PLANS FOR ORBITAL STUDY OF RAT BIORHYTHMS* RESULTS OF INTEREST BEYOND THE BIOSATELLITE PROGRAM

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1. Introduction

The Biosatellite Program of the National Aeronautics and Space Administration was intended to investigate effects of an extraterrestrial space environment on terrestrial life forms. This program called for a series of 6 earth-orbital missions to study organisms as varied as bacteria, plants and primates (Dyer, 1969). Only 3 of the planned missions were launched, the remainder being deferred.

One of the latter missions was to have included a study of circadian and other rhythms** now known to persist at least for a week or so in men in an earth orbit

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** Desiderata discussed elsewhere (Halberg, 1969) for rendering objective the techniques of rhythmometry prompt us in this paper to adhere to the following considerations: To qualify as a *rhythm* a set of biological changes must recur systematically according to a (algorhythmically) formulatable 'pattern' or waveform which is validated by inferential *statistical* means. We tend to reserve *biorhythm* for cases meeting (2 added *biological criteria*). First, if it is amenable to frequency synchronization with an environmental cycle, the institution of an abrupt (single cycle) shift by 90° or more in the environmental cycle should be followed by only gradual (rather than within a single cycle) adjustment. Second, the biorhythm should persist as a statistically significant entity (validated, for instance, by a test showing that its amplitude is not zero) for 2 or more cycles after elimination of the environmental cycle.

The minimal and/or average number of (a) transient cycles before resynchronization after phase-shifts of the environmental cycle and of (b) persisting periods after removal of that environmental periodic input, as well as the duration and extent of any damping noted during either the phase-shift or the persistence test, also should be indicated for a biorhythm.

As a basic example, the circadian rhythm in intraperitoneal temperature, telemetered at $\sim 10'$ intervals for 116 days from 6 blinded mature female inbred Minnesota Sprague-Dawley rats kept singly housed with food freely available at 24 ± 0.5 °C environmental temperature, in light and darkness alternating at 12-h intervals, persisted with a 24.3 ± 0.03-h period and a 0.57 ± 0.03 °C amplitude. An amplitude of 0.61 ± 0.02 °C at a $24 \pm < 0.01$ h period was found in 5 concomitantly evaluated intact (rather than blinded) rats, kept under the same conditions.

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(Halberg *et al.*, 1970). The purpose of this particular investigation on 8 rats during a 21-day flight was to determine under especially designed test conditions whether characteristics of physiologic rhythms in mammals are measurably influenced by an exposure to 'zero gravity'* away from the earth for three weeks. To achieve the nearly constant, nearly 'zero gravity' the satellite was to orbit in a circular, equatorial path at an altitude of approximately 200 nautical miles. Under these conditions any periodic changes within the vehicle caused by the configuration of the orbit would occur with a period of 1.5 h. Considerations of economy and efficiency required that a number of different biologic experiments share a single space craft with a common life-support system and that compatible experiments be combined so that positive results from an experiment would not be totally contingent upon successful recovery of the test-organism from space; in this particular case, a study of body composition in the rat was to be combined with a study of telemetered biorhythms.

Although the project could not be completed, preparations briefly summarized in this report may be of general interest to those concerned with planning similar missions in the future or engaged in other rhythmometry involving applied or basic problems on Earth as well as in aerospace (Halberg, *et al.*, 1970).

These preparations included:

(1) The development of computerized methods for objective characterization of biorhythms (Halberg, 1968, 1969);

(2) Studies to evaluate the compatibility of hardware and mission plan with the requirements of biorhythm investigation;

(3) Studies pertaining to the design of an efficient biorhythm 'test sequence'.

2. Materials and Methods

A. TEST ORGANISM: SPECIES, SEX, NUMBER

Special considerations having a decisive bearing on the choice of the albino rat as the test organism were related to a study of body composition to have been performed by one of us (G.C.P.) at the University of Virginia on rats recovered from the Biosatellite. The Sprague-Dawley rat (Minnesota inbred strain – MSD) was picked not only because it is usually used in research and is genetically standardized but also because it is well characterized in terms of classical pathology (and, through the present studies, in terms of its rhythms).

Females were chosen in preference to males because of:

(1) their lighter weight, an important factor in meeting vehicle payload limitations;

(2) the additional possibility of obtaining information on biorhythms related to the estrous cycle.

As a concession to payload limitations, the number of rats to be flown was set at 8. Data from this group in orbit could be compared with those from a

* i.e., zero net radial acceleration. This qualification serves as a reminder that biologic effects from gravity as such need not necessarily be identical to those from changes in velocity (acceleration). Hereafter this qualification is indicated by quotes around 'zero gravity'.

much larger number of earth-based controls for the statistical evaluation of rhythm parameters.

B. METHODS OF DATA ACQUISITION

'State of the art' systems for obtaining data from rats by telemetry included miniaturized completely-implantable, battery-powered temperature transensors, (Table 1) along with associated devices for data reception and recording. Although the AEL-TTX was used in earlier investigation, including the study of light intensity (Section 4A), the VI-XLL-type, long-life transensor was selected for the Biosatellite mission and was used for most of the studies reported herein. This transensor was implanted into each rat's peritoneal cavity using sterile techniques. Antennae for the individual

TABLE I

Illustrative characteristics of implantable temperature transensors used

Device designation	AEL-TTX	IV-XLL
Manufacturer	American Electronics Laboratory Colmar, Pa.	Franklin Institute Philadelphia, Pa.
Characteristics		
Mode of operation	FM: $\Delta f = 0.64$ kHz/°C	Self-quenching blocking oscillator
Carrier frequency (nominal)	263 kHz at 38 °C	400 ± 15 kHz
Pulse repetition rate at 30 °C		340 pps
Pulse repetition rate at 38 °C		680 pps
Slope per 1 °C between 30° and 33 °C		19.4 pps
Slope per 1 °C between 37.5 and 40.8 °C		24.3 pps
Equilibration time to 4° thermal step (time constant):	< 90 sec	< 36 sec
Anticipated *in situ* lifetime:	6 months	> 18 months
Signal stability over 6 months (drift from original calibration)	⩾ 1 °C	± 0.04 °C/month
Weight	10 g	4.4 ± 0.2 g
Volume	5 ml	1.8 ml
Shape	cylindrical	disc
Diameter	1.2 cm	1.8 cm
Height or thickness	5.1 cm	0.7 cm
Capsule	teflon (coated prior to use with Tissuemat®)	paraffin (Tissuemat®)

cages monitored the radio frequency signal from the transensor for readout at predetermined intervals (e.g., every 10 min). Data from 8 rats thus recorded and stored in digitized form in the satellite could in turn be relayed to Earth on command. In addition to direct information on intraperitoneal temperature, data indicative of gross body movements could be obtained through monitoring of changes in signal-voltage in one of the antennae. Feeding activity also could be monitored. A stretch of data on all 3 variables from a single randomly-picked rat during the 'test sequence of lighting regimens' is shown in Figure 1.

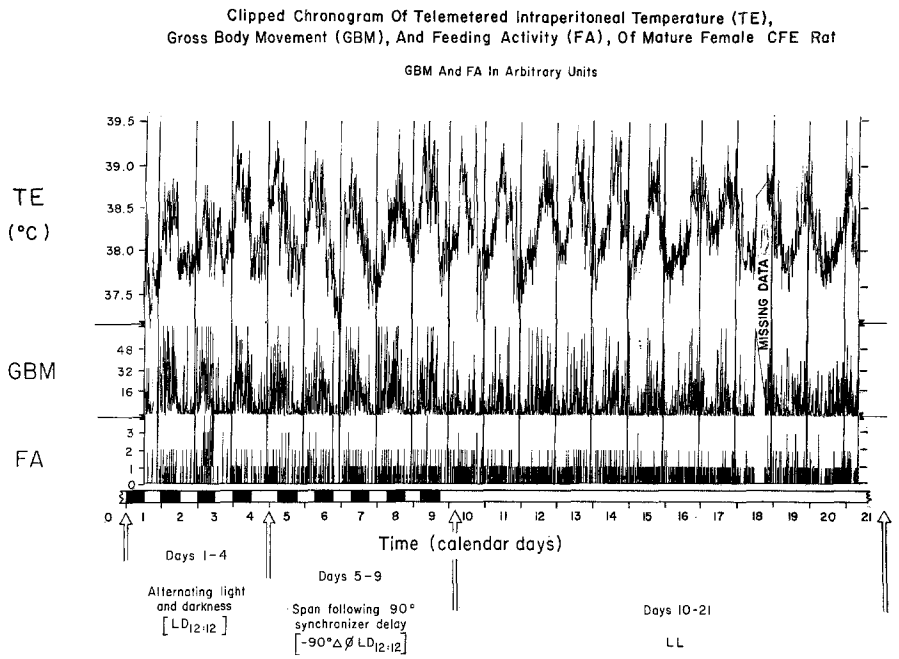


Fig. 1. Circadian rhythms of three physiologic variables in a mature, female inbred Minnesota-Sprague-Dawley rat monitored every 10 min for 21 days stand out with different prominence in time plots; their characteristics are not readily quantified in any such chronograms. 'Clipped' refers to the automatic omission of any data point outside ± 3 standard deviations from the computed mean.

