same cohort and schedule; this was done because huddling was considered an important factor in energy balance and so could affect survival, especially for meal-fed mice.

RESULTS

Data from the four cohorts were pooled to summarize food intake and body weight changes during the first year of study (Fig. 1). These results are from the same mice throughout this span. Spotchecks at intervals thereafter revealed no appreciable departure from the intergroup relations in Fig. 1, despite the influence of deaths on group composition. When expressed as a percentage of food consumption by the unshifted AL group, the across-cohort means (±S.E.) of average weekly values for food intake during the entire first 46 weeks of study (after which weekly food weighings were discontinued) were 102.0±1.8, 75.4±1.0, 74.2±0.9 and 76.2±0.4 for groups 2, 5, 6 and 7, respectively. Analysis of variance [5] indicated that differences among the means of the meal-fed groups were not statistically significant, $F(2,9)=1.64, p>0.05$. The mean body weight of the AL group subjected to weekly LD shifts beginning early in life (group 2) was consistently lower than that of its unshifted control, although its average food intake tended to be greater. At the end of the first year, the difference between the body weight means of these two groups was of borderline statistical significance, $t(153)=2.01$, $p\approx0.05$. Differences among the body weight means of the three meal-fed groups at this time were statistically highly significant, $F(2,264)=9.02$, $p<0.01$. The meal-fed group subjected to shifts of both LD and mealtime (group 7) weighed, on the average, consistently less than the other restricted groups, despite a similar or perhaps slightly higher average food intake.

Graphs of mean hourly intraperitoneal temperature values (Fig. 2) show the responses of groups 2, 6 and 7 to two successive shifts. These results were from mice of cohort A when they were about 4 months old and had been subjected to weekly shifts for over 2 months. Essentially similar results were obtained throughout the span of temperature monitoring, with ages ranging from 15–70 weeks and the number of shifts from 8–63. As in previous studies [20], meal-fed mice exhibited an increased temperature range in comparison with mice feeding ad lib.

The temperature rhythm of mice feeding ad lib (group 2) responded to each LD shift by a gradual phase delay, with-

TABLE 1

LIFESPAN OF FREELY-FEEDING MICE SUBJECTED TO WEEKLY 12-HOUR SHIFTS OF DAILY LD REGIMEN BEGINNING AT 3 DIFFERENT AGES, AS COMPARED TO UNSHIFTED CONTROLS

	Survival-time, weeks (mean ± S.E. (N)*)			
		Shifted, starting at age (weeks)		
Cohort	Controls (no shift)	7	20	52
A	112.9 ± 2.7 (48)	115.6 ± 3.5 (27)	120.6 ± 3.8 (27)	113.6 ± 4.1 (26)
B	115.9 ± 3.4 (46)	122.0 ± 3.5 (26)	112.2 ± 3.6 (27)	117.5 ± 4.5 (27)
C	120.7 ± 2.9 (38)	112.3 ± 5.2 (27)	106.0 ± 4.3 (27)	113.1 ± 3.1 (27)
D	119.1 ± 3.8 (36)	118.6 ± 6.0 (18)		
Overall	116.8 ± 1.6 (168)	116.9 ± 2.2 (98)	112.9 ± 2.3 (81)	114.7 ± 2.3 (80)

*(N)=number of mice.

out marked changes in pattern, and appeared to regain its pre-shift relation to the LD schedule by the fourth or fifth day (Fig. 2, top row). In the meal-fed groups, on the other hand, the pattern of daily temperature variation changed appreciably after each shift. This was especially noticeable in the case of group 7 (Fig. 2, bottom row). Considering first the response to the shift (of both LD and mealtime) on day 7, one sees a change from the single pronounced peak (associated with mealtime and the onset of darkness) on days 5 and 6 to a pattern of two peaks per 24 hours, which persisted for several days. One of these two peaks remained associated with mealtime and darkness while the other occurred during the daily L-span. The same kind of change characterized group 7 after the shift on day 1. Thus, group 7 responded in a similar way regardless of whether the LD shift was from L(0600–1800) to L(1800–0600) or the reverse, presumably because its mealtime also shifted.

