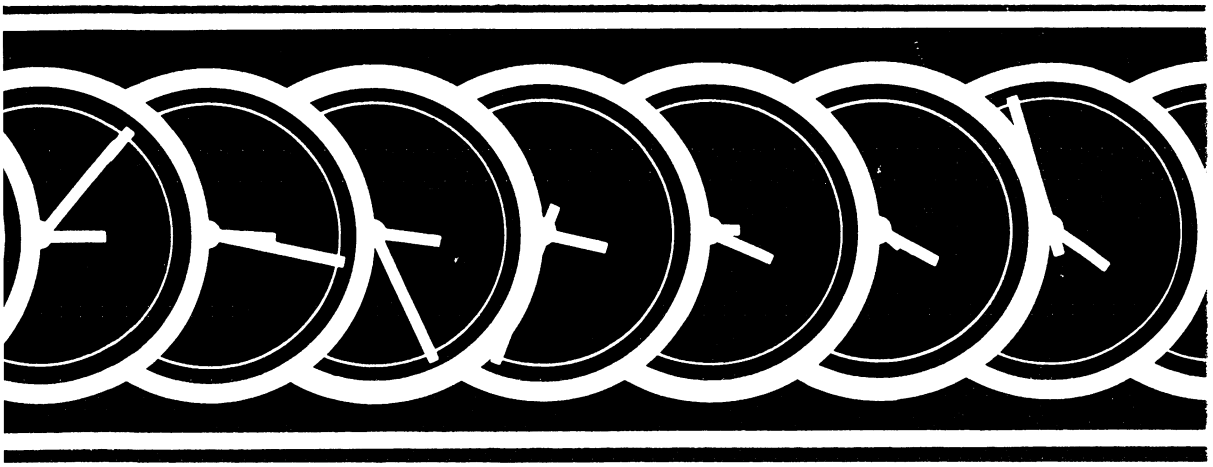

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THE INFLUENCE OF TIMESHIFT ON CIRCADIAN RHYTHM OF SENSITIVITY TO X-IRRADIATION IN MICE*

A. L. Gerbes † and B. J. Arbogast‡

†Medical Department II, Klinikum Großhadern, University of Munich, Marchioninistr. 15, D-8000 Munich 70, Federal Republic of Germany
‡Institute of Social Pediatrics, University of Munich, Lindwurmstr. 131, D-8000 Munich 2, Fed. Republic of Germany

Abstract—For two groups of male C3H mice an eastbound transmeridional flight was simulated by inducing a time shift of the L:D schedule of 8 hr. The assumed flight brought about a maximal reduction of the daily light and dark span, respectively. A third group remained unshifted. At seven different times during the following day, subgroups of the time shifted mice as well as of the group with unchange schedule were exposed to whole body X-irradiation. Mortality and body 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 during the second half of the daily light span, confirming earlier reports by other authors. Well defined effects of the time shift and a corresponding shift of the acrophase of radioresistance could be demonstrated. There was no significant difference between the two time shifted groups, but there was a consistent slight trend towards an advantage for the group whose L:D shift resulted in a maximally reduced dark span.

Key words—Time shift, acute radiation injury, circadian rhythms, radioresistance, mice, body temperature.

Introduction

To date there have been numerous investigations dealing with the question if mammalian radiation response depends upon the phase of circadian systems at exposure time (1–4). After some contradictory results (5, 6) it now seems generally accepted that animals such as rats (7, 8) and mice (9–11) show lower radiation susceptibility during the daily light/rest as compared to the daily dark/activity span. Most of the authors simply regarded lethality or mean survival time as indicators for radiation injury, some more recent reports however even found bone marrow cell-cycle dependence of X-radiosensitivity (12, 13). Transmeridional flights with resulting time shift of synchroniser phase are known to impose considerable stress to biological systems (14–18). Stress during acute radiation sickness reportedly increases lethality (19), whereas corticosteroids (20) or skin lesions in the combined injury model (21) could decrease radiation sensitivity in mice when applied shortly before exposure.

Considering the aforementioned reports, it seemed worthwhile to evaluate the circadian rhythm of radioresistance, recording additional parameters such as rectal temperature and to investigate the influence of a sudden time shift shortly before irradiation.

