OFFICIAL ORGAN OF THE RADIATION RESEARCH SOCIETY

RADIATION RESEARCH

EDITOR-IN-CHIEF: DANIEL BILLEN

Volume 99, 1984

ACADEMIC PRESS, INC.

San Diego Orlando New York London
Toronto Montreal Sydney Tokyo

0033-7587/84 $3.00

MADE IN THE UNITED STATES OF AMERICA

RADIATION RESEARCH

OFFICIAL ORGAN OF THE RADIATION RESEARCH SOCIETY

VOLUME 99, 1984

Councilors, Radiation Research Society 1984–1985

PHYSICS

G. L. Brownell, Massachusetts General Hospital

C. C. Ling, George Washington University

BIOLOGY

D. B. Leeper, Thomas Jefferson University

E. A. Blakely, University of California, Berkeley

MEDICINE

M. A. Bagshaw, Stanford University

L. J. Peters, University of Texas

CHEMISTRY

J. E. Biaglow, Case Western Reserve University

G. E. Adams, Institute of Cancer Research, Sutton, Surrey, England

AT-LARGE

J. D. Chapman, Cross Cancer Institute, Edmonton, Alberta, Canada

E. A. L. Travis, University of Texas

CONTENTS OF VOLUME 99

NUMBER 1, JULY 1984

NUMBER 2, AUGUST 1984

SYMPOSIUM: *In Vitro* Oncogenic Transformation

REGULAR ARTICLES

CORRESPONDENCE

NUMBER 3, SEPTEMBER 1984

The Subject Index for Volume 99 will appear in the December 1984 issue as part of a cumulative index for the year 1984.

RADIATION RESEARCH 99, 285–293 (1984)

Acute Radiation Injury of Mice and the Influence of Sudden Time Shift[1]

ALEXANDER L. GERBES,* BERNHARD ARBOGAST,† PETER SCHICK,* AND OTFRIED MESSERSCHMIDT*

*Laboratories of Experimental Radiology, Sanitätsakademie der Bundeswehr, Ingolstädter Landstrasse 2, 8042 Neuherberg, Federal Republic of Germany
†Institute of Social Pediatrics, University of Munich, Lindwurmstrasse 131, 8000 Munich 2, Federal Republic of Germany

GERBES, A. L., ARBOGAST, B., SCHICK, P., AND MESSERSCHMIDT, O. Acute Radiation Injury of Mice and the Influence of Sudden Time Shift. *Radiat. Res.* **99**, 285–293 (1984).

The daily light–dark regimen for two groups of mice was advanced by 8 hr. A third group remained in unchanged lighting conditions. At seven different times within the following day subgroups of the time-shifted mice as well as of the group with unchanged time schedule were exposed to whole-body X irradiation. Mortality, body weight, and temperature of each animal were registered for 30 days following exposure and were regarded as indicators of radiation response. Radioresistance was found to be highest after two-thirds of the daily light span, confirming earlier reports by other authors. Well-defined effects of time shift and a corresponding shift of the maximum of radioresistance could be demonstrated. With the individual body weight as an independent variable, mathematical formulas for survival prognosis could be established.

INTRODUCTION

In the course of evolution on our rotating planet, circadian rhythms may have developed as an adaption to many variations in the environment following an estimated 24-hr cycle (*1, 2*). The extent of an organism's response to drugs or harmful agents in many cases also shows diurnal rhythms (*3–5*). To date there have been numerous investigations on the possible dependence of mammalian radiation response on the phase of circadian systems at exposure time (*6–8*). After some contradictory results (*9, 10*), it now seems generally accepted that animals such as rats (*11, 12*) and mice (*13–15*) exhibit lower radiation susceptibility during the daily light compared to the daily dark span.

Sudden time shifts of synchronizer phase, e.g., after transmeridional flights, are known to bring about considerable internal desynchronization of biological systems (*16–18*). However, to date there have been no investigations on the influence of time shift on radiation susceptibility. Considering the aforementioned reports it seemed worthwhile to evaluate the circadian rhythm of radioresistance, to investigate the influence of sudden time shift before irradiation, and to establish possible mathematical models of survival prognosis.

[1] The authors dedicate this paper to Professor Dr. Gustav Paumgartner in honor of his fiftieth birthday.

