Constant Light Extends Life in Hamsters with Heart Disease (43513)

BENJAMIN H. NATELSON,[1] WALTER N. TAPP, SUSAN DRASTAL, JENNIFER GROSS, AND JOHN E. OTTENWELLER
Neurobehavioral Unit, Veterans Affairs Medical Center, East Orange, New Jersey 07019 and Department of Neurosciences, New Jersey Medical School, Newark, New Jersey 07103

Abstract. **Our previous work has shown that constant light can prolong the life of hamsters with heart disease. Although we have seen this result several times, constant light was not protective in our most recent experiment. We undertook this study because we had changed some conditions. As in previous experiments, we found that life in constant light extended life for cardiomyopathic hamsters as compared with others living in a 12:12-hr light:dark environment. A second factor affecting survival was the number of hamsters in a group. Hamsters housed in groups of five lived longer than those housed in groups of two under the same lighting conditions. Data from one experiment suggested that a short photoperiod (6:18 light:dark) was also protective. Although these experiments indicate that the protective effects of different light:dark schedules are not simple ones, they are important because their use may prove to be a helpful adjunct in the treatment of congestive heart failure.** [P.S.E.B.M. 1993, Vol 202]

The decade of the 1980s has opened new therapeutic vistas using light as an adjuvant. Specific treatments include the use of bright light to treat seasonal affective disorder (1) and dermatologic problems (2). In addition to these uses in human medicine, we have been exploring the use of constant exposure to bright light as an adjunct in treating experimental congestive heart failure. Congestive heart failure occurs when the heart can no longer compensate for damage. It is a disabling syndrome producing shortness of breath and fluid retention and is the most common cause for hospitalization of patients over 65 years of age; only 50% of patients are still alive 5 years after the onset of signs and symptoms (3). Unfortunately, this poor outcome occurs despite the use of the best available medicines and so there is a critical need to develop other treatments that could diminish disability and death from heart failure.

Prior to our initiating our studies, we assumed that living in a constant light environment devoid of time cues would prove detrimental to an animal's health and that animals living under such conditions would die sooner than appropriate controls. We based that belief on the notion that jet lag was debilitating and that shift work was often accompanied by health problems (4). Jet lag and shiftwork produce disruptions in entrainment of the body's circadian clock, and we reasoned that constant light would be an extreme way to produce such disruptions.

The idea that life in non-24-hr light:dark schedules was deleterious to health was supported by life-span studies in insects that frequently showed a shortened life-span (5). However, other studies also done on insects have shown that life in non-24-hr light:dark schedules can be lengthened (6). Work in insects as subjects has been extended to simulated jet lag also (7, 8) and has shown that life can be extended dependent on the frequency and time of the simulated jet lag. These data have led to the hypothesis that interactions among rhythms of different phases may be responsible for the increase in life-span seen (9).

Obviously, insects had been chosen for this line of research because of their relatively short life-span, but data on insects may have little applicability to questions of longevity in mammalian species. Unfortunately, when similar studies were undertaken on laboratory rodents, inconclusive effects of living in conditions that produce disruptions in circadian rhythms were found, with some experiments showing prolongation of life (10), some showing no effect on longevity (11), and others showing a detrimental effect (12).

[1] To whom request for reprints should be addressed at Department of Neurosciences, New Jersey Medical School, 88 Ross Street, East Orange, NJ 07018.

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In thinking about these results, we reasoned that these disparate effects might have occurred for one of two reasons: (i) the effects of living in non-24-hr light:dark schedules were not dangerous to health, or (ii) the effects of life in these schedules were beneficial in some disease states and detrimental in others. To evaluate the latter possibility, we decided to do our studies on animals whose life ended due to a unitary disease—heart failure due to progressive heart disease. A secondary advantage of this choice was that the duration of any one experiment would be shorter due to the shorter life-span produced by the endogenous disease.