Materials and Methods

Animals

Male C3H mice with an age of 3 weeks were purchased from GSF, Munich and housed five animals per Makrolon® cage under standard laboratory conditions (24±1°C room temperature, 65% humidity, Altromin® hard role food and tap water *ad libitum*). A group of 80 mice (group I) was kept on an illumination regimen L 0600–2100, D 2100–0600, corresponding to the natural day–night ratio during the time of the experiment.

*The experiments were performed at the Laboratories of Experimental Radiology, SANAk BW, Ingolstädter Landstr. 2, D-8042 Neuherberg, Federal Republic of Germany.

Time shift

For two groups of 160 mice in total (groups II, III) the lighting cycle was shifted by 120° (8 hr) to L 1400–0500, D 0500–1400 3 weeks before irradiation. 2 weeks after this 8-hr shift of synchronizer phase, rectal temperature of every mouse was taken at seven about equidistant times during one day (Telethermometer 43TF, temperature probe 520, Yellow Springs Instrument Co., Ohio, U.S.A.) to make sure that there had been a corresponding shift in acrophase of body temperature and metabolism. One week later, an eastbound transmeridional flight of 8-hr duration was simulated, with a maximal reduction of the daily dark (for group II) and light span (for group III), respectively: at 0930, after only 4.5 hr of darkness, the mice of group II and at 2130, after only 7.5 hr of light period, the mice of group III were transferred to a room with the L:D schedule of group I. The next morning, each animal's body temperature was recorded and seven (Nos 1–7) separate sets of 10 mice each of groups I–III were irradiated once at one of seven different times over the 24 hours, each being at about 3.5-hr intervals from another. Age of the animals at this time was about 10 weeks.

Irradiation

The unanesthesized mice were exposed to whole body X-irradiation (X-ray unit MG 300, C.H.F. 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, Freiburg, P.T.W., FRG). For 30 days following irradiation, rectal temperature was recorded daily at fixed times.

Statistical analysis

The data were analysed on a CYBER 175 computer using SPSS library routines as well as own FORTRAN programs for linear rhythm estimation. From the raw data the following variables were calculated as indicators for radiosensitivity:

$TEMP_{min}$: Minimal body temperature measured during observation,

$TEMP_{diff}$: Maximal reduction of body temperature compared to day before irradiation and

SVT : Mean survival time after irradiation (observation span was 30 days).

Means and standard deviations for all variables were calculated within each of the 7 subgroups of groups I–III and were displayed in chronograms (cf. Figure 2). 2-way analyses of variance were calculated for the dependent variables $TEMP_{min}$, $TEMP_{diff}$ and SVT, using time of irradiation and time shift (I, II or III) as independent variables. Weight before irradiation and age were first included as covariates, then removed from the model after showing no significant effects. Subgroups of high and low response, respectively, were identified by multiple classification analysis. Linear contrasts and *t*-tests calculated for $TEMP_{min}$, $TEMP_{diff}$ and SVT showed significant differences between subgroups with high (group I: subgroups 1, 5, 6, 7; groups II, III: 1, 2, 3, 7) and low (group I: subgroups 2, 3, 4; groups II, III: 4, 5, 6) radiosensitivity in all cases.

Finally, more accurate estimates of parameters of radioresistance rhythm were obtained by COSINOR analysis using the subgroup means of the variables $TEMP_{min}$, $TEMP_{diff}$, SVT and $TEMP_{diff}$/SVT.

Results

(1) *Presynchronization*

Two weeks past the 8-hr shift of the L:D schedule, every animal's circadian profile of rectal temperature was taken. Population Mean Cosinors (Figure 1) demonstrate an average 8-hr shift of acrophase in groups II and III, indicating the effects of presynchronization. There are no significant differences of mesors and amplitudes between the groups, which proves good randomization of the animals.