0033-7587/84 $3.00

MATERIALS AND METHODS

Animals. Male C3H mice were purchased from GSF, Munich, and housed five animals per cage (Macrolon) under standard laboratory conditions (24 ± 1°C temperature, 65% humidity, Altromin hard role food and tap water *ad libitum*). The illumination regimen was L (light) 0600–2100, D (dark) 2100–0600 for group I, corresponding to the natural day–night ratio during time of experiment and L 1400–0500, D 0500–1400 for groups II and III. Each group consisted of 70 mice. Age of the animals at time of irradiation was about 10 weeks.

Time shift. One week before irradiation the rectal temperature of every mouse was taken at seven approximately equidistant times during 1 day (Telethermometer 43 TF, temperature probe 520, Yellow Springs Instrument Co., Ohio). Best fitting cosine curves (Fig. 1) demonstrate an average 8-hr phase difference between temperature rhythms of group I and groups II and III, corresponding to the different lighting schedules. One week later groups II and III were time shifted: At 0930, after half of their daily dark span, the mice of group II and at 2130, after half of their daily light period, the mice of group III were transferred to a room with the L–D schedule of group I. The bar diagram of Fig. 2 explains the lighting regimens and the time shift manipulations. The next morning groups I to III were divided in seven subgroups each. Subgroups 1 to 7 of each group were irradiated at seven approximately 3.5-hr intervals: subgroup 1 at 1000, subgroup 2 at 1330, subgroup 3 at 1700, and so on.

Irradiation. The unanesthetized mice were exposed to whole-body X irradiation (X-ray unit MG 300, CHF Müller, Hamburg, FRG; 250 kV, 12 mA, half-value layer 1.9 mm Cu, dose rate 88 cGy/min). At a focus mouse distance of 40 cm the total dose was 640 cGy (simultaneous dosimetry with Duplex Dosimeter, PTW, Freiburg, FRG). For 30 days following irradiation, rectal temperature and body weight of each animal were recorded daily at fixed times.

Statistical analysis. The statistical analysis was done on a CYBER 175 computer using SPSS library routines as well as our own FORTRAN programs. From the raw data the following variables were calculated as indicators of radiosensitivity:

$TEMP_{min}$	minimal rectal temperature recorded during observation
$TEMP_{diff}$	$TEMP_0 - TEMP_{min}$, where $TEMP_0$ means normal temperature at day before irradiation; all temperature measurements were performed at corresponding times of day
$WEIGHT_{diff}$	$\log(WEIGHT_0/WEIGHT_{min})$, where $WEIGHT_0$ means body weight at 1 day before irradiation and $WEIGHT_{min}$ means minimal weight recorded during observation
MST	mean survival time after irradiation in days
Percentage survivors:	percentage of surviving animals per subgroup

Means and standard deviations for all variables were calculated within each of the seven subgroups of groups I to III separately. Subgroups with high and low radiation response, respectively, were identified by multiple classification analysis. Linear contrasts and *t* tests showed significant differences between high (group I: 1, 5, 6, 7—groups II, III: 1, 2, 3, 7) and low sensitivity subgroup categories (group I: 2, 3, 4—groups II, III: 4, 5, 6).

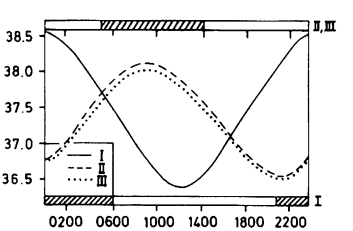

FIG. 1. Best fitting cosine curves for circadian rhythms of rectal temperature (°C) in groups I to III before time shift. Bars at the bottom and at the top indicate L–D schedules for group I and groups II and III, respectively.

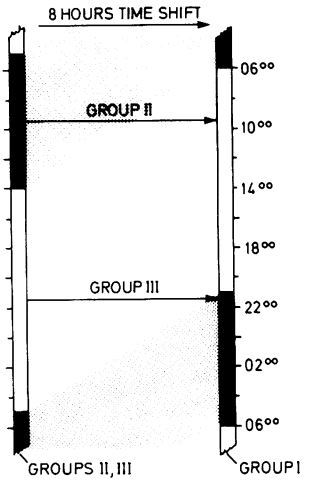

FIG. 2. L–D schedules and manipulations to induce time shift. Dotted lines indicate times of transfer for groups II and III to L–D regimen of group I.