In contrast, the response of group 6 (middle row, Fig. 2) to these opposite LD shifts was appreciably different, presumably because of its fixed mealtime. With mealtime occurring in early D (after the shift on day 7), group 6 exhibited two major temperature peaks per 24 hours for several days, one peak associated with mealtime and the other with the L-span—a response similar to that shown by group 7 to its shifts. After the shift on day 1, on the other hand, the daily span of food accessibility for group 6 occurred in early-L and the 24-hr temperature pattern changed from that of a single large peak toward one characteristic of mice habituated to meal-feeding in early-L [20]. That is, by the fourth or fifth day after the shift on day 1, there is a major peak associated with food accessibility and a secondary peak near the span of darkness.

These differences in response of the three shifted groups were quantified by fitting a mathematical model consisting of combined 24-hour and 12-hour cosine curves [3] to each 24-hour span of the three data sets shown in Fig. 2. This model was statistically significant ($p<0.001$) in all but one case (day 1 of group 7) for which $p=0.006$, a value considered to be high in view of multiple testing. For each group and each day, the temperature waveform was then roughly characterized by computing the ratio between the amplitudes of the 12-hour and 24-hour components (i.e., the amplitude ratio, A_{12}/A_{24}). Daily ratios are shown in parentheses at the top of each row in Fig. 2. These results are consistent with impressions gained from the plots of temperature means. That is, the ratio was low on all twelve days for the group feeding ad lib whereas both meal-fed groups showed a high ratio (a prominent 12-hour component) on the shift-days and for a day or two thereafter. In addition, group 6 had an elevated ratio for two days before the shift on day 7.

The response of the temperature rhythm in AL mice to weekly LD shifts beginning at one year of age (group 4; results not shown) was similar to that of mice beginning such shifts at 7 weeks of age (group 2). The patterns of circadian temperature variation in unshifted AL and MF groups were essentially stable throughout the age span examined herein. Support for this statement can be found in a separate report on a concurrent investigation into the effects of different feeding schedules on circadian rhythms and lifespan (in press, *J Nutr*).

Survival times for the AL groups are summarized in Table 1 as a function of schedule and cohort. Following a demonstration by Bartlett's test [24] of variance homogeneity, $\chi^2(13)=16.8$, $p>0.05$, analysis of variance indicated no statistically significant variation among the 14 cell means in this table, $F(13,413)=1.25$, $p>0.05$. This result supports the conclusion that weekly 12-hr shifting of the LD schedule had no effect on lifespan of female CD2F$_1$ mice feeding ad lib, regardless of cohort (season) or the age at which shifting began. Pooled data from the AL groups of the 4 cohorts are summarized as survival curves in Fig. 3 and as overall means and standard errors in the bottom row of Table 1.

Table 2 summarizes data on survival times for the meal-fed animals. Comparison with results from mice feeding ad lib (Table 1) reveals the expected increase in lifespan attributable to food restriction as such [17]. Bartlett's test indicated homogeneity of variance among the 12 cells in Table 2, $\chi^2(11)=9.33$, $p>0.05$. Analysis of variance then indicated that neither cohort nor schedule had a statistically significant effect on mean survival time, $F(11,325)=1.04$, $p>0.05$.

Survival curves of the MF groups are presented in Fig. 4, based on pooled data from the four cohorts. Overall means and standard errors are included in Table 2. In the case of the unshifted control group, there was no statistically significant difference in mean survival time between the subgroup receiving its daily meal in early darkness and that fed in early light, $t(182)=1.04$, $p>0.05$.

The data summarized in the tables and in Figs. 3 and 4 include values from mice which bore a transensor at some stage of their lives. This load had no statistically significant