For our animal model, we chose the cardiomyopathic hamster (CMH), an animal that inherits a process that produces multiple areas of myocardial necrosis that lead to congestive heart failure. The CMH lives about half as long as healthy hamsters (13). Contrary to our initial expectation, in four experiments (14, 15), we found that constant light extended the lives or ameliorated the pathology of CMH as compared with CMH kept on a 12:12-hr light:dark schedule. Thus, the effect of this "phototherapy" was not simply to extend the life of sick animals but also to retard the pathologic process; this suggests that the treatment led to an improved quality of life. A subsequent experiment was designed to begin evaluating the reasons for the life-extending effects of constant light. To begin this analysis, we put the CMH in different light:dark (LD) schedules (16). That study was the first in which we did not find a life-extending effect of constant light. Instead, we found modest life-extending effects of two non-24-hr light:dark schedules—LD 12:13 and LD 6:30. However, because we had not replicated our major effect, we were quite tentative about the significance of these results.

In evaluating that experiment, we found two differences from our prior work. First, because of an equipment failure, we did not put the hamsters into their lighting conditions until 2–3 months later than had been the case in the previous experiments. And second, we housed our animals five to six per cage (i.e., gang-housed) rather than in pairs (i.e., pair-housed), as in our earlier work.

Because we had to determine whether the loss of the effect of constant light was real or not, we immediately initiated this current study in which we sought to compare the effects of life in constant light with life in LD 12:12 and several other lighting conditions.

Materials and Methods

Male CMH (strain CHF-147; Canadian Hybrid Farms, Nova Scotia, Canada) were 1 month old upon arrival in the laboratory. They were pair-housed in "shoe box" cages (12 in × 9 in × 6 in) with Sanicell bedding and had *ad libitum* access to food (Purina mouse chow) and water. Cages were kept on shelves in light-tight closets. Above each shelf was a 4-foot fluorescent lamp (Vitalite; Durotest, North Bergen, NJ) that produced about 1700 lux at the cage floor when illuminated.

Hamsters were allowed 2 weeks to acclimate to our animal facility. Then, on July 5, 1989, they were assigned to one of four experimental groups: constant light ([LL] $n = 24$), LD 12:12 ($n = 24$), LD 6:30 ($n = 24$), and LD 6:18 ($n = 22$). The LL and LD 12:12 groups were done to replicate our earlier finding; this was also the case for the LD 6:30 group. We had originally run that group because it was a non-24-hr LD schedule that was reported to produce entrainment to 24 hr; in addition, this photoperiod is known to stimulate gonadal function (17), i.e., it is photostimulatory. We ran the LD 6:18 group because it had the same 6 hr of light as 6:30, but is a "short day" photoperiod that is known to inhibit gonadal function, i.e., it is photoinhibitory. Previously, we had run the opposite condition of LD 18:6 in our last experiment (16) and had found it to be no different from LD 12:12. A fifth group was housed five per cage in larger shoe box cages measuring 18 in × 9.5 in × 6 in ($n = 30$). These animals lived under the same conditions as the pair-housed group living in LD 12:12 and thus allowed a comparison between the two groups that was different only in the number of CMH living in a cage.

Hamsters were checked daily. Dates of death were logged, and testes were removed and weighed. Testes weights were compared for statistical significance by one-way analysis of variance to determine whether the different lighting conditions altered testes weight at the time of death from heart failure. Kaplan-Meier estimates (18) of the conditional probability of survival to a particular day were computed and are depicted in the survival curves. This technique takes into account the fact that, statistically, the odds of an animal's dying at any given time change as other animals die in the study. Statistical significance was determined on these probabilities by log-rank tests (18). The log-rank test is the appropriate way of comparing survival in different treatments because it provides comparison across the entire survival curve rather than at any single point, as would be the case with χ^2 tests at any single death age.