C. BIOSATELLITE HARDWARE

Figures 2 and 3 show views of the Biosatellite rat cage assembly. The 8 cages are arranged in a 'pie' formation around a central unit from which liquid diet is dispensed in known volume of 0.75 ± 0.03 ml as each rat actuates its feeding lever. The number of lever actuations per unit of time is recorded to indicate feeding activity. Associated systems maintain the environmental temperature, humidity and gas composition at desired values. Lights in the ceiling of each cage are controlled by an ON-OFF programmer which can be overridden by command from Earth.

D. BIORHYTHMS OPERATIONS PLAN

Figure 4 summarizes the plan of operations for the biorhythms study. Included are criteria for changing the lighting regimen according to a test sequence. The effects of the different lighting regimens on biorhythms constitute the major feature of the study and were to be investigated on all rats (including controls) at least once prior to the flight, as well as during the flight. The different stages in this test sequence and criteria to be used in determining their durations were as follows:

Stage I: $LD_{12:12}$ – 12 h light alternating with 12 h darkness. This regimen was to be maintained for at least 24 h after lift-off in order to collect sufficient data for group

analyses of circadian rhythm parameters and until any one of the following conditions was met:

(1) the transients associated with lift-off had worn off, i.e., as soon as rhythm parameters correspond to those determined prior to lift-off and/or to those of concomitantly monitored controls;

(2) a statistically significant difference in rhythm parameters between controls and

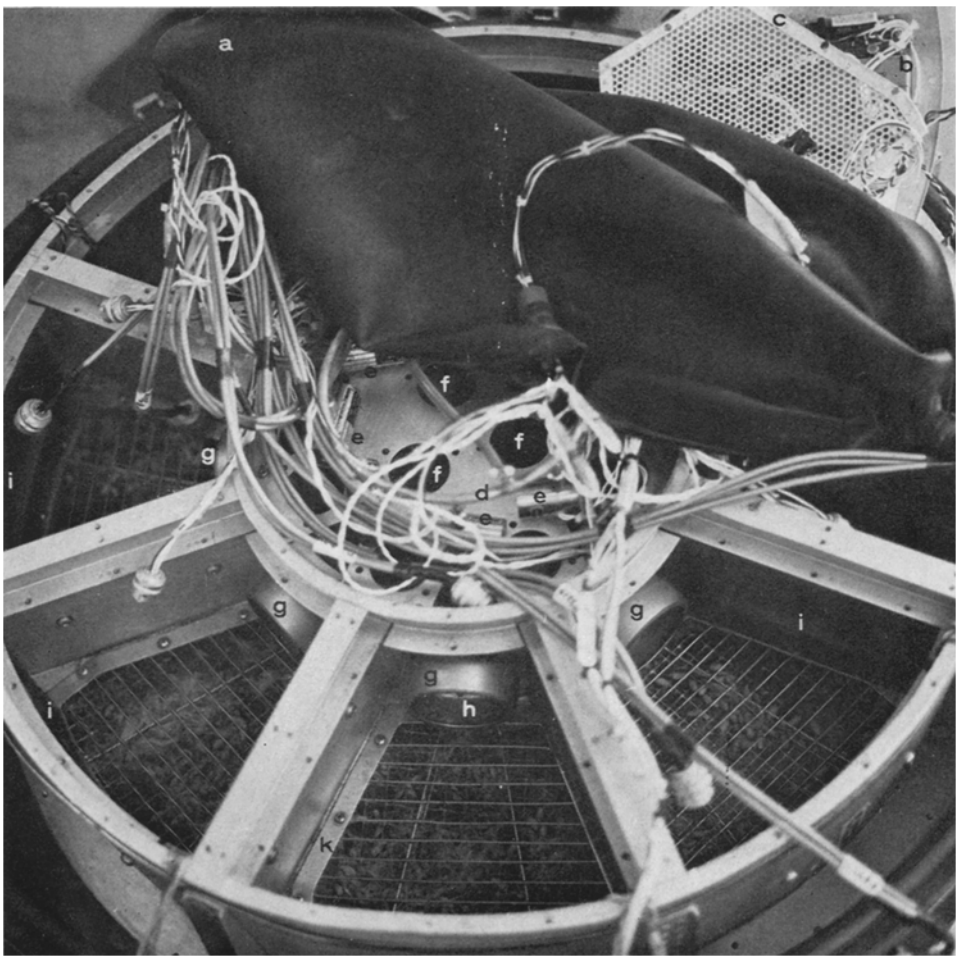


Fig. 2. Top view into rat cage assembly of the developmental hardware for the 21-day Biosatellite after a 3-week test operation with rats, showing: (a) gasket-cover for hermetically sealing the access area of the assembly; (b) compartment cover with telemetry receiver; (c) perforated cage ceiling with cage light; (d) distribution manifold for liquid diet; (e) valves for delivery of liquid diet 'on demand'; (f) mounting holes for pressurized food cannisters; (g) feeder cups with (h) rat-operated feeding lever and delivery nozzle; (i) telemetry antennas, as far as visible; (j) cage bottom grid, positioned above debris trap and bacterial filter. The ventilating air is distributed among the cages between compartment cover b and cage ceiling c, entering the cages through perforations in c and thus causing waste deposition on the debris trap.

experimentals had been ascertained and had been confirmed on each of three consecutive days; or

(3) after one week,

whichever span was shorter.

$LD_{12:12}$ was chosen as the initial regimen for several reasons. First, any transient effects of lift-off, *per se*, could be expected to wear off more rapidly in the presence of a synchronizer than under other conditions. Whether or not this were the case, standardization in $LD_{12:12}$ is needed in order to fix the circadian system phase of the Biosatellite rats at lift-off – a phase which would hold from day to day if a launch date had to be reset for logistic reasons.