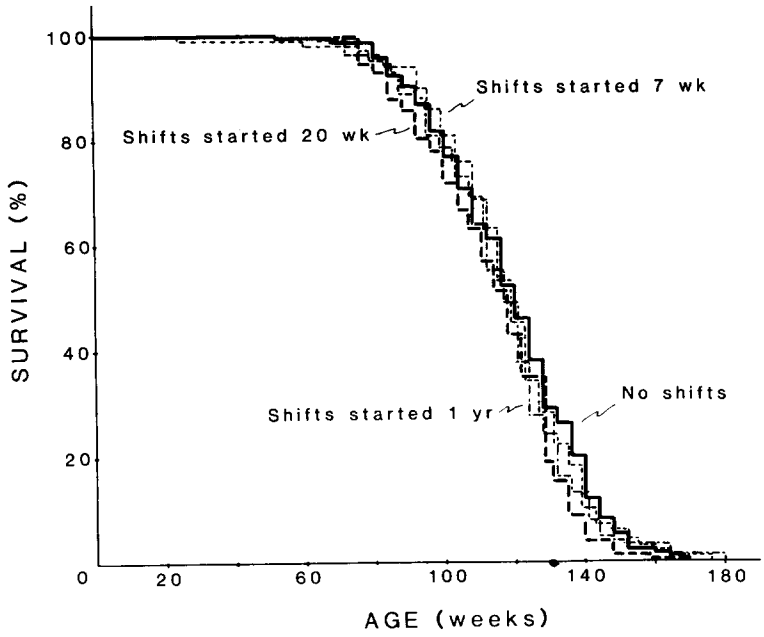

FIG. 3. Survival curves of freely-feeding mice subjected to weekly 12-hr shifts of daily lighting schedule beginning at age 7 weeks (group 2), 20 weeks (group 3) or 1 year (group 4), as compared to unshifted controls (group 1). Data pooled across cohorts from totals of 168, 98, 81 and 80 mice in groups 1–4, respectively. Curves stepped at 4-week intervals rather than at each occurrence of death(s) on weekly basis.

effect on mean lifespan in either the combined AL or the combined MF groups, $t(425)=0.16$, and $t(335)=0.79$, respectively.

In Fig. 4 the divergence of survival curves at later stages prompted the testing of tenth-decile survivorship, a measure of longevity that is relatively unaffected by accidental factors (such as infection) which can alter the overall mean survival time [23]. Application of the Kruskal-Wallis test [24] indicated statistically significant differences in tenth-decile survival times among the three MF groups, $H(2)=9.18$, $0.01<p<0.05$. The tenth-decile means±S.E. (with number of mice in parentheses) were 183.6±2.6 (18), 193.0±1.0 (8) and 184.5±1.6 (8) weeks for groups 5, 6 and 7, respectively.

DISCUSSION

The observation that the mean body weight of freely-feeding mice subjected to weekly LD shifts (group 2) tends to be lower than that of unshifted control animals is consistent with a similar finding on rats [6]. In the present case the slower weight gain was seen to occur despite a slightly greater food intake. Meal-fed mice subjected to weekly shifts of both LD and mealtime (group 7) also exhibited a lower body weight and a slightly greater food intake than did unshifted controls. In considering these results, it must be noted that the day of weighing, in relation to a shift, can affect the result. Thus, weighing mice of shifted groups 2 and 7 on the day immediately before a shift—as was done for the results in Fig. 1—tended to yield lower values than those observed if weighing was done on the day after a shift. The former values were considered to be better measures of true body weight because there is reason to expect that data obtained soon after a shift were influenced by temporarily greater food consumption. Just after a shift, the freely-feeding mice of group 2 presumably continued to feed according to a rhythm synchronized by the preceding LD schedule and so probably were eating up until the time of weighing, toward the end of the light span. The meal-fed mice of group 7 could also be expected to have an elevated body weight soon after a shift because the method of shifting allowed them two meals about 8 hr apart during the shift, as compared to the usual interval of about 20 hr. Such artifactual effects of schedule-shifting on body weight were minimized by weighing late in the week following a shift.

For several days after a shift of both LD schedule and mealtime, the mice of group 7 exhibited two well-defined temperature peaks per 24 hours, one peak at the new mealtime and the second during the new L-span (Fig. 2, bottom row). The timing of this second peak suggests that it relates to a circadian food-anticipatory activity rhythm [22] which, over the course of 4–5 days after a shift, changes its ties from one mealtime to the other. The gradual diminution and delay of the L-associated peak could be indicative of this change. The results from group 6 (Fig. 2, middle row) appear to require a different interpretation. Thus, although the temperature pattern after the LD shift on day 7 is quite similar to that of group 7, the L-associated peak in this case apparently represents a circadian rhythm shifting in response to the changed LD schedule. The markedly different response of group 6 to the LD shift on day 1, as compared to that on day 7, suggests that the results from this group overall reflect changes in the extent of coupling between a food-anticipatory rhythm and the circadian system influenced by the LD schedule.