(2) *Circadian Rhythm of Radiosensitivity*

Lethality as well as mean survival time within

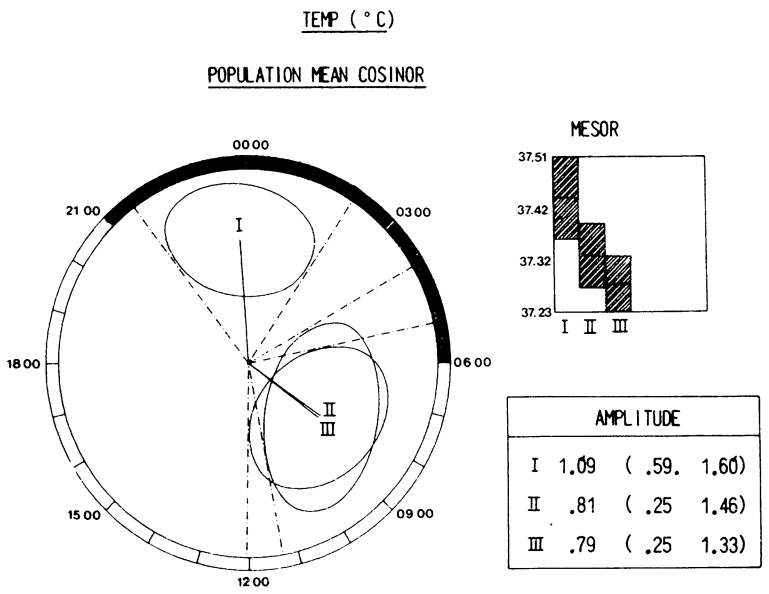


Figure 1. Population Mean Cosinor of circadian temperature rhythm in groups I, II, III 2 weeks after 8-hr shift of the L:D regimen for groups II and III. Population rhythms were significant at $P<.02$. 95% confidence intervals are indicated for mesor (upper right-hand portion of figure) and amplitude (lower right-hand portion of figure).

30 days post irradiation are generally regarded as indicators of radiation effects in mice. Figure 2(a) shows the mean survival time of groups I–III (SVT) as a function of the time of exposure (subgroups 1–7). For group I, mean survival time proved to be significantly higher in animals irradiated during the light span. Due to the fact that a time shift had not been performed until shortly before irradiation, radioresistance in groups II and III was found to be significantly higher in animals irradiated at times corresponding to the light span in their presynchronization schedule.

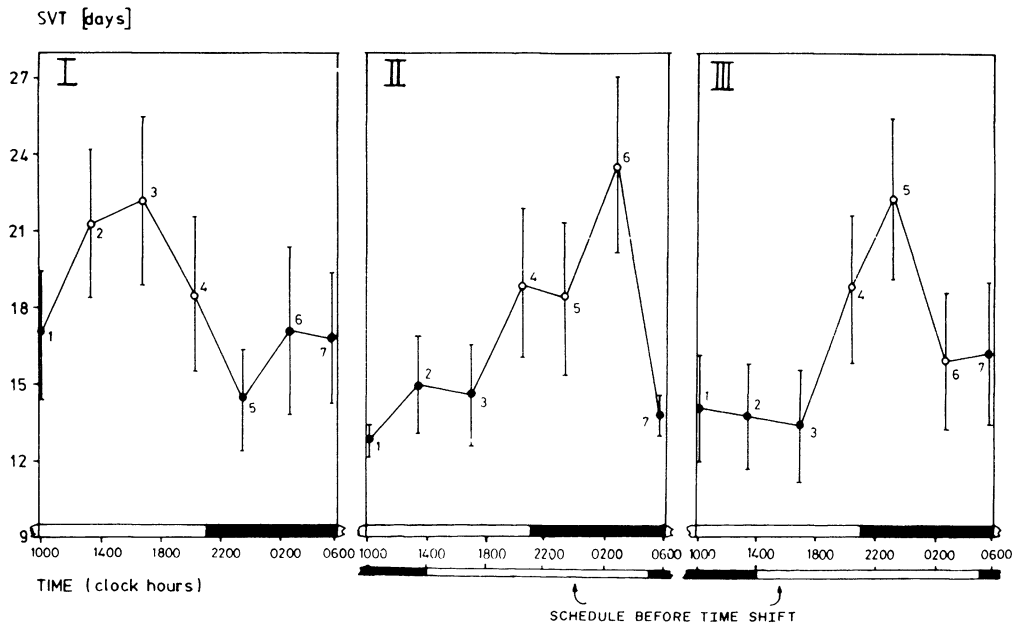
During acute radiation sickness with the so-called "Hematopoetic syndrom" (22), the second and third week after exposure were to be regarded as the critical period. In many animals body temperature as a marker of metabolic activity sank considerably, in prefinal stages even to as low as 34°C. The lowest rectal temperature of each animal during the time of observation [$TEMP_{min}$, Figure 2(b)] as well as the difference between the individual normal body temperature before irradiation and the lowest temperature—at corresponding times of the day—[$TEMP_{diff}$, Figure 2(c)] were regarded as suitable indicators of radiation susceptibility. Again, highly significant advantages were to be seen for the mice irradiated during the light phase.