Second, the parameters of a 24-h (*LD*)-synchronized circadian rhythm describe

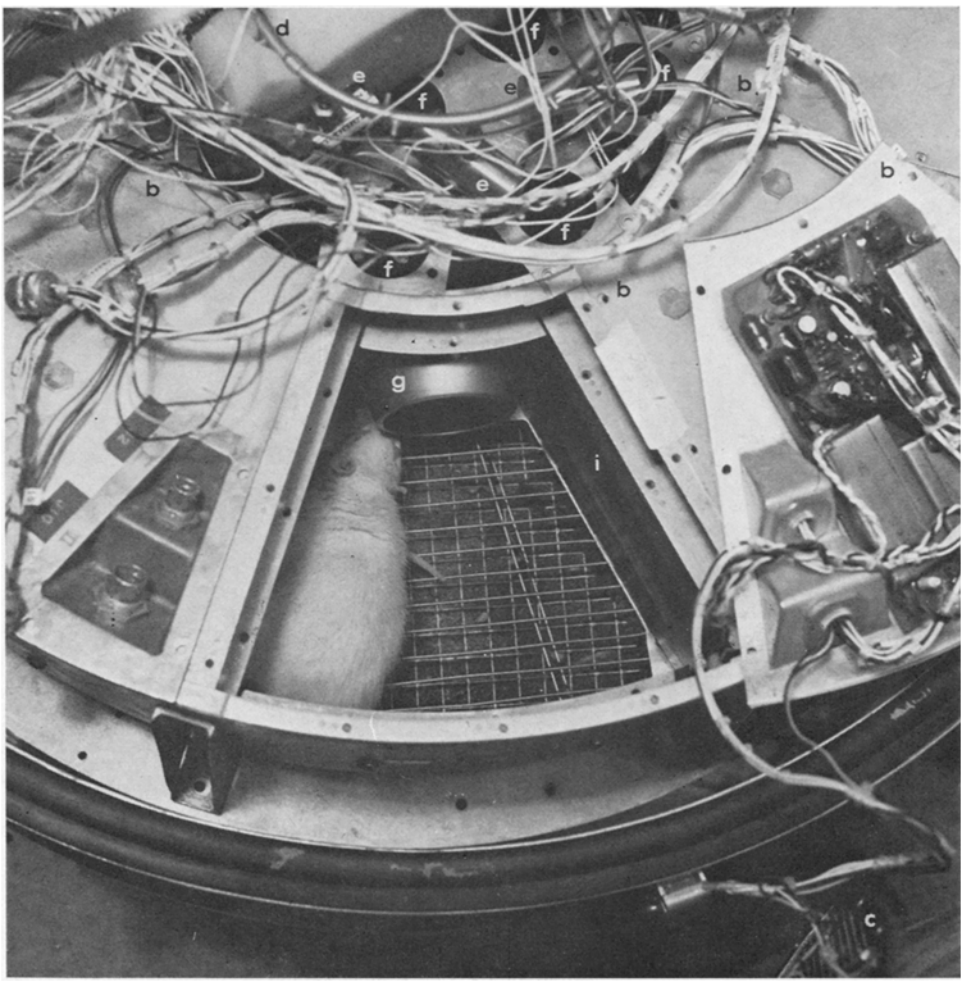


Fig. 3. Close-up view into a rat cage compartment opened at the end of a laboratory test, illustrating its relative size and structural detail. Labelling of components is identical to that in Figure 2.

organisms under conditions that approximate those to which most life forms on Earth are exposed – except for the environments prevailing, e.g., at the poles, at the bottom of the seas or in caves.

Stage II: $-90°\Delta\phi LD_{12:12}$ – the temporal placement of the alternating 12-h spans of light and darkness being changed by the lengthening of a single light span from 12 to 18 h. The new placement of the $LD_{12:12}$ was to be maintained:

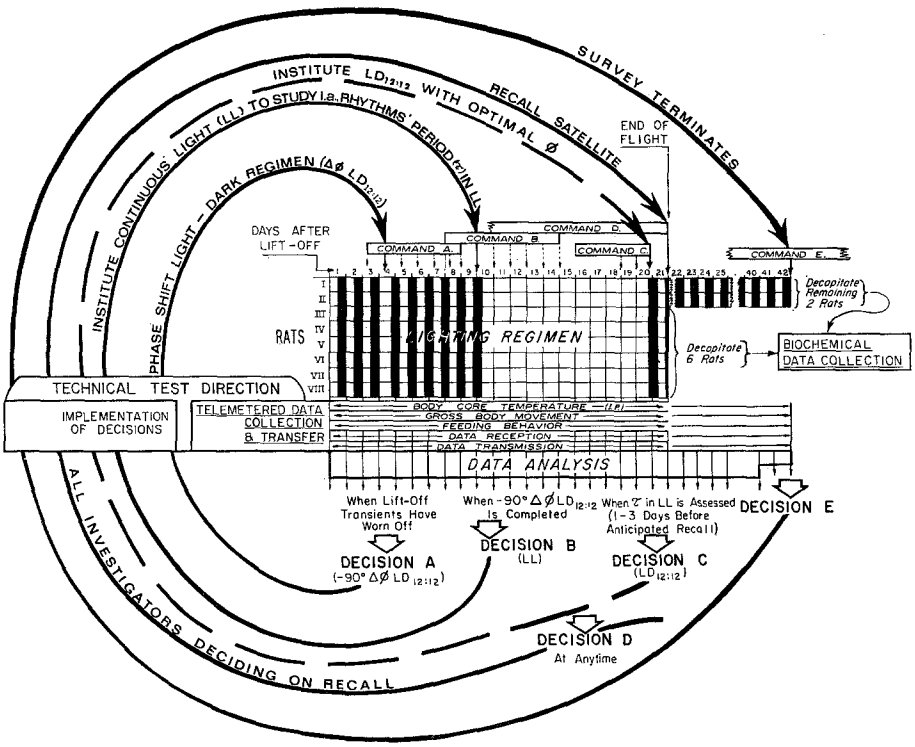


Fig. 4. Operations plan for 21-day rat Biosatellite – sequence of lighting regimens to be instituted after lift-off at times determined by results from 'as-you-go' analyses of the data. The same as-you-go analyses may be useful for assessing rhythm characteristics in experimental work on Earth and conceivably in the clinic.

(1) until the circadian rhythms' shift in acrophase had been demonstrated to be complete according to inferential statistical criteria *or*

(2) for one week,

whichever span was shorter. Any effects of orbital flight on the resynchronization of circadian rhythms could be evaluated during this stage by reference to quasi-currently obtained results from analyses of control animals on the ground, subjected to the same conditions.

Stage III: *LL*-continuous light (approximately 30 lx) – applied in order to evaluate the characteristics of circadian rhythms desynchronized from both the solar and

lunar day. Stage III was to last until the 19th day of the 21-day flight or for a minimum of 7 days.

Stage IV: $LD_{12:12}$ – reapplied with such timing that circadian rhythms are resynchronized in the shortest possible span. Stage IV was to be omitted:

(1) if, during the last few days prior to recall of the satellite, the results in *LL* suggested but did not as yet establish a difference between 'flyers' and concomitant controls, or

(2) if the recall had to be implemented on short notice before the 21st day.

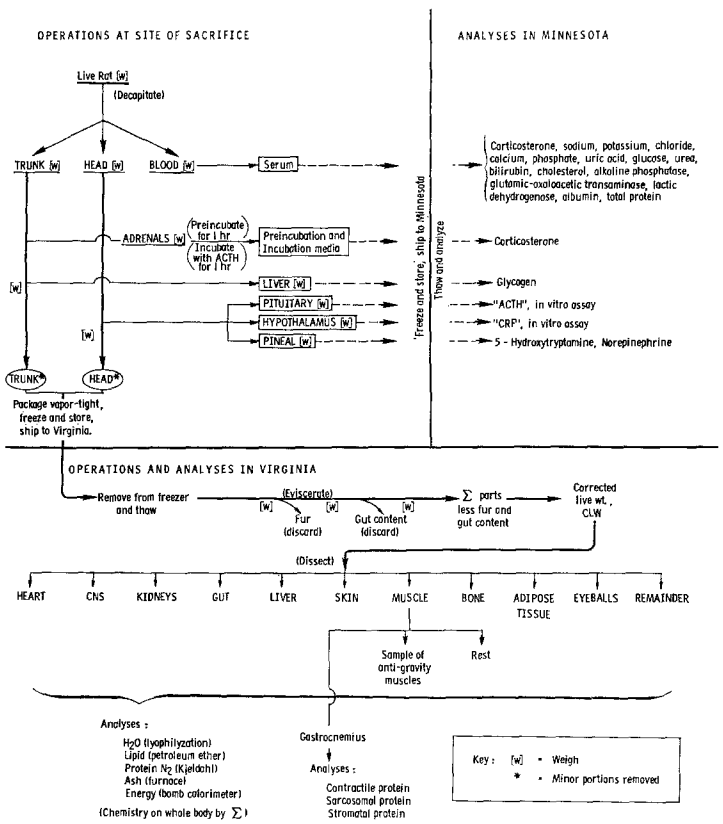


Fig. 5. Biochemical operations visualized after Biosatellite recovery; the same procedures were to be applied to 'flight rats' and to a much larger number of control rats (see text). Such integrated plans may be modified and extended by including many other variables for work in a Skylab or with organisms kept consistently on Earth.

Immediately following recovery, 6 of the 8 flight rats and an optimal number of control animals were to be sacrificed for biochemical studies, including a detailed analysis of body composition according to Figure 5. The remaining 2 flight-rats and their controls were to undergo a post-flight exposure to the same test sequence as a further examination of possible flight effects.