RESULTS

Circadian Rhythm of Radiosensitivity

Lethality as well as mean survival time (MST) within 30 days postirradiation is generally regarded as indicators of radiation effects in mice. Figure 3a exhibits MST of groups I to III as a function of exposure time (subgroups 1 to 7). In group I MST proved to be significantly higher in animals irradiated during the light compared to the dark span. Since the time shift was not performed until shortly before irradiation, the radioresistance of groups II and III was found to be significantly higher in animals irradiated at times of day corresponding to the light span during their previous lighting schedule. The same held true for the overall lethality (see Table I).

During acute radiation sickness with the "hematopoietic syndrome" (*19*), the critical period is the second and third weeks after exposure. In many animals body temperature as a marker of metabolic activity sank considerably, in prefinal stages even to as low as 34°C. The lowest rectal temperature of each animal during the time of observation ($TEMP_{min}$, Fig. 3b) and the difference between the individual normal body temperature before irradiation and the lowest temperature ($TEMP_{diff}$, Fig. 3c) were regarded as suitable indicators of radiation susceptibility (see Material and Methods). Again highly significant advantages were seen for the mice exposed during the light phase. Changes in body weight usually are considered to be concomitant with alterations of murine state of health. The analysis of weight loss ($WEIGHT_{diff}$, Fig. 3d), however, showed no significant differences between the high and low resistance categories. Observation of weight loss on a day to day basis, e.g., of group II, on the other hand, showed significant advantages for the high resistance subgroups after the onset of radiation sickness (Fig. 4).

To define the most resistant/sensitive time more precisely, the data of Fig. 3 were subjected to approximation by cosine functions. MST and $TEMP_{min}$ (Figs. 5a, b)