Repeated shifting of daily schedules may affect health and

TABLE 2

LIFESPAN OF MEAL-FED MICE SUBJECTED TO WEEKLY 12-HOUR SHIFTS OF DAILY LD REGIMEN ONLY OR OF BOTH LD AND MEALTIME, AS COMPARED TO UNSHIFTED CONTROLS

	Survival-time, weeks (mean ± S.E. (N)*)		
		Shifted	
Cohort	Controls (no shift)	LD only	LD and mealtime
A	132.9 ± 3.1 (59)	138.1 ± 7.0 (21)	130.0 ± 6.7 (21)
B	135.9 ± 5.3 (42)	140.2 ± 7.7 (21)	145.0 ± 6.6 (20)
C	143.4 ± 4.5 (47)	150.6 ± 6.4 (21)	146.6 ± 7.2 (21)
D	140.0 ± 4.9 (36)	145.5 ± 9.3 (13)	139.7 ± 7.7 (15)
Overall	137.7 ± 2.2 (184)	143.4 ± 3.7 (76)	140.3 ± 3.5 (77)

*(N)=number of mice.

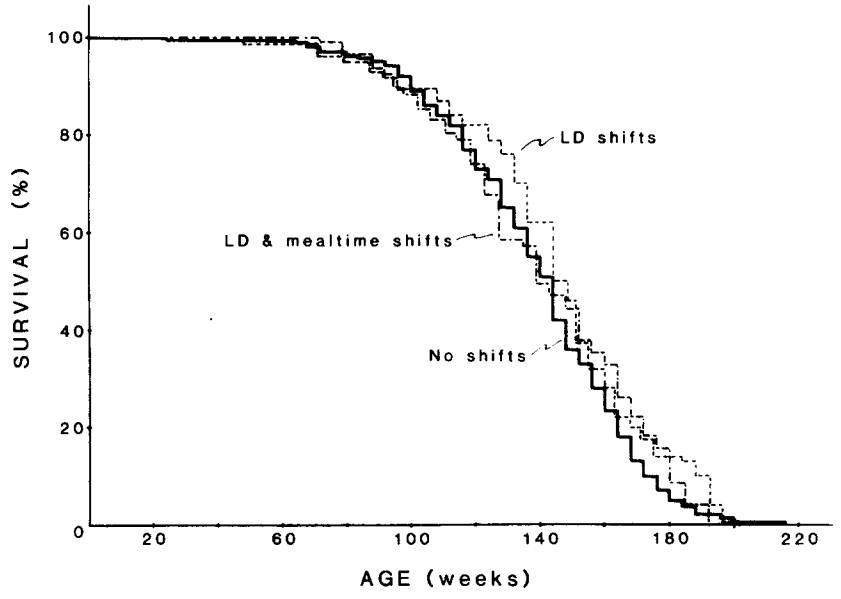

FIG. 4. Survival curves of meal-fed mice subjected to weekly 12-hr shifts of daily LD schedule alone (group 6) or of both LD schedule and mealtime (group 7), as compared to unshifted controls (group 5). Data pooled across cohorts from totals of 184, 76 and 77 mice in groups 5–7, respectively. Curves stepped at 4-week intervals rather than at each occurrence of death(s) on weekly basis.

survival only under certain conditions. Results from earlier separate studies on BALB/c mice suggested that age at the onset of shifting may be important. This could not be confirmed by the present internally-controlled investigation on $CD2F_1$ mice: there was no statistically significant effect of weekly LD shifts on the survival of freely-feeding mice regardless of whether shifting began when the animals were immature, in young adulthood or near middle age. A recent study on rats also failed to find an effect of weekly LD shifting, beginning either early or late in life, on lifespan [6]. The initial observation of a slight adverse effect of LD shifting on survival of BALB/c mice [11] may have been a chance occurrence, although the possibility of a genetic influence should be considered.