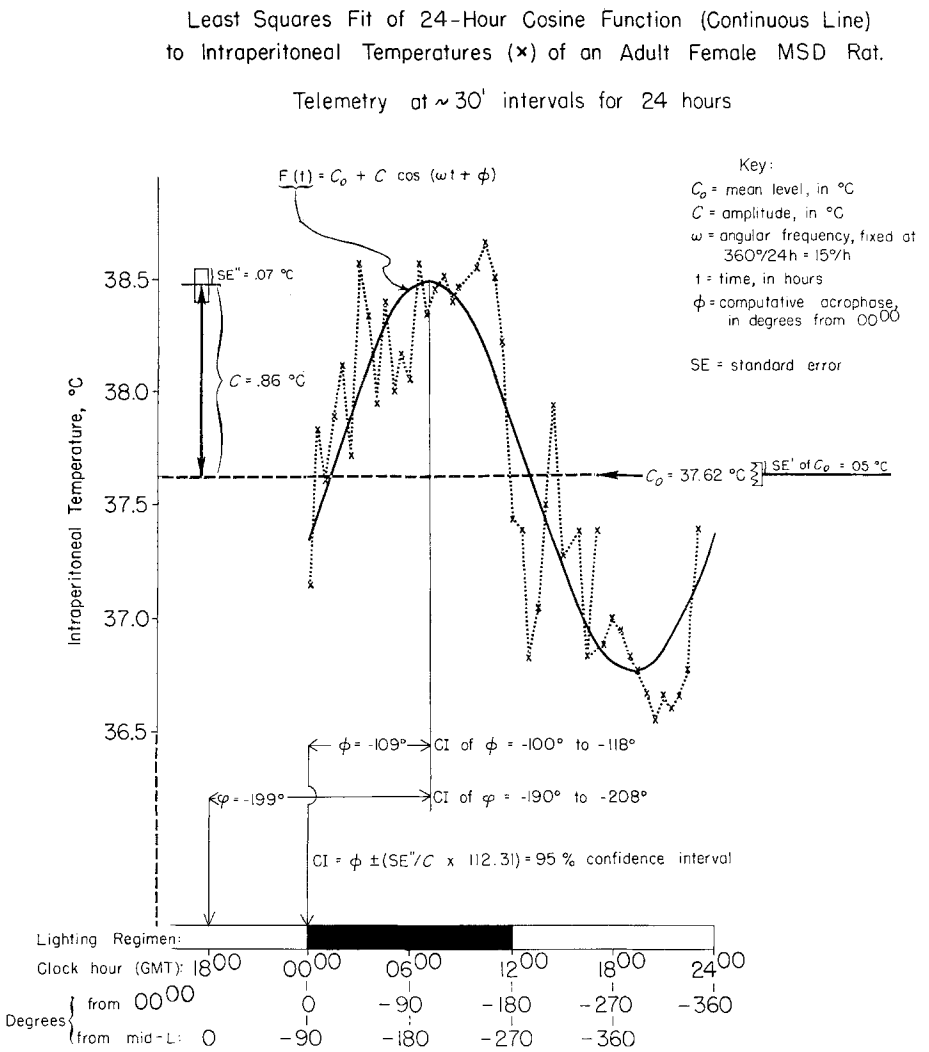


Fig. 6. Imputation of level, amplitude and acrophase by the least squares fit of a cosine curve – as a first step in the cosinor procedure. Such imputations are intermediate computations rather than being endpoints in themselves (see legend to Figure 7). Note especially the different types of acrophases, computative (ϕ) and external (φ).

E. METHODS OF DATA ANALYSIS

The development of methods for the objective evaluation of biorhythms was critical for the purposes of the proposed study, since it was presumed that the effects of orbital flight would be quantitative rather than qualitative; i.e., it did not seem likely that biorhythms would be obliterated in extraterrestrial space (Halberg *et al.*, 1970) but rather that some of the biorhythm characteristics might be altered.

Over the span of several years, a number of computerized methods were developed and tested, including:

ABSTRACT ILLUSTRATIONS OF COSINOR PROCEDURE

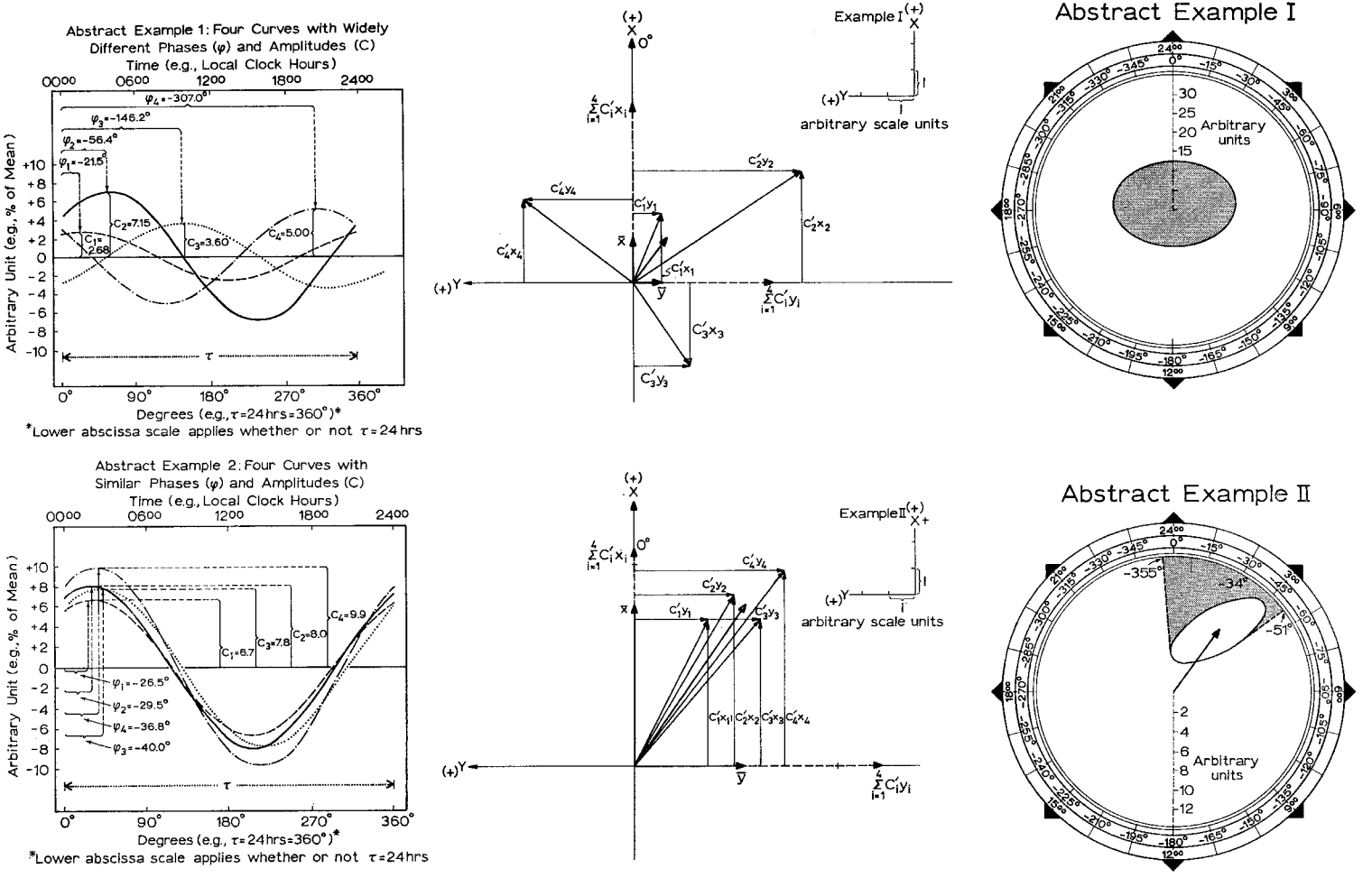


Fig. 7.

(1) the least squares fitting of cosine functions to a time series yielding point and interval estimates of rhythm levels (C_0), amplitudes (C) and acrophases (ϕ) – the application of this method to telemetered data on intraperitoneal temperature of a rat during a single 24-h span being depicted in Figure 6;

(2) the cosinor method of combining individual estimates of C and ϕ for a statement of group rhythm characteristics (Halberg *et al.*, 1967) – the conceptual steps in this method being summarized in Figure 7.