Stratification is a frequently used technique to examine portions of a survival curve where the hazard function is heterogeneous or discontinuous (18). This is done even when the cause of the change in hazard function is unknown. One example of this is outcome after tumor surgery where differences are found between early and late survival; stratification is commonly used in evaluating data sets such as these. We noticed such heterogeneity in several of the curves. To analyze these differences, we followed the tactic used in evaluating the postoperative tumor outcome data, stratified at the median survival point, and then applied the log-rank test.

Results

Figure 1 shows the survival curves comparing hamsters in constant light with those in LD 12:12; hamsters living in constant light lived significantly longer (χ^2 = 3.9; $P < 0.049$); the protective effect was exerted over the entire survival curve (χ^2 = 4.5 and 5.3 for first and second half of curve, respectively; $P = 0.033$ and 0.021). Median life-span was 13% longer in LL (401 days) than in LD 12:12 (355 days). Hamsters in LD 12:12 had a 50% chance of living 355 days, whereas those in LL had a 70% chance of living that long.

Figure 2 compares the LD 12:12 group with animals living in an entrained short-photoperiodic day, LD 6:18. No significant difference was found in survival across the entire curve; however, the short day condition did protect hamsters in the first half of the curve (χ^2 = 8.9; $P < 0.003$). In the first half of the survival curve, median survival was 11% longer in LD 6:18 (356 days) than in LD 12:12 (320 days). No significant differences existed in the survival curves between the LD 12:12 hamsters and those living in LD 6:30.

No significant difference in survival was noted across the entire survival curves for gang-housed hamsters living in LD 12:12 compared with pair-housed hamsters in the same condition, but gang-housed hamsters lived significantly longer in the second half of the curve (χ^2 = 4.1; $P < 0.043$; Fig. 3). In the second half of the curve, median survival was 16% longer in gang-housed CMH (481 days) than in paired CMH living in LD 12:12 (416 days).

Testes weight ordered from heaviest to lightest as follows: LD 6:18 > LL > LD 12:12 (pairs) > LD 6:30 > LD 12:12 (groups of five). The only significant difference was between the LD 6:18 (mean ± SE, 1.46 ± 0.18 g) and the gang-housed groups (0.80 ± 0.13 g; $P < 0.006$). These data are difficult to interpret in terms of photoperiodic effects because they also reflect marked inhibition of reproductive function by a lethal disease process.

Discussion

A major finding in this paper was the replication of our original basic finding that constant light extends life in CMH. That raises the question of why we failed to replicate this effect in our last report (16). In that