In order to define the most resistant and the most sensitive phases more precisely, the data of Figure 2 were put to COSINOR analysis. SVT and $TEMP_{min}$ [Figures 3(a) and 3(b)] showed radioresistance to be highest at about 1500 (group I) and 2400 (groups II, III), respectively; in other words rather exactly after two thirds of the daily light span. Cosinors of $TEMP_{diff}$ and $TEMP_{diff}/SVT$ [Figures 3(c) and 3(d)] with acrophases at about 0300 and 1230, respectively, indicated highest susceptibility to X-irradiation to be during the last third of the daily dark span.

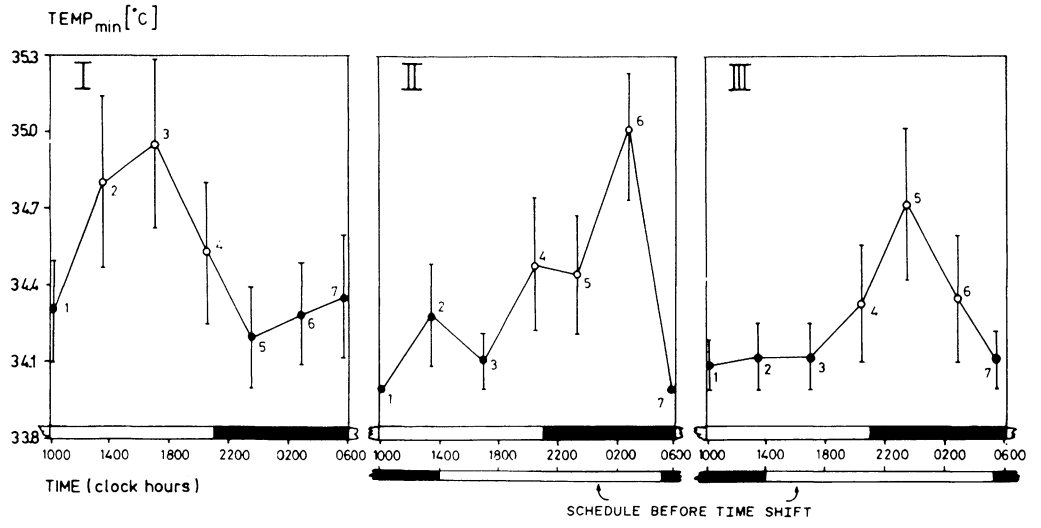
(3) *Effects of time shift*

Cosinor analysis quite clearly demonstrates a shift of the acrophase of radioresistance approximately corresponding to the presynchronization time shift of groups II and III. There is a general tendency towards lower resistance of groups II and III as compared to

(a)


SVT [days]
I
II
III
27
24
21
18
15
12
9
1000 1400 1800 2200 0200 0600
TIME (clock hours)
SCHEDULE BEFORE TIME SHIFT

(b)


TEMP min [°C]
I
II
III
35.3
35.0
34.7
34.4
34.1
33.8
1000 1400 1800 2200 0200 0600
TIME (clock hours)
SCHEDULE BEFORE TIME SHIFT