The value of this analytic approach can be appreciated by inspecting daily cosinor results from a group of 7 rats during consecutive days in $LD_{12:12}$ (Figure 8) and following changes in lighting regimen (Figure 9). The relative stability of the group acrophase in $LD_{12:12}$ can readily be differentiated from the changes in acrophase after regimen shifts or in continuous light.

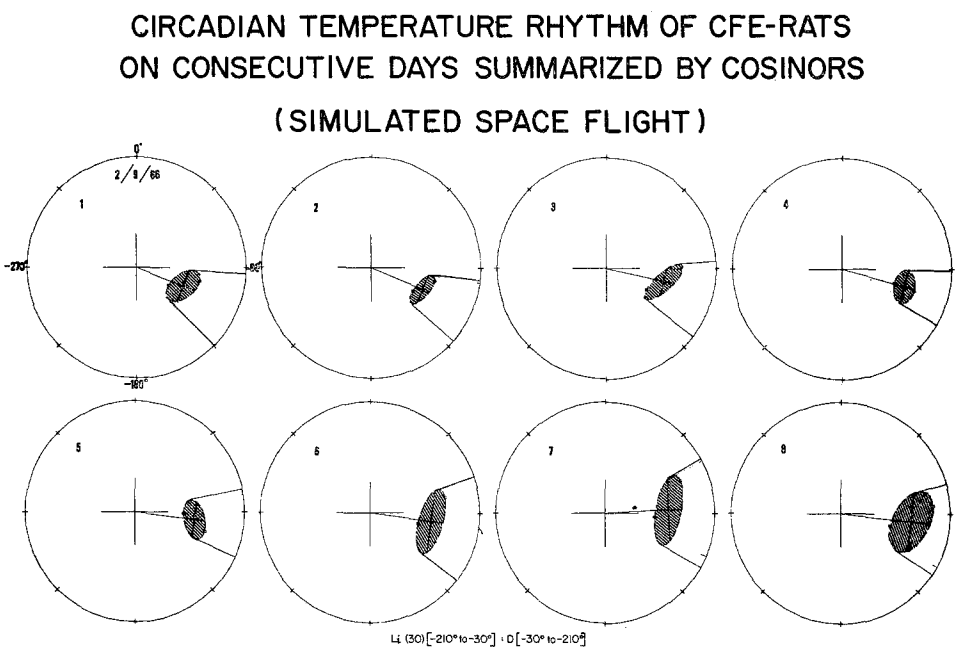


Fig. 8. As-you-go cosinor analyses of circadian rhythm in intraperitoneal temperature of mature female CFE rats during a span involving exposure to a regimen of alternating light and darkness at 12-h intervals.

Fig. 7. Abstract illustration of cosinor procedures. As opposed to the display exclusively in time – the chronograms on the left allowing only a macroscopic view of rhythms – the cosinor summaries at the right involve first a vectorial transformation – the imputation (Halberg *et al.*, 1967b) – shown in the middle. The summary of imputed vectors is displayed in a polar graph in the cosinor. Note that the cosinor at the top, based upon four curves with widely differing acrophases and amplitudes, overlaps the pole – a finding indicating that no phase and frequency-synchronized rhythm has been detected. By contrast, the cosinor ellipse at the bottom does not cover the pole; a rhythm has been detected in this case, and one then proceeds to the estimation of amplitude and acrophase with their statistical confidence intervals.

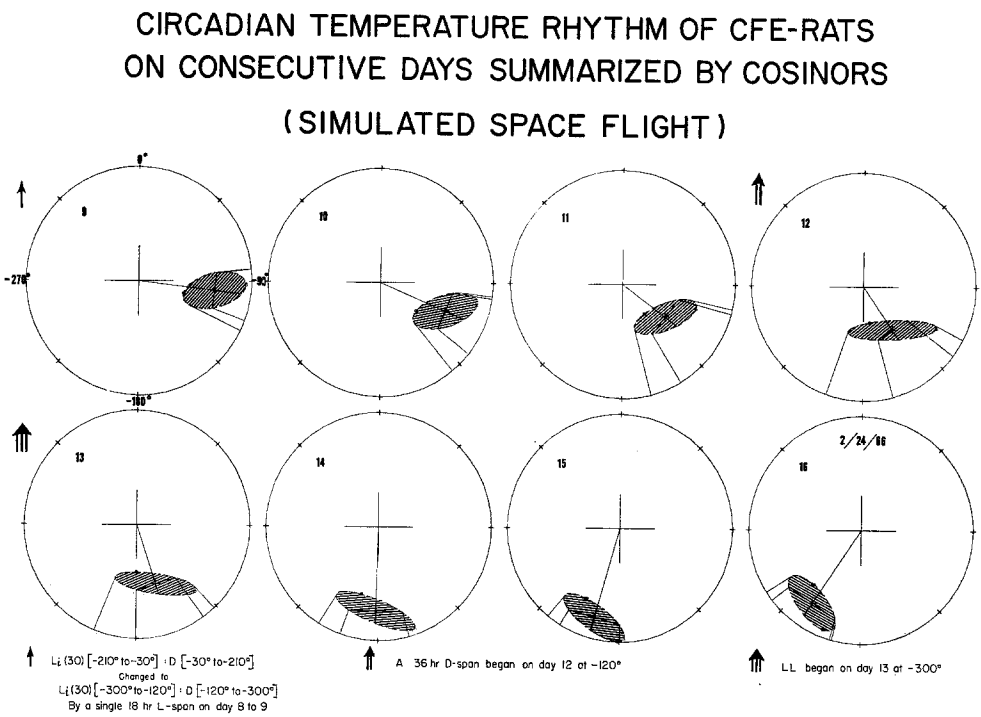


Fig. 9. As-you-go cosinor analyses of intraperitoneal temperature data from mature female CFE rats following a single delay of the synchronizing lighting regimen on Day 9 and the institution of continuous light on Day 13; on Day 12 occurrence of a 36-h span of darkness was unintentional and did not appreciably alter results.

The cosinor procedure has a proven ability to provide consistent estimates of time relations among rhythms in data which may confound the unaided eye. This conclusion was validated when time plots of data on intraperitoneal temperature, gross motor activity and feeding of a number of rats over a 7-day span were examined by

TABLE II

Disagreement, evaluated by x^2, of results from rating macroscopic (Halberg, 1969) physiologic records of gross motor activity, feeding and body temperature

Variables and criterion rated	Disagreement P
Activity precedes temperature	< 0.01
Temperature precedes activity	< 0.01
No difference detected, or unable to evaluate	< 0.01
Insufficient data for evaluation	> 0.10

Data telemetered from > 24 rats every 10′ during 7 days.
Agreement on (1) inability to interpret timing in any and all records of feeding activity (not shown) and (2) that certain records are insufficient for evaluation (last row). (cf. Halberg, 1969.)
Analyses by Professor George P. H. Styan, formerly of the Department of Statistics, University of Minnesota, Minneapolis.

TABLE III

Internal circadian acrophases of intraperitoneal temperature (T), gross body movement (A) and feeding (F)[a]

1968 Date	N of rats (range)	Internal timing		
		$\Phi_A(T)$	$\Phi_F(T)$	$\Phi F^{(A)}$
5/6	24–35	0	+19	+19
5/7	31–34	0	+21	+21
5/8	31–35	+ 7	+33	+26
5/9	28–32	+ 7	+25	+18
5/10	24–35	+ 3	+10	+ 7
5/11	31–35	+10	+14	+ 4
5/12	24–29	+ 1	+13	+12

[a] Cosinor summaries of data at 10′ intervals for consecutive days on groups of mature female CFE rats; internal acrophase reference is external acrophase of rhythm identified in ( ).

a panel of five senior research personnel (Maynard *et al.*, 1969) to determine, with the unaided eye, whether the circadian activity and feeding rhythms preceded or followed that of intraperitoneal temperature. These 'judges' were unable to interpret timing of feeding activity; moreover, their independent interpretations as to whether the temperature or activity peak occurred earlier did not agree (Table II). On the other hand, analysis of the data with the cosinor procedure indicated that the acrophase for feeding was consistently earlier than that for temperature or activity. The acrophase of activity preceded that of temperature on 5 of the 7 days examined, with no difference on the other 2 days (Table III)*.