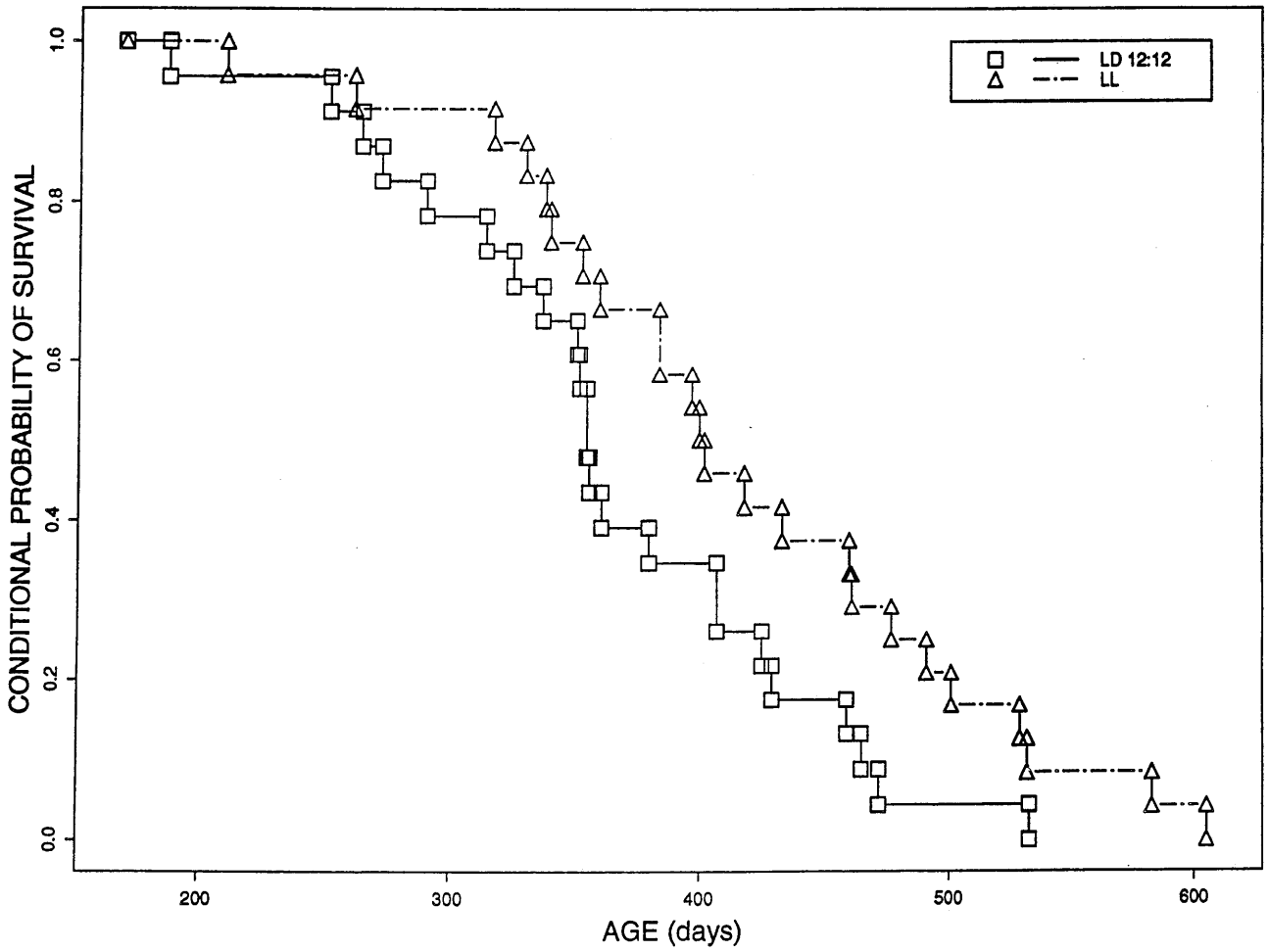

Figure 1. Survival curves for CMH living in constant light and 12:12-hr light:dark. Hamsters living in constant light lived significantly longer than those in LD 12:12.