Shifting of the lighting schedule alone may not always be sufficient to alter relations among circadian rhythms in mice [14]. This could explain the absence of an effect on survival in the AL groups even though the LD shift produced the expected response of the temperature rhythm (Fig. 2, top row). Meal-feeding imposed a second schedule which either counteracted (group 6) or reinforced (group 7) shifts of the lighting regimen. Neither of these extreme kinds of weekly schedule shifting throughout life had a statistically significant effect on the mean lifespan of meal-fed mice, despite the considerable alteration of circadian rhythms suggested by the temperature results in Fig. 2. On the other hand, an effect on tenth-decile survival times was indicated, with the shifted groups exhibiting an increased tenth-decile mean. There is,

however, reason to doubt that shifting could have altered this index of maximal lifespan without also affecting the overall mean. Thus, results from the cited tests (all with $p>0.05$) indicate that the three complete data sets portrayed in Fig. 4 have the following characteristics in common: (1) a normal distribution (Kolmogorov-Smirnov test [16]); (2) similar variances (Bartlett's test); and (3) similar means (analysis of variance). Given this information, derived from all of the data, one would not expect to observe differences among the tenth deciles except by chance. It should be noted in this regard that, although the tenth-decile means were greater in the shifted groups, the mouse living by far the longest of all (214 weeks) was in the unshifted group. The rationale for considering data on the oldest survivors as being more meaningful than the complete data set also seems weakened in the present case inasmuch as there was no cohort-related effect to indicate the operation of infectious agents on mean survival time.

In any event, there was no indication of a *harmful* effect from any of the shift schedules employed in this investigation; if anything, there may have been a slight increase in longevity for the meal-fed mice, especially those with a fixed mealtime (group 6). A beneficial effect from such a schedule, as compared to that imposed on group 7, could related to the greater irregularity of the 24-hour temperature pattern from week to week (Fig. 2), suggesting a difference in response of circadian rhythms, as discussed above. Another consideration is the possibility that differences in the relation between nutrient intake and body weight gain (Fig. 1) were responsible for any differences in lifespan among the meal-fed groups. This is contraindicated by the following observations: (1) In comparing the three meal-fed groups, the body weight gain and food intake of group 7 were the most unlike those of the unshifted control group, whereas the lifespan indices of these two groups were the most similar; and (2) A comparable effect of shifting on the food intake and body weight gain of mice feeding ad lib was not accompanied by an effect on lifespan.

Studies on insects have indicated that the effect of repeated schedule shifts on survival may be negative, absent or positive, depending on the extent, direction and frequency of shifting [2,13]. Apart from a limited comparison of weekly and twice-weekly shifts [11], none of these factors has yet been tested for effects on the lifespan of rodents. Nevertheless, results of the present study accentuate doubts as to the significance of internal circadian organization for health and longevity [4], at least of rodents and perhaps of other endothermic animals. A similar conclusion can be reached from the observation that mice on different meal-feeding schedules, which produce markedly different relations among circadian rhythmic variables, exhibited no statistically significant differences in mean or tenth-decile lifespan (in press, *J Nutr*). These findings of course do not dispute the importance of circadian rhythms as such. It is well-known that their relation to external events can be crucial for survival. For example, an animal may live or die depending on the circadian stage of exposure to a toxic agent [21].

The external timing of a particular rhythm may be readily interpreted as being optimal for certain biological functions, e.g., in the case of behavioral rhythms related to reproduction or to predation [4]. In view of evidence that circadian rhythms are endogenous, it seems reasonable to expect that there should also be an optimal relation *among* these rhythms, influenced by various physiological and biochemical control mechanisms. The results herein, when considered with others cited, do not support this homeostatic expectation. An explanation for these findings may come from recent chronobiologic investigations revealing novel mechanisms of internal temporal coordination [8, 9, 12].