* It should be emphasized that since the acrophase, ϕ, describes the timing of the cosine curve approximating all data best, this endpoint cannot be used to examine any question relating to single characteristic points on a curve such as an activity onset. Hence, the activity ϕ can lag slightly behind the feeding as it does in the case here examined even though the animal usually will have had to move first to go to the feeding place. Moreover, if the animal was resting close to the feeding place, no movement will be recorded as activity with the system here employed since it is based on relatively large horizontal displacement. It is also conceivable that, apart from acrophase relations, an initial about-daily temperature rise following the longest about-daily rest span may well precede that in body activity and that the onset of body activity may precede the initiation of feeding – even if the overall curves are timed differently. An almost certain finding based upon the results obtained herein on acrophase is that circadian rhythms in feeding, body temperature and activity are extremely closely timed. Thus the major point here concluded objectively without much bias, if any, is that the activity acrophase on the average was within 10 deg from the temperature acrophase and the feeding acrophase within 33 deg on the average from that of temperature. Clearly such results will have to be complemented by a study of the waveform which remains beyond the scope of this first publication but constitutes a major consideration in any follow-up.

The value of consistent estimates from mathematical analyses may be emphasized by an analogy to histology. In the latter science, it is often quite difficult to distinguish artifact from structure and critics have referred to histology as the study of reproducible artifacts obtained with denatured material. Nevertheless, histologic techniques are of unquestionable value to medicine.

When time series are analyzed by the cosinor method, for instance, artifacts can indeed appear. The need for 'standards' and 'blanks' has been emphasized elsewhere (Halberg, 1968) as a routine test for artifacts due to the method of data analyses. With proper attention to such procedures the different statistical analyses of rhythms can resolve new dimensions in time hopefully as significant as those revealed in space by the methods of histology.

3. Compatibility of Hardware and Mission Design in the Planned Biorhythms Investigation

A. HARDWARE SUITABILITY, INCLUDING EFFECTS OF SIMULATED LIFT-OFF AND REENTRY TRANSIENTS.

Would the design and life-support system of the proposed 21-day Biosatellite package be compatible with the health of experimental animals and with the demonstration of circadian rhythm characteristics? This question was answered favorably by a study conducted on 7 rats housed in a Biosatellite 'developmental' hardware during a 3-week simulation of the mission, including exposure to expected lift-off and reentry transient levels in acceleration, vibration and noise. The rats gained weight and survived in good condition. It was also possible to demonstrate (by applying to telemetered data the analytic procedures described above) a synchronized circadian rhythm in intraperitoneal temperature while the rats were under an $LD_{12:12}$ regimen in the hardware (Figure 8), as well as the expected acrophase changes of the rhythm in response to changes in the lighting regimen (Figure 9).

B. PERIODIC ENVIRONMENTAL TEMPERATURE CHANGES

The 21-day Biosatellite on its intended orbit at 200 nautical miles altitude could not be expected to maintain ideally constant internal environmental characteristics, the cage climate being generated by a complex interaction of factors both inside and outside the spacecraft. Since the period of one orbital pass was to be 1.5 h, most environmental changes could be expected to synchronize with this periodicity. It had been estimated that, during each orbital pass, the rat cage temperature might vary about $\pm 3\,^{\circ}C$ and the relative humidity about $\pm 15\%$, due to orbital fluctuations in heat exchange.

Studies were carried out to test the effects of such environmental changes on rhythms in the rat. A 90-min cycle in environmental temperature, from 19° to 25 °C associated with inversely varying relative humidity from 59% to 45%, was imposed on a group of 11 rats bearing intraperitoneal temperature transensors while they were exposed to the lighting regimen test sequence planned for the 21-day Biosatellite (Figure 4). Comparison of daily cosinor results from this group with those from a concurrently monitored control group of 12 rats maintained at constant environmental temperature and humidity indicated no appreciable effect of cycling temperature on the shift-time of the circadian temperature rhythm in response to a 90° delaying shift in the lighting regimen (Figure 10).

The period of the free-running circadian temperature rhythm in *LL* was also similar for the two groups, as indicated by computing the number of days required for a 360° drift of the circadian acrophase.

C. PERIODIC NOISE

Before flight, little can be ascertained about 'background' changes in mechanical vibrations and acoustics to be expected on board the orbiting satellite. Limited

measurements under crudely simulated orbital conditions indicated a fluctuation of background noise from 54 to 68 dB (sound pressure level) in the band from 100 to 1500 Hz. In addition there occurred a 100-min span of intermittent noise up to about 86 dB at 12-h intervals, associated with operation of cameras and solenoids on board the 21-day Biosatellite for other experiments.

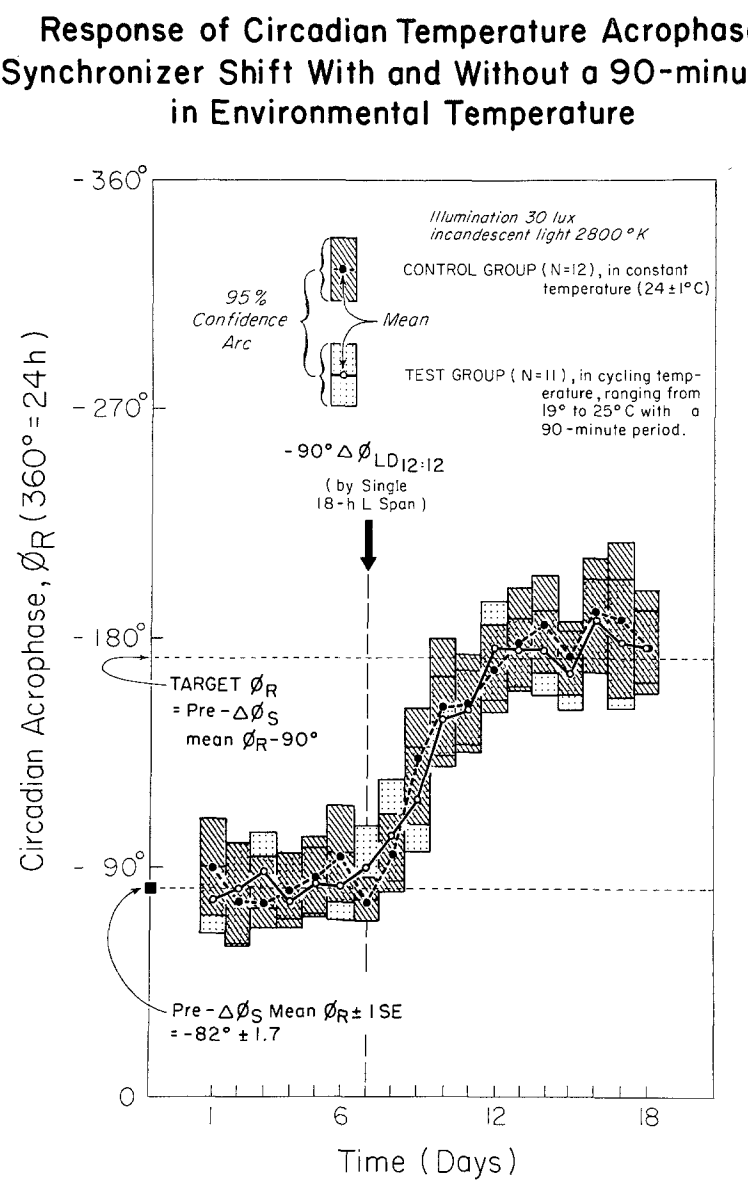


Fig. 10. Similar acrophases and shift rates of circadian rhythm in intraperitoneal temperature of mature female inbred Minnesota Sprague-Dawley rats; confidence intervals of acrophases overlap and both groups reach resynchronized acrophase at about the same time despite exposing the test group to a 1.5-h cyclic 6°C change of cage temperature.