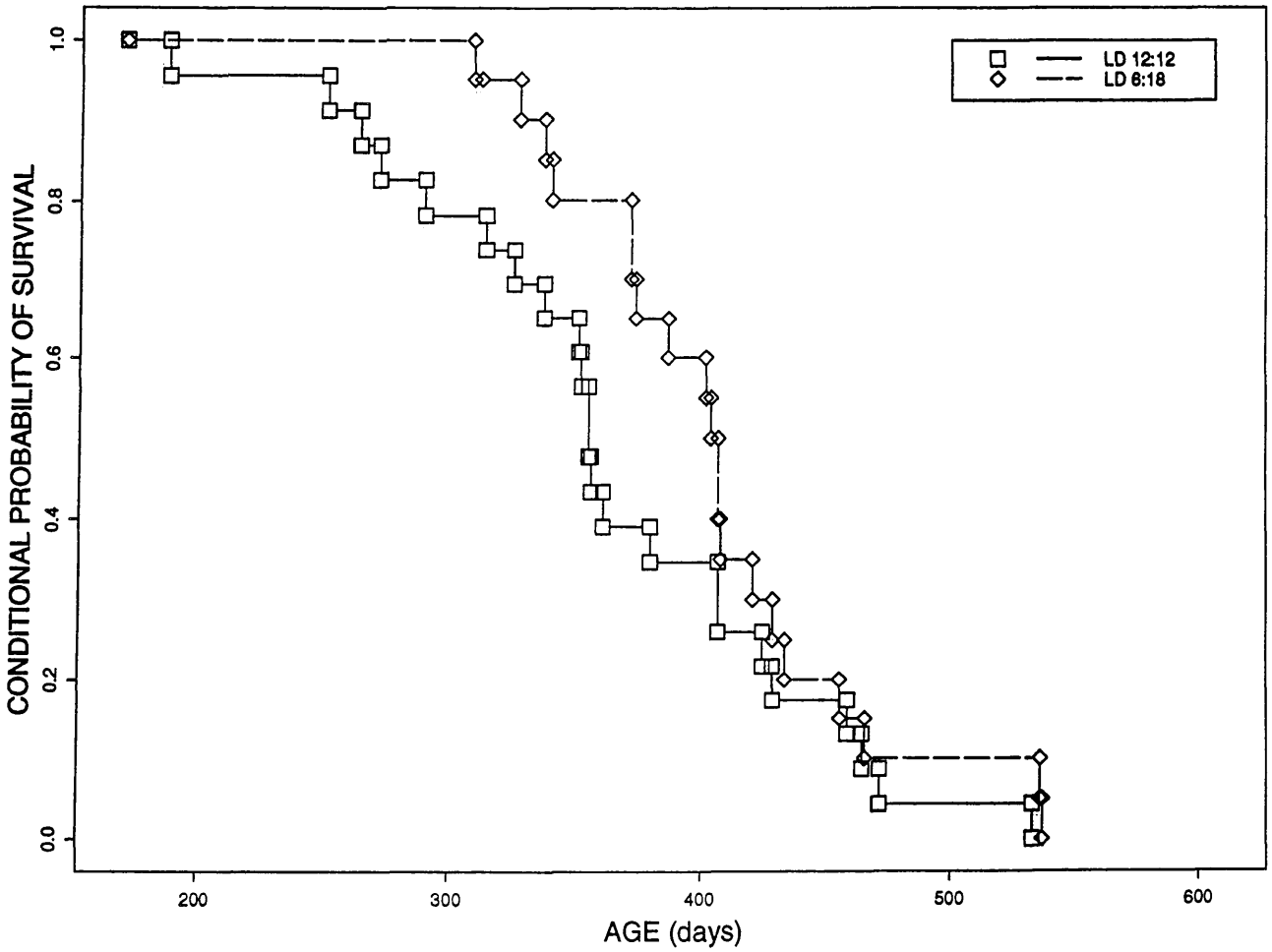

Figure 2. Survival curves for CMH living in LD 12:12 and LD 6:18. Although overall survival curves were not different between the two groups, hamsters living in LD 6:18 lived longer than those in the other lighting condition during the first half of the survival curve.

experiment, we noted that CMH lived much longer than in our earlier studies (14, 15). The median survival of CMH living in LD 12:12 in that study was 465 days, whereas the longest median survival in LD 12:12 hamsters from all our previous studies was 358 days. Our survival data for LD 12:12 CMH from this study (i.e., 355 days) support our belief that the longer life-spans shown in our last study (16) somehow interfered with the life-extending effect of life in constant light.

Although one explanation for the longer life-spans in the previous study might have been "batch-to-batch" variation as we suggested, the data from this experiment point to an additional possibility. In order to increase sample size with the housing available in our last experiment, we gang-housed our hamsters in groups of five rather than in pairs, as we had done originally. To evaluate the effect of living in larger groups, we compared the life-span of gang-housed CMH living in LD 12:12 to that of pair-housed CMH living in the same conditions. Although a significant difference was not found across the entire survival curve, gang-housed CMH did live longer than the pair-housed animals during the second half of their survival curve. Although carefully controlled studies of housing density on life-span have not been done, Masoro (19) notes that gang-housed, barrier-bred rats live longer than singly housed rats, and Ratcliffe *et al.* (20) reported that coronary arteriosclerosis is more advanced in pair-housed than in grouped swine. Although further research will be needed to determine the role of housing density in affecting life-span, our data plus the references cited support the idea that at least part of the reason for our not seeing an effect in our last study was related to our use of gang housing.