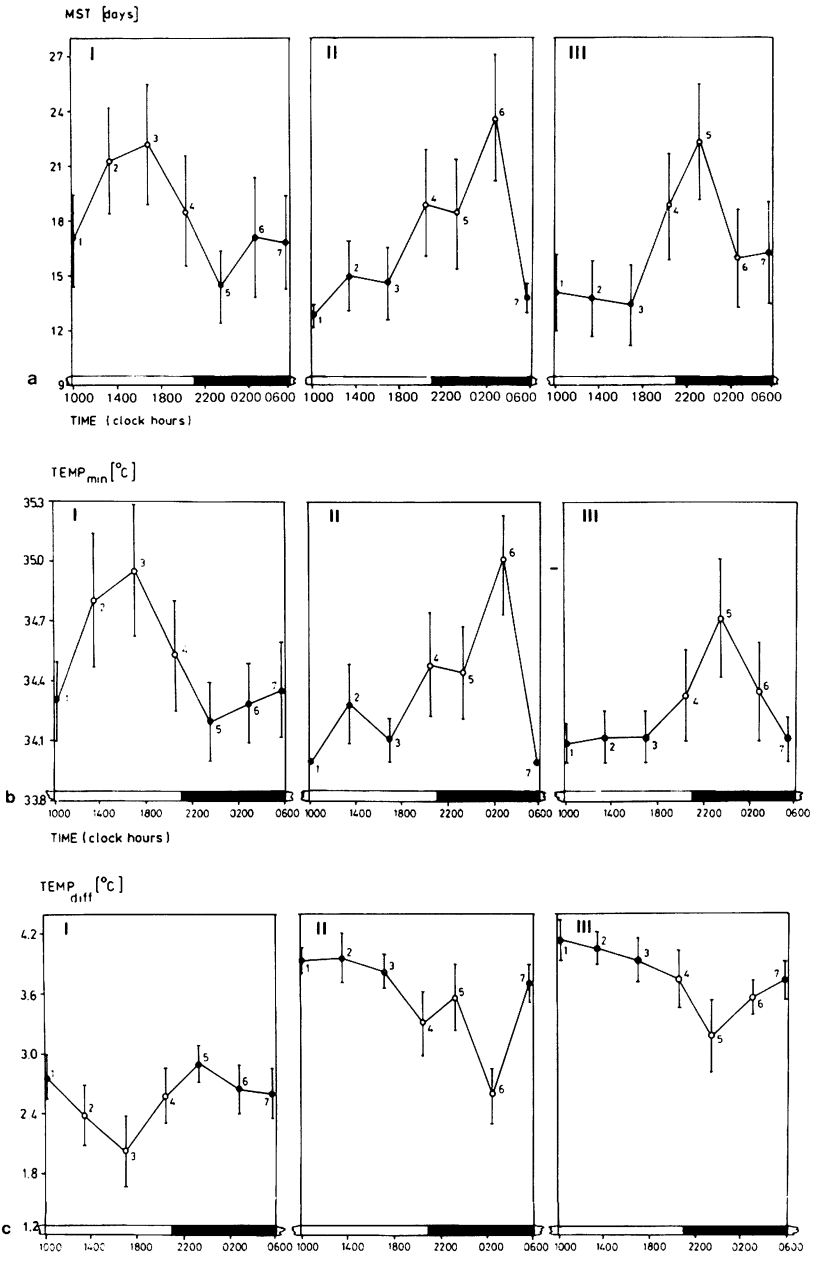

FIG. 3. Means and standard deviations of subgroups 1 to 7 plotted against the corresponding times of irradiation. The bars at the bottom of each plot display the general L–D schedule, being unchanged for group I only. Subgroups with high radiosensitivity are marked by solid circles, subgroups with low radiosensitivity are shown by open circles. As a consequence of time shifting groups II and III show a corresponding shift of radiation response. Significance levels of difference between high and low sensitivity categories were <0.05 in groups I to III for all variables except $WEIGHT_{diff}$. (a) MST—mean survival time, (b) $TEMP_{min}$—lowest rectal temperature, (c) $TEMP_{diff}$—maximal reduction of body temperature, (d) $WEIGHT_{diff}$—logarithm of maximal weight reduction.

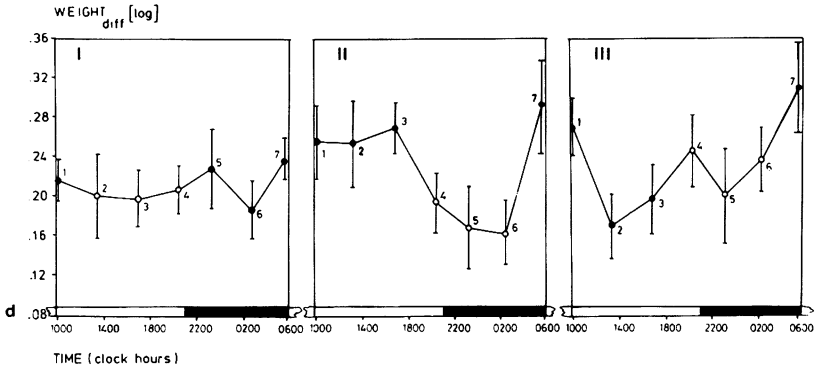

FIG. 3—*Continued.*

demonstrated radioresistance to be highest at about 1500 (group I) and 2400 (groups II, III), respectively, rather exactly after two thirds of the daily dark span. Cosine curves of $TEMP_{diff}$ with maxima at about 0300 and 1230 (Fig. 5c) indicated highest susceptibility to X irradiation to be during the last third of the daily dark span.

Effects of Time Shift

The best fitting cosine curves (cf. Fig. 5) quite clearly demonstrate a shift of the rhythm of radioresistance in groups II and III approximately corresponding to their previous time shift. There is also evidence for lower resistance of the time shifted groups compared to group I: the variable $TEMP_{diff}$ showed significantly higher radiosensitivity of groups II and III (analysis of variance, $P < 0.05$). However, for MST and $TEMP_{min}$ the groups did not differ significantly. Between the shifted groups II and III there were no significant differences.

TABLE I

Overall Lethality (Percentage) within 30 Days Postirradiation as a Function of Time of Exposure

Subgroup	*Group I*	*Group II*	*Group III*	*Approximate time of irradiation*
1	[80]	[100]	[90]	1000
2	60	[90]	[90]	1330
3	50	[90]	[90]	1700
4	70	70	70	2030
5	[90]	70	50	2330
6	[70]	40	80	0300
7	[80]	[90]	[80]	0630
High sensitivity category	80	93	88	
Low sensitivity category	60	60	67	

Note. High radiosensitivity categories comprise subgroups 1, 5, 6, 7 for group I and 1, 2, 3, 7 for groups II and III; low sensitivity categories subgroups 2, 3, 4 for group I and 4, 5, 6 for groups II and III. High sensitivity subgroups are framed.