Possible effects of such noises were studied on a test group of 8 rats during a 21-day verification test of the Biosatellite hardware, with all experiments 'on board'. A concomitant control group of 8 rats was housed in a similar hardware but without the presence of other experiments. Analyses of telemetered data by group cosinors indicated similar changes in timing of the circadian temperature rhythm in response to the test sequence of lighting changes for both the control and test groups, despite exposure of the latter group to the periodic noises referred to above (Figure 11). Furthermore, a least squares spectrum analysis of temperature data from the experi-

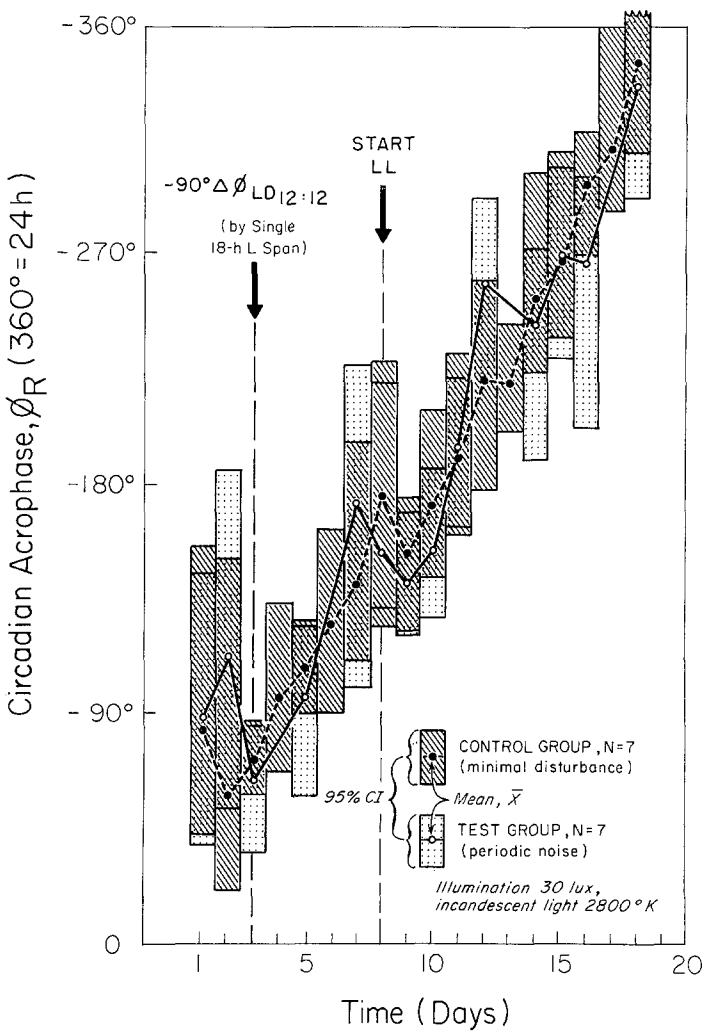


Fig. 11. Shift and drift rates of the circadian body temperature acrophases of mature female inbred Minnesota Sprague-Dawley rats remain similar in the presence of cyclic intermittent noise – the test group being exposed to a noise continuum varying between 54 to 68 dB with 12-h periodic increases to 86 dB for 100 min. Because of system malfunctions, data were available from only 7 of the 8 rats in each group.

mental rats in *LL* revealed no component with a period of 12 h, so there was no apparent synchronization by the 12-h periodic noise of 100-min duration.

D. 'CROSS-TALK'

Another point of concern was the possibility of biological 'cross-talk' among rats in the satellite. Even though the animals were to be individually housed, the food control system, as well as other properties of the cage design, presented possible opportunities for communication by sound and odor. Any such animal interactions might affect the interpretation of results, especially in comparisons with those control animals housed in more conventional facilities.*

As a test of such possible effects, data on temperature, activity and feeding were obtained from 8 rats housed in a single Biosatellite developmental hardware. All 8 rats had been on a regimen of continuous light (*LL*) for about a week prior to start of the 'cross-talk' test. At this time an $LD_{12:12}$ regimen was imposed on 4 of the rats while the remaining 4 continued in *LL*. Analyses of data by daily group cosinors revealed a rapid shift of rhythms in the *LD* group from the phase observed on the last day in *LL* to a new and apparently stable phase imposed by the $LD_{12:12}$ regimen. Cosinors from the *LL* group, on the other hand, indicated a continuing gradual phase-drift at a rate similar to that previously observed for rats in *LL*. Thus 'cross-talk' among rats housed individually but within the same hardware unit was not sufficient for circadian rhythm synchronization. (Maynard *et al.*, 1969).

E. PRESENCE OF TRANSENSOR IN PERITONEAL CAVITY

The use of a miniature implantable transensor was based on the desire to obtain nearly continuous information on undisturbed and unrestrained animals. Possible effects of such chronic implants must be born in mind.

In the light of the literature (Bischoff and Bryson, 1964; Brand *et al.*, 1967, 1968; Johnson *et al.*, 1970), tumorigenesis from the paraffin-base encapsulant of the transensors appeared unlikely within the implantation span of these studies. Systematic autopsies covering implantation times up to a year revealed no such tumors. In some animals the 'peritonealization' of the free implants led to formation of massive fibrotic capsules. Such effects were seen with the use of the relatively large and heavy AEL device and were absent after implantation with the smaller IV-XLL transensor (Table I). Histologic spotchecks, kindly carried out by Dr. K. G. Brand, Department of Microbiology, University of Minnesota, confirmed the absence of tumorigenesis for the span of observation.

A previous report from our laboratories (Pitts *et al.*, 1969) dealt with the effects of a rather large ($\sim$10 g) intraperitoneally-implanted mock-AEL-transensor on body composition of rats. A principal finding was that the presence of such an object for two to three months resulted in an apparent decrease in carcass fat content and an

* For reasons of economy, the number of planned rat assembly replicas was to be smaller than was needed to accommodate the larger group of control rats needed for biochemical and physiological studies.

increase in visceral water content. These results are probably due to the effect of the object as a physical load and as a local irritant, respectively. It was also noted that implantation of the mock-transensor resulted in a sustained reduction in body weight. A more recent study has essentially confirmed this effect of the large mock transensor on body weight, while showing that a smaller unit approximating in dimensions, weight and coating the transensor ultimately selected for the Biosatellite study – see

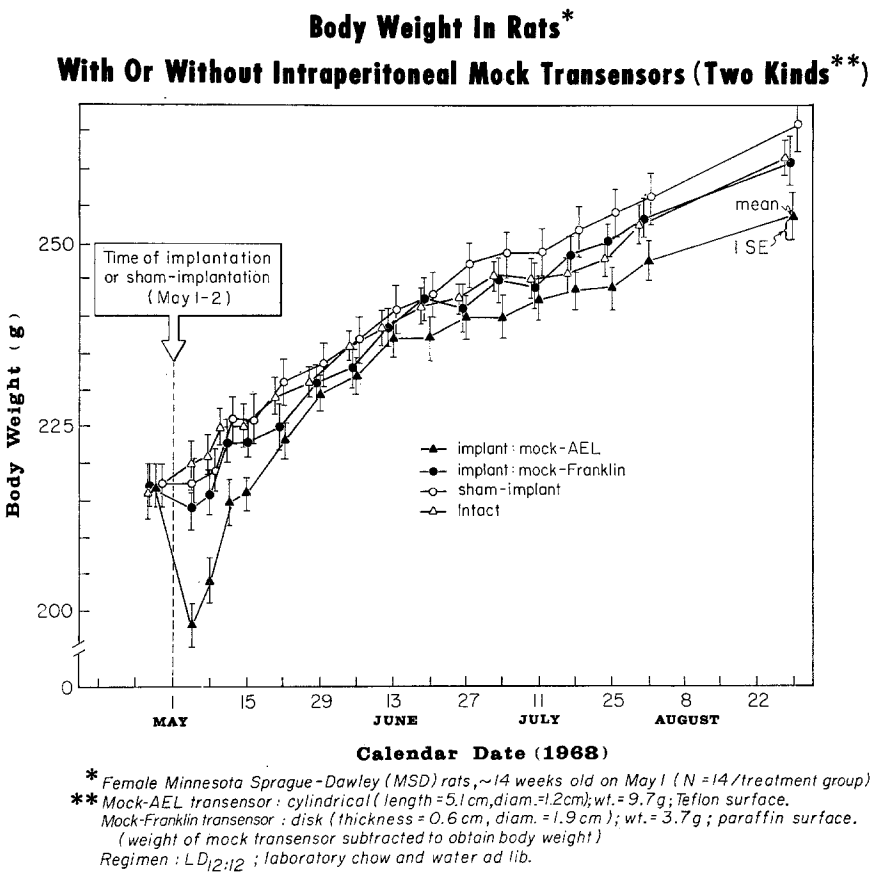


Fig. 12. The intraperitoneal implantation of a light-weight (3.7 g) temperature telemeter into mature rats is well-tolerated.