A second finding in this experiment was that a short photoperiod (i.e., LD 6:18) protected CMH for the first half of their survival curves as compared with another lighting schedule (LD 12:12) which we have shown also acts as a short photoperiod. These results were surprising to us in that they could in no way explain the enhanced survival produced by life in constant light. The LD 6:18 group was interesting in that it had the largest testes of all groups studied in this experiment. This may be important because hamsters are different from other mammals in that gonadectomy shortens life (21), and some of our previous data have

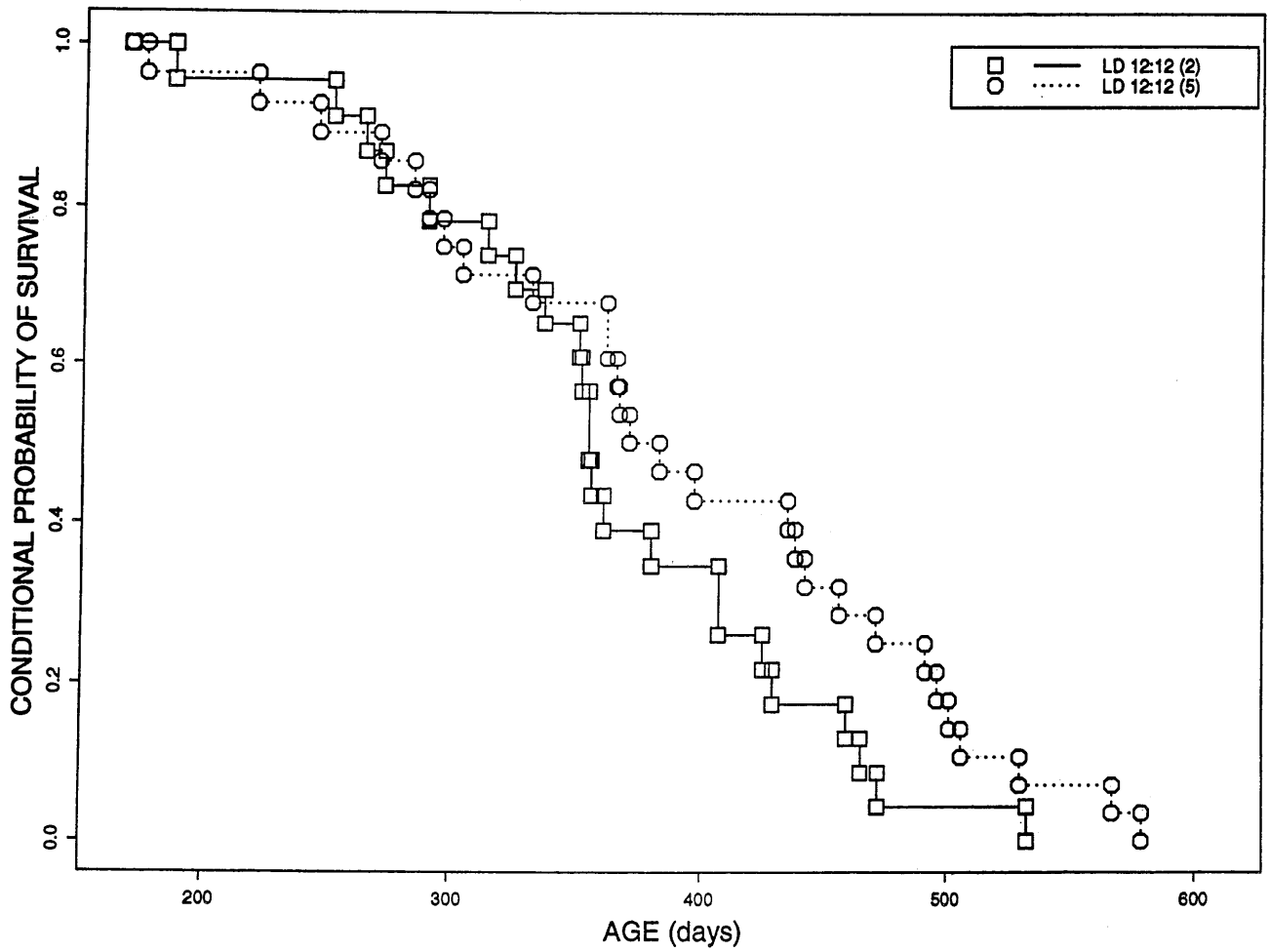

Figure 3. Survival curves for CMH living in LD 12:12 in pairs and in groups of five. Although overall survival curves were not different between the two groups, gang-housed CMH lived longer than pair-housed CMH in the latter half of the curve.

suggested that life in constant light could exert its effect through the testes (15). Thus, the marginally prolonged life in this group could relate to enhanced testicular activity. However, before additional explanations are given for the protective effect of short photoperiods, we believe these data must be replicated and testes size determined throughout the hamsters' lives rather than just terminally.

Our work makes it clear that replication is critical in exploring the life-extending effects of life in different LD schedules. Constant light has been protective in five of six studies. In contrast, in the one study where constant light was not protective, we found that CMH living in LD 6:30 lived longer than our LD controls. Because of the loss of our main effect, however, we were unsure of the reliability of the LD 6:30 effect and so were loathe to consider mechanism of protection at that time. That caution is reinforced by the results of this experiment, where we found that CMH living in LD 6:30 do not live longer than others housed in LD 12:12.