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REFERENCES

1. Aschoff, J., K. Hoffman, H. Pohl and R. Wever. Reentrainment of circadian rhythms after phase-shifts of the Zeitgeber. *Chronobiologia* **2:** 23–78, 1975.
2. Aschoff, J., U. v. Saint Paul and R. Wever. Die Lebensdauer von Fliegen unter dem Einfluss von Zeit-Verschiebungen. *Naturwissenschaften* **58:** 574, 1971.
3. Bingham, C., B. Arbogast, G. Cornélissen Guillaume, J. Lee and F. Halberg. Inferential statistical methods for estimating and comparing cosinor parameters. *Chronobiologia* **9:** 397–439, 1982.
4. Daan, S. and J. Aschoff. Circadian contributions to survival. In: *Vertebrate Circadian Systems: Structure and Physiology*, edited by J. Aschoff, S. Daan and G. A. Gross. Berlin/Heidelberg: Springer-Verlag, 1982, pp. 305–332.
5. Dixon, W. and F. Massey. *Introduction to Statistical Analysis*. New York: McGraw-Hill, 1969.
6. Finger, F. Does repeated internal desynchronization shorten life? *Chronobiologia* **10:** 127, 1983.
7. Folkard, S., D. S. Minors and J. M. Waterhouse. Chronobiology and shift-work: current issues and trends. *Chronobiologia* **12:** 31–54, 1985.
8. Halberg, F. Quo vadis basic and clinical chronobiology: promise for health maintenance. *Am J Anat* **168:** 543–594, 1983.
9. Halberg, F., F. Guillame, S. Sánchez de la Peña, M. Cavallini and G. Cornélissen. Cephalo-adrenal interactions in the broader context of pragmatic and theoretical rhythm models. *Chronobiologia* **13:** 137–154, 1986.
10. Halberg, F. and W. Nelson. Chronobiologic optimization of aging. In: *Aging and Biological Rhythms*, edited by H. V. Samis and S. Capobianco. New York: Plenum Press, 1978, pp. 5–56.
11. Halberg, F., W. Nelson and L. Cadotte. Living routine shifts simulated on mice by weekly or twice-weekly manipulation of light-dark cycle. Proc XII Int Soc Chronobiol, 1977, pp. 133–138.
12. Halberg, F., S. Sánchez de la Peña and G. Cornélissen. Circadian rhythms and the central nervous system. In: *Circadian Rhythms in the Central Nervous System*, edited by P. H. Redfern, I. Campbell, J. A. Xavier and K. F. Martin. London: Macmillan, 1985, pp. 237–248.
13. Halberg, J., E. Halberg, D. Hayes, R. Smith, F. Halberg, C. Delea, R. Danielson and F. Bartter. Schedule-shifts, life quality and quantity-modeled by murine blood pressure elevation and arthropod lifespan. *Int J Chronobiol* **7:** 17–64, 1979.
14. Haus, E. and F. Halberg. Phase-shifting of circadian rhythms in rectal temperature, serum corticosterone and liver glycogen of the male C-mouse. *Rass Neur Veg* **23:** 83–112, 1969.

15. Knutsson, A., B. Johnson, T. Åkerstedt and K. Orth-Gomér. Increased risk of ischaemic heart disease in shift-workers. *Lancet* **ii:** 89–92, 1986.
16. Lilliefors, H. W. On the Kolmogorov-Smirnov test for normality with mean and variance unknown. *J Am Statist Assn* **62:** 399–402, 1967.
17. McCay, C. M., M. F. Crowell and L. A. Maynard. The effect of retarded growth upon the length of lifespan and upon the ultimate body size. *J Nutr* **10:** 63–79, 1935.
18. Nelson, W., R. Fundakowski, J. Baer, L. Cadotte and F. Halberg. An apparatus for automatically timing access to food by mice. *Lab Anim Sci* **32:** 66–69, 1982.
19. Nelson, W. and F. Halberg. Effects of a synchronizer phase-shift on circadian rhythms in response of mice to ethanol or ouabain. *Space Life Sci* **4:** 249–257, 1973.
20. Nelson, W., L. Scheving and F. Halberg. Circadian rhythms in mice fed a single daily meal at different stages of lighting regimen. *J Nutr* **105:** 171–184, 1975.
21. Reinberg, A. and F. Halberg. Circadian chronopharmacology. *Annu Rev Pharmacol* **11:** 455–492, 1971.
22. Rosenwasser, A. M., R. J. Pelchat and N. T. Adler. Memory for feeding time: Possible dependence on coupled circadian oscillators. *Physiol Behav* **32:** 25–30, 1984.
23. Smith, G. S., R. L. Walford and M. R. Mickey. Lifespan and incidence of cancer and other diseases in selected long-lived inbred mice and their F_1 hybrids. *J Natl Cancer Inst* **50:** 1195–1213, 1973.
24. Sokal, R. and F. Rohlf. *Biometry*. San Francisco: W. H. Freeman & Co., 1969.
25. Taylor, P. J. and S. J. Pocock. Mortality of shift and day workers 1956–1968. *Br J Ind Med* **29:** 201–207, 1972.