Table I – had no observable effect on body weight over a four-month span of observation (Figure 12). It appears safe to conclude, with present information, that chronic intraperitoneal presence of the small Franklin Institute transensor is well-tolerated by rats for the span here investigated.

F. DIET

The Biosatellite hardware-designers first considered the rat as a biting animal and

designed a food delivery system which was activated by a bite. However, on this system the rats starved. Subsequent slow motion cinematography revealed that the rat first uses its incisors to rasp food loose and thereafter licks up the particles. Based on this observation a satisfactory system was devised. Rats were easily trained to operate a lever dispensing liquid diet into a feeder cup from which the animal could lick (g in Figure 2 and 3).

A comparison of rats consuming rat chow (Ralston Purina, St. Louis, Missouri) plus water and rats consuming the liquid diet planned for the Biosatellite mission (Codelid Diet-15, Schwarz Bioresearch, Orangeburg, New York) indicated a significantly greater weight gain and body fat content for the latter. However, estimates of period and amplitude of the circadian temperature rhythm of rats in *LL* were similar regardless of diet (Figure 13). On the basis of this and numerous other studies, it was concluded that this liquid diet was satisfactory from the standpoint of the biorhythm study.

G. CIRCADIAN PHASE AT LIFT-OFF

Numerous studies have revealed circadian rhythms in the susceptibility of animals to noxious agents, including noise (Halberg, 1962; Pauly and Scheving, 1964; Reinberg and Halberg, 1971). Such information suggested that the success of the 21-day Biosatellite mission might depend, in part, on the circadian system of the rats at the time of exposure to the trauma associated with rocket launching. To test this possibility, four groups of 8 adult female rats, maintained on a regimen of *L* (0600–1800): *D* (1800–0600), were exposed to simulated Biosatellite lift-off transients (noise, vibration and acceleration), each group at one of 4 different clock h (0600, 1200, 1800 and 2400) corresponding to different circadian system phases of the synchronized animals.

Following such exposure each group was placed in a developmental hardware. A control group of 8 rats was treated in the same manner as the above except that it experienced no lift-off transients. Data on intraperitoneal temperature, gross body movement and feeding activity were recorded at 10-min intervals before and during a 51-day span following the exposure to launch transients. During this span all rats were on a regimen of *L*(0600–1800):*D*(1800–0600) for the first three weeks. Then all were subjected to a 6-h delaying shift of the regimen to *L*(1200–2400):*D*(0000–1200).

Analysis of data by the cosinor method has revealed no appreciable difference among study groups with respect to:

(1) estimates of circadian level, amplitude and acrophase of the three variables studied during the $LD_{12:12}$ regimen;

(2) rate of resynchronization of these rhythms in response to the 6-h delaying shift in lighting regimen.

Although more frequent tests or a more detailed analysis of the data may yet reveal differences dependent upon the circadian system phase at exposure to lift-off conditions, it appears that the effects here tested would be slight and that no biological constraints would need to be placed on the timing of actual lift-off, unless the launch conditions here tested were exceeded.

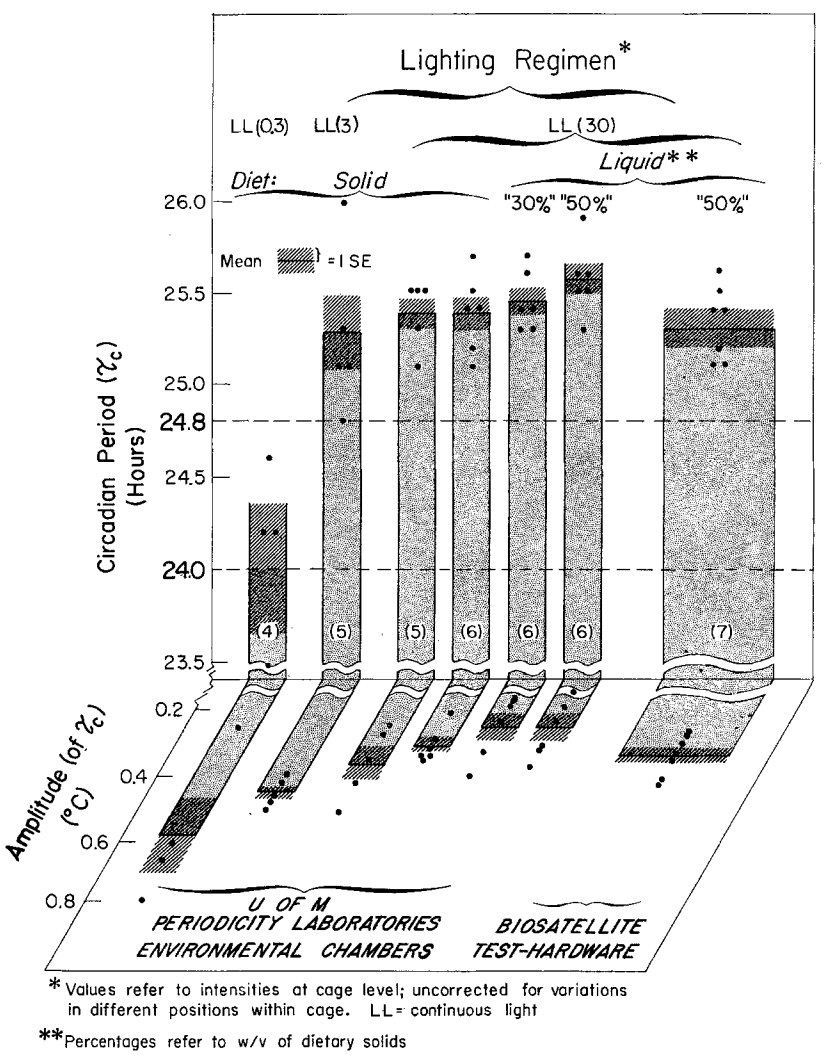


Fig. 13. Continuous light of about 30 lx intensity is specified for certain rat strains as a condition associated with a prominent circadian period differing from both the solar and the lunar day length (Halberg, 1966). It will become apparent from the last five columns on the right of the figure, summarizing time series from rats kept in continuous light of 30 lx intensity, that a non-24-h, non-24.8-h average circadian τ is highly reproducible, whether the animals are fed Purina Laboratory Chow, with water freely available, or are maintained on liquid fully-synthetic diets with 30 or 50% weight per volume solids. The data in the last column were obtained at the Ames Research Center of NASA at Moffett Field, California, with the cooperation of G. Dale Smith, T. N. Edwards, P. Sebesta and T. Jackovich.

4. Studies Associated with Design of Operations Plan (Figure 4)

A. LIGHT INTENSITY IN *LL*

One of the early problems faced in preparation for the 21-day Biosatellite was the specification of light intensity for the rat cages. Aschoff (1959, 1969) had reported for the mouse that the period of the circadian rhythm in gross motor activity, desynchronized from the 24-h schedule on a regimen of *LL*, increases as light intensity increases. Since similar information was not available for the rat, a study was made of the relation between characteristics of the circadian rhythm in the telemetered intraperitoneal temperature of rats and the intensity of continuous illumination over a range from zero (*DD*) to about 500 lx using incandescent bulbs operating at 2800 K color temperature. Figure 14 presents the results of this study comparing circadian period and amplitude of the synchronized rhythm (rats in $LD_{12:12}$) with corresponding measures of the desynchronized rhythm (rats in *DD* or in *LL* of different intensities). Each estimate was made from data collected nearly continuously from 8–14 rats over a span of at least 2 weeks.

For stage III of the test sequence (i.e., of the intended flight profile, namely the *LL* span), interpretation of results would be facilitated if it were possible to obtain, at least for control animals on earth, a desynchronized circadian period different from both the solar day (24 h) and the lunar day (24.8 h). By applying this desideratum to the results shown in Figure 14, and to results from other similar studies, the light intensity for rat cages was specified at 30 lx, rather than at higher level, i.a., in order to save electrical power.