In summary, the data from this experiment again show the life-prolonging effect of living in constant light, an environment without time cues. Therefore, although the effects of using different lighting schedules to alter the hazard of medical disease are not simple, once again we find them to be ameliorative in this animal model of disease. With our understanding of the consequences of gang housing CMH, we can now return to our prior conditions of pair housing to define further those conditions that accentuate and produce the photobiologic therapeutic effect of life in constant light.

In addition to this line of research, we have begun to speculate as to mechanisms by which constant light produces its therapeutic effect. The first of these has to do with biological rhythms per se. One possibility is that the CMH's free-running rhythm is more healthful than a 24-hr entrained rhythm. Another possibility is that rhythms of different periods exist under constant conditions and that the interactions among these rhythms can be healthful; Halberg and colleagues have termed such a mechanism as "feedsideward," as opposed to feedback or feedforward (9). In unpublished work in nonhuman primates, we have found that temperature rhythms continue to free run in LL, whereas

activity rhythms lose their circadian envelope and appear "broken down" or ultradian. Finding similarities in the CMH would support the Halberg hypothesis. A second set of possibilities has to do with endocrine factors. One of these has to do with increased testicular function, a possibility discussed in another part of this paper. A second possibility has to do with the effects of constant light on the pineal gland. Bright light functions to turn off the pineal, and data exist that lack of melatonin could extend life (22).

We are currently designing experiments to test these various hypotheses. Continuing this line of research will be important because our work suggests that photobiologic treatments may be important adjunct therapies in the treatment of congestive heart failure—a common medical diagnosis with a lethal outcome.