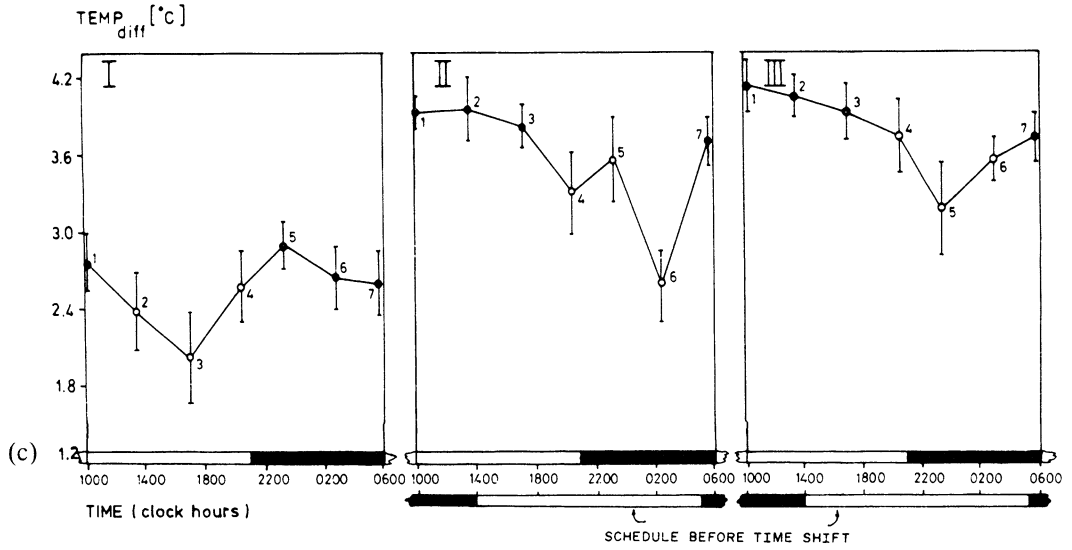


Figure 2. Subgroup means ± S.E.M. (subgroups 1–7) plotted vs the corresponding times of irradiation, separately for groups II–III. The bars at the bottom of each plot display the general L:D regimen after irradiation which is unchanged for group I only, and the L:D schedule immediately before time shift. As a consequence of presynchronization, groups II and III exhibit a corresponding shift of radioresistance. Significance levels (*t*-test) of difference between subgroups of high and low radiosensitivity were $P<.05$ for groups I–III. Subgroups with high radiosensitivity (group I:1, 5, 6, 7; groups II, III:1, 2, 3, 7) are marked by open circles, subgroups with low radiosensitivity (group I:2, 3, 4; groups II, III:4, 5, 6) are marked by solid circles. (a) Mean survival time. (b) Lowest body temperature during observation span. (c) Maximal reduction of body temperature.

group I, demonstrated by the lower mesors of SVT and $TEMP_{min}$. But only $TEMP_{diff}$ and $TEMP_{diff}/SVT$ show a significantly higher radiosensitivity of the time shifted groups (Analysis of variance, $P<0.05$, see also mesor plots in Figure 3). There are very slight trends towards greater radiation injury in group III as compared to group II for all variables, but differences between the two time shifted groups were not significant (cf. Figure 3).

Discussion

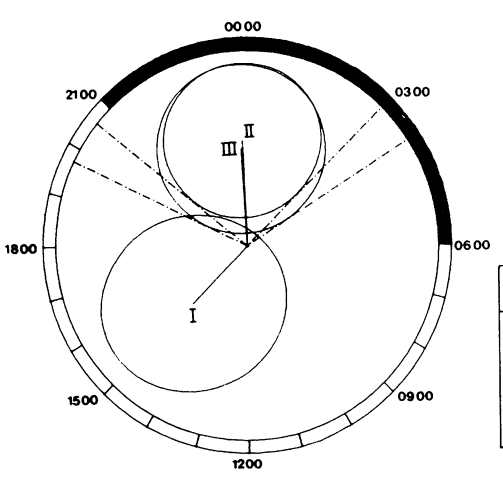
In earlier years, some authors obtained contradictory answers to the question of circadian rhythms of radiosensitivity. This fact might be explained by undefined light schedules or seasonal conditions or the use of female animals with possible cyclic interference of radioprotective sexual hormones. Considering not only lethality and mean survival time, but also regarding body temperature, this report confirms more recent investigations (12, 23) that defined the second half of the daily light span as the time of highest radioresistance in mice (L:D 12:12). With a lighting regimen of 15:9 (L:D) corresponding to the natural seasonal conditions during the time of experimentation, highest and lowest radioresistance were found to be after two thirds of the light span and in the last third of the dark span, respectively. This can be closely correlated with the circadian rhythms of general activity (24), body temperature (25), metabolic (26) and mitotic (12) activity.

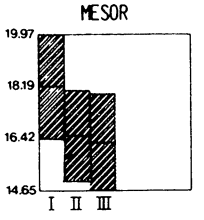
The corresponding shift in the acrophase of radioresistance following a sudden time shift supports the above mentioned diurnal rhythms. The stress of a sudden time shift shortly before X-irradiation did not reduce the effects of radiation injury; there were even strong and partly significant signs of an aggravation of

(a)

SVT (days)