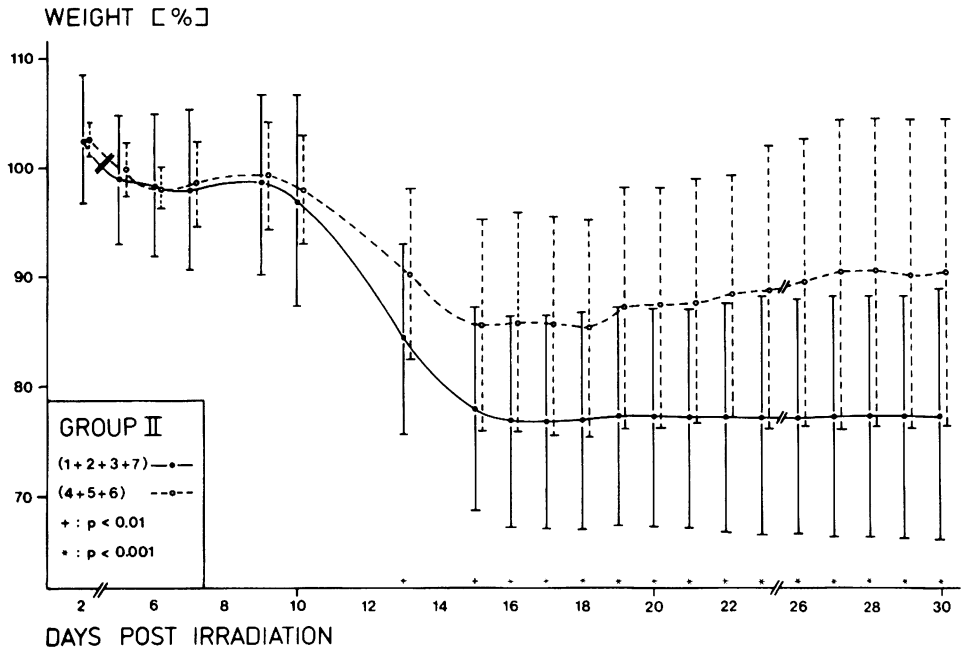

FIG. 4. Changes of body weight in high and low sensitivity categories of group II, shown as percentage of the weight before irradiation. *P* indicates level of significance (*t* test) for difference.

Survival Prognosis

As body temperature decreases very rapidly and to a marked extent often not before prefinal stages, it was not considered to be helpful for prediction of survival chances. Survival rates plotted against maximum weight loss, however, could be approximated by simple logarithmic regression (Fig. 6). With the help of the resulting formula,

$$\text{maximal survival chance} = e^{(0.04-6.08\times \text{WEIGHT}_{\text{diff}})} - 0.05,$$

the maximal survival chance for a given weight can be calculated if the weight before irradiation is known. Considering the day after exposure as a second independent

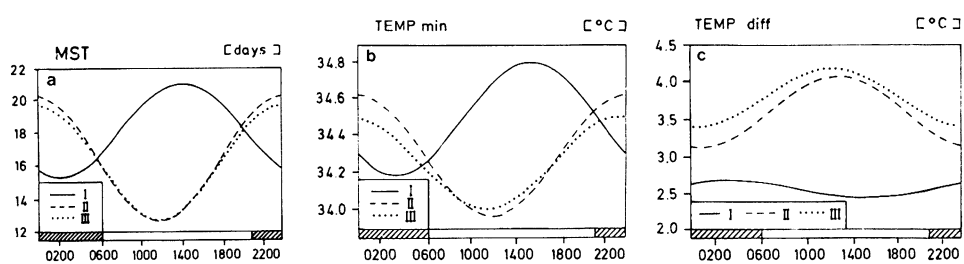

FIG. 5. Estimated rhythms of radioresistance, based on best fitting cosine curves for data of Fig. 2. (a) MST, (b) TEMP_{min}, (c) $\text{TEMP}_{\text{diff}}$.