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1. Rosenthal NE, Sack DA, Skwerer RG, Jacobsen FM, Wehr TA. Phototherapy for seasonal affective disorder. J Biol Rhythms **3**:101–120, 1988.
2. Parrish JA, Rosen CF, Gange RW. Therapeutic uses of light. Ann NY Acad Sci **453**:354–364, 1985.
3. Francis GS. Heart failure in 1991. Cardiology **78**:81–94, 1991.
4. Tasto DL, Colligan MJ, Skjei EW, Polly SJ. Health consequences of shift work (NIOSH. SRI Project URU-4426, Contract No. 210-75-0072). Washington, DC: U.S. Government Printing Office, 1978.
5. Pittendrigh CS, Minis DH. Circadian systems: Longevity as a function of circadian resonance in *Drosophila melanogaster*. Proc Natl Acad Sci USA **69**:1537–1539, 1972.
6. von Saint Paul U, Aschoff J. Longevity among blowflies *Phormia terraenovae* R.D. kept in non-24-hour light-dark cycles. J Comp Physiol **127**:191–195, 1978.
7. Hayes DK, Halber E, Cornelissen G, Shankarajah K. Frequency response of the face fly, *Musca autumnalis* (Diptera: Muscidae), to lighting-schedule shifts at varied intervals. Ann Entomol Soc Am **79**:317–323, 1986.
8. Marques MD, Cutkomp LK, Cornelissen G, Marques N, Halberg F. Life span of springtail on 12-hr shifts at varying intervals of 12-hr alternation in ambient temperature. In: Pauly JE, Scheving LE, Eds. Advances in Chronobiology, Part A. New York: Alan R. Liss, pp257–264, 1987.
9. Nelson W, Halberg F, Shankarajah K. Rhythm scrambling: Good, bad or indifferent. Chronobiologia **14**:291–295, 1987.
10. Nelson W, Halberg F. Schedule-shifts, circadian rhythms, and lifespan of freely-feeding and meal-fed mice. Physiol Behav **38**:781–788, 1986.
11. Finger FW. Effects of atypical illumination schedules. In: Brown FM, Graeber RC, Eds. Rhythmic Aspects of Behavior. Hillsdale, NJ: Erlbaum Associates, pp345–362, 1982.
12. Brown FM. Two-factor illumination effect hypothesis for viability decreases in animals under altered 24-hour days. In: Halberg F, Scheving L, Powell I, Hayes DK, Eds. Chronobiology: Proceedings of the 13th International Conference, Pavia. Milan: II Ponte, pp91–96, 1981.
13. Ottenweller JE, Tapp WN, Chen TS, Natelson BH. Cardiovascular aging in Syrian hamsters: Similarities between normal aging and disease. Exp Aging Res **13**:73–84, 1987.
14. Tapp WN, Natelson BH. Life extension in heart disease: An animal model. Lancet **1**:238–240, 1986.
15. Tapp WN, Natelson BH, Khazam C, Ottenweller JE. Gonadal function during prolongation of life produced by constant light in hamsters with heart failure. Physiol Behav **40**:243–246, 1987.
16. Tapp WN, Ottenweller JE, Natelson BH. Effect of different light-dark schedules on survival from heart failure. Life Sci **46**:1739–1746, 1990.
17. Elliott JA. Circadian rhythms and photoperiodic time measurement in mammals. Fed Proc **35**:2339–2346, 1976.
18. Kalbfleisch JD, Prentice RL. The Statistical Analysis of Failure Time Data. New York: Wiley, 1980.
19. Masoro EJ. Mortality and growth characteristics of rat strains commonly used in aging research. Exp Aging Res **6**:219–233, 1980.
20. Ratcliffe HL, Luginbuhl H, Schnarr WR, Chacko K. Coronary arteriosclerosis in swine: Evidence of a relation to behavior. J Comp Physiol Psychol **68**:385–392, 1969.
21. Kirkman H, Yau PKS. Longevity of male and female, intact and gonadectomized, untreated and hormone-treated, neoplastic and non-neoplastic Syrian hamsters. Am J Anat **135**:205–220, 1972.
22. Hoffman RA, Johnson L, Brown G. Growth and development of golden hamsters: Influence of the pineal gland, melatonin, and photic input. In: Brown GM, Wainwright SD, Eds. The Pineal Gland: Endocrine Aspects. New York: Pergamon Press, pp289–293, 1985.