SINGLE COSINOR



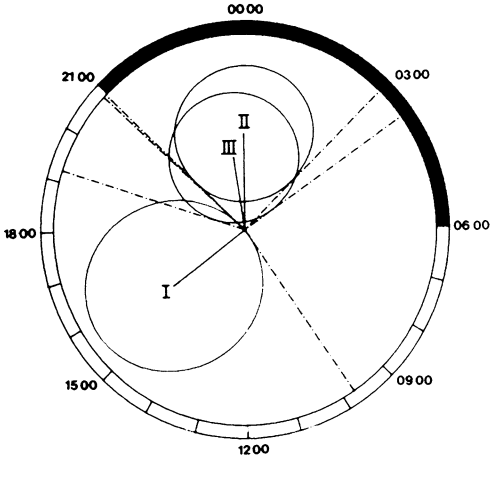


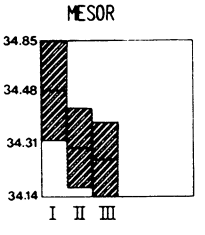
AMPLITUDE		
I	2.84	(.51. 6.20)
II	3.77	(1.01. 6.52)
III	3.47	(.45. 6.45)

(b)

TEMP min (°C)

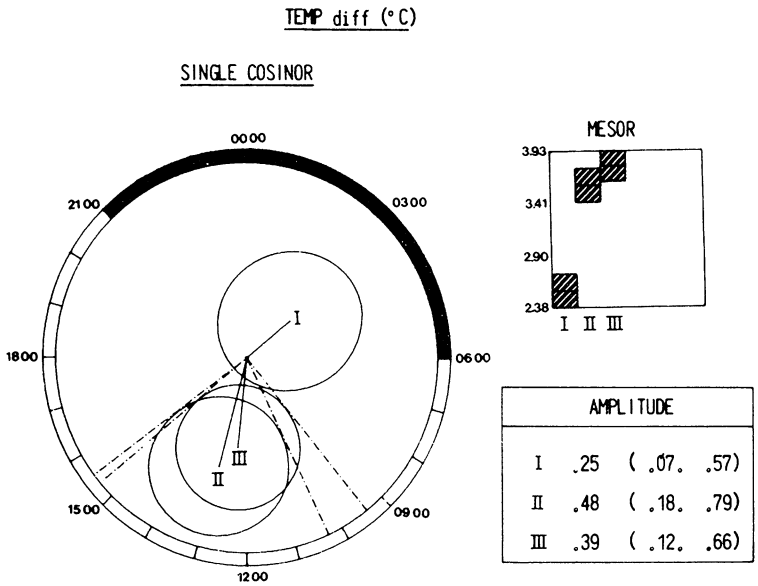
SINGLE COSINOR





AMPLITUDE		
I	.31	(.00. .62)
II	.33	(.10. .56)
III	.25	(.03. .47)

(c)



(d)

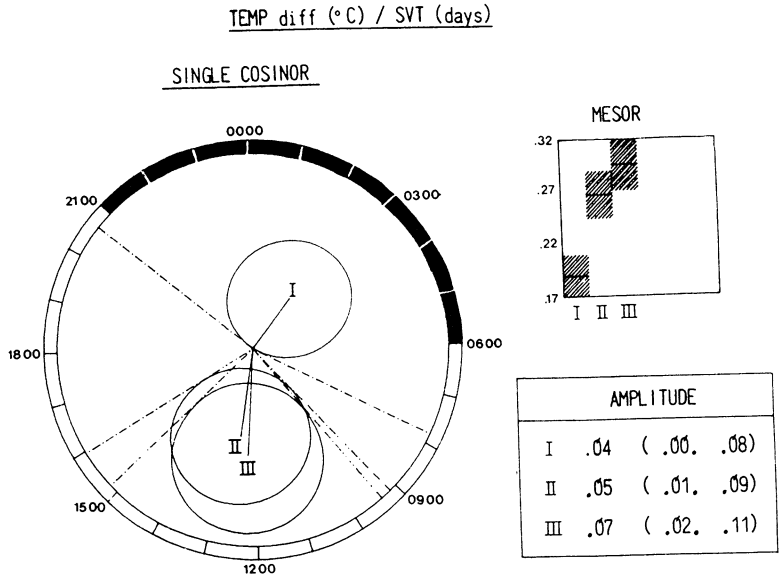


Figure 3. Cosinor analysis of the data in Figure 2. Plots with 95% significance level: (a) SVT; (b) $TEMP_{min}$; (c) TEM_{diff} and (d) $TEMP_{diff}$/SVT.

radiation sickness. Group II with shortening of the dark/activity span by time shifting showed very slight but not significant advantages as compared to group III with reduced light/rest span.

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