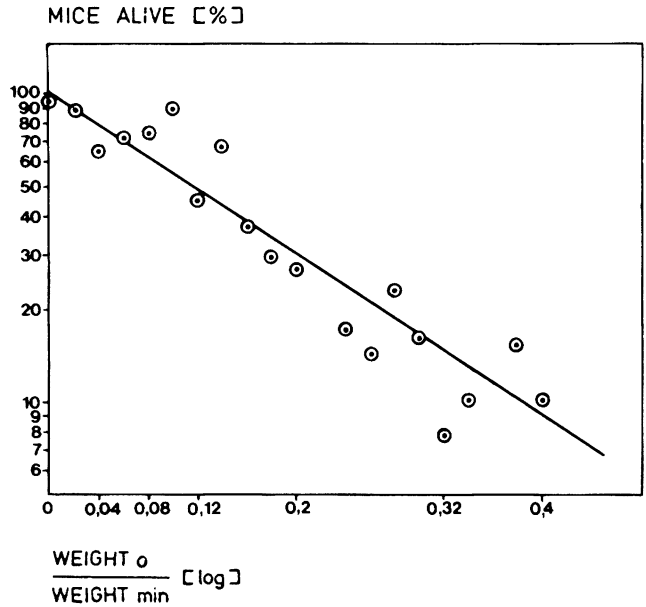

FIG. 6. Double-logarithmic display of correlation between loss of body weight and lethality. Each data point was computed regarding all animals with a logarithmic weight loss within an abscissa interval of 0.02.

variable, the overall survival chance at a given day with known weight loss can be calculated using the following formula found by multiple logarithmic regression:

$$\text{survival chance} = e^{(-0.65-\text{WEIGHT}_{\text{diff}}\times 5.3+\text{DAY}\times 0.038)} - 0.05.$$

Sixty-two percent of the overall variability could be explained by this mathematical model. As could be expected, the quality of prognosis was found to be best for data from the second and third weeks after exposure, after the beginning of considerable weight loss in the critical period of the hematopoietic syndrome.

DISCUSSION AND SUMMARY

Undefined light schedules or seasonal conditions, inadequate caging or laboratory conditions, or the use of female animals with possible cyclic interference of radioprotective sexual hormones may explain contradictory answers to the question of circadian rhythms of radiosensitivity in earlier years. Considering not only lethality and mean survival time, but also body weight and temperature, this report confirms more recent investigations (*20, 21*) that defined the second half of the daily light span (L–D 12–12) as the period of highest radioresistance in mice. With a light regimen of 15–9, corresponding to the natural seasonal conditions during the time of experiment, least and most sensitive times were found to be after two-thirds of the daily light span and in the last third of the daily dark span, respectively. This result can be closely correlated with the circadian rhythms of general activity (*22*), body temperature (*23*), metabolic (*24*), and mitotic activity (*21, 25*).

The corresponding shift of rhythm of radioresistance following sudden time shift supports the above-mentioned diurnal rhythms. Sudden time shift shortly before irradiation led to a significantly greater reduction of body temperature, possibly indicating an aggravation of radiation sickness. There was, however, no significant effect on survival time. The mice with shortened dark and activity span by time shifting (group II) showed no significant differences compared to the animals with reduced light and rest span (group III).

Using the body weight as a variable, mathematical models of survival prognosis and of the overall survival probability on a given day after irradiation could be established. An even better model of survival prediction might be found regarding the changes of parameters of circadian rhythms, e.g., the amplitudes of body temperature.

ACKNOWLEDGMENTS

Parts of this work were supported by a grant from the Stiftung Maximilianeum, Munich. We thank Dr. Nüssel, GSF Munich, for his help in preparing the time shift procedures and Mr. Bitter, Dr. Jacob, Mrs. Krienke, Mr. Sedlmeier, and Mrs. Schleippfer for helpful discussions.

RECEIVED: April 5, 1983; REVISED: October 20, 1983

REFERENCES

1. F. HALBERG, The 24-hour scale: A time dimension of adaptive functional organization. *Perspect. Biol. Med.* **3,** 491–527 (1960).
2. F. HALBERG, Chronobiology. *Annu. Rev. Physiol.* **31,** 675–725 (1969).
3. A. REINBERG, The hours of changing responsiveness or susceptibility. *Perspect. Biol. Med.* **11,** 111–126 (1967).
4. J. P. CONCANNON, M. H. DALBOW, C. WEIL, and S. E. HODGSON, Radiation and actinomycin D mortality studies: Circadian variations in lethality due to independent effects of either agent. *Int. J. Radiat. Biol.* **24,** 405–411 (1973).
5. F. HALBERG, When to treat. *Indian J. Cancer* **12,** 1–20 (1975).
6. T. H. RODERICK, The response of twenty-seven inbred strains of mice to daily doses of whole-body X-irradiation. *Radiat. Res.* **20,** 631–639 (1963).
7. H. LACH and Z. SREBRO, Changes in the diurnal rhythm of neurosecretory activity in the mouse following X irradiation. *Folia Biol.* **12,** 289–297 (1971).
8. W. LAPPENBUSCH, Effect of circadian rhythm on the radiation response of the Chinese hamster. *Radiat. Res.* **50,** 600–610 (1972).
9. R. RUGH, V. CASTRO, S. BALTER, E. V. KENNELLY, D. S. MAROSDEN, J. WARMUND, and M. WOLLIN, X-rays: Are there cyclic variations in radiosensitivity? *Science* **142,** 53–56 (1963).
10. R. F. NELSON, Variation in radiosensitivity of mice with time of day. *Acta Radiol. Ther.* **4,** 91–96 (1966).
11. D. J. PIZZARELLO, R. L. WITCOFSKI, and E. A. LYONS, Variations in survival time after whole-body radiation at two times of day. *Science* **139,** 349 (1963).
12. K. FOCHEM, W. MICHALICA, and E. PICHA, Über tages- und jahreszeitliche Unterschiede der Strahlenwirkung bei Versuchstieren und die Möglichkeit einer pharmakologischen Beeinflussung (ein zusammenfassender Bericht). *Strahlentherapie* **133,** 256–261 (1967).
13. D. J. PIZZARELLO, D. ISAAK, and K. E. CHUA, Circadian rhythmicity in the sensitivity of two strains of mice to whole-body radiation. *Science* **145,** 286–291 (1964).
14. K. FOCHEM. W. MICHALICA, and E. PICHA, Zur Frage tagesrhythmischer Unterschiede in der Strahlensensibilität bei Tumormäusen und Vergleichstieren. *Strahlentherapie* **130,** 590–594 (1966).
15. E. HAUS, F. HALBERG, M. COHEN, and Y. S. KIM, Circadian rhythmometry of mammalian radio-

sensitivity. In *Space Radiation Biology and Related Topics* (C. A. Tobias and P. Todd, Eds.), pp. 435–474. Academic Press, New York, 1971.

16. J. LAVERNHE, Wirkungen der Zeitverschiebung in der Luftfahrt auf das Flugpersonal. *Muench. Med. Wochenschr.* **39,** 1746–1752 (1970).
17. G. T. HAUTY, Phase shifts of the human circadian system and performance deficit during the periods of transition. *Aerospace Med.* **37,** 1027–1033 (1966).
18. K. E. KLEIN and H. M. WEGMANN, Das Verhalten des menschlichen Organismus beim Zeitzonenflug. Teil 1: die zirkadiane Rhythmik und ihre Desynchronisation. *Fortschr. Med.* **93,** 1407–1414 (1975).
19. V. P. BOND, T. M. FLIEDNER, and J. O. ARCHAMBEAU, *Mammalian Radiation Lethality.* Academic Press, New York, 1965.
20. F. HALBERG, E. HAUS, S. CARDOSO, L. E. SCHEVING, J. F. W. KÜHL, R. SHIOTSUKA, G. ROSENE, J. E. PAULY, W. RUNGE, J. F. SPALDING, J. K. LEE, and R. A. GOOD, Toward a chronotherapy of neoplasia: Tolerance of treatment depends upon host rhythms. *Experientia* **29,** 909–934 (1973).
21. E. HAUS, F. HALBERG, and M. K. LOREN, Circadian susceptibility-resistance cycle of bone marrow cells to whole body x-irradiation in BALB/C mice. In *Chronobiology* (L. E. Scheving, F. Halberg, and J. E. Pauly, Eds.), pp. 115–122. Thieme, Stuttgart, 1974.
22. J. ASCHOFF and J. MEYER-LOHMANN, Die Aktivität von Nagern im künstlichen 24 Studen Tag. *Z. Vergl. Physiol.* **37,** 107 (1955).
23. J. M. DE CASTRO, Diurnal rhythms of behavioural effects on core temperature. *Physiol. Behav.* **21,** 883–886 (1978).
24. Y. MORIMOTO, U. ARISUE, and Y. YAMAMURA, Relationship between circadian rhythm of food intake and that of plasma corticosterone and effect of food restriction on circadian adrenocortisol rhythm in the rat. *Neuroendocrinology* **23,** 212–222 (1977).
25. A. VACEK and D. ROTKOVSKA, Circadian variations in the effect of x irradiation on the hematopoietic stem cells of mice. *Strahlentherapie* **140,** 302–306 (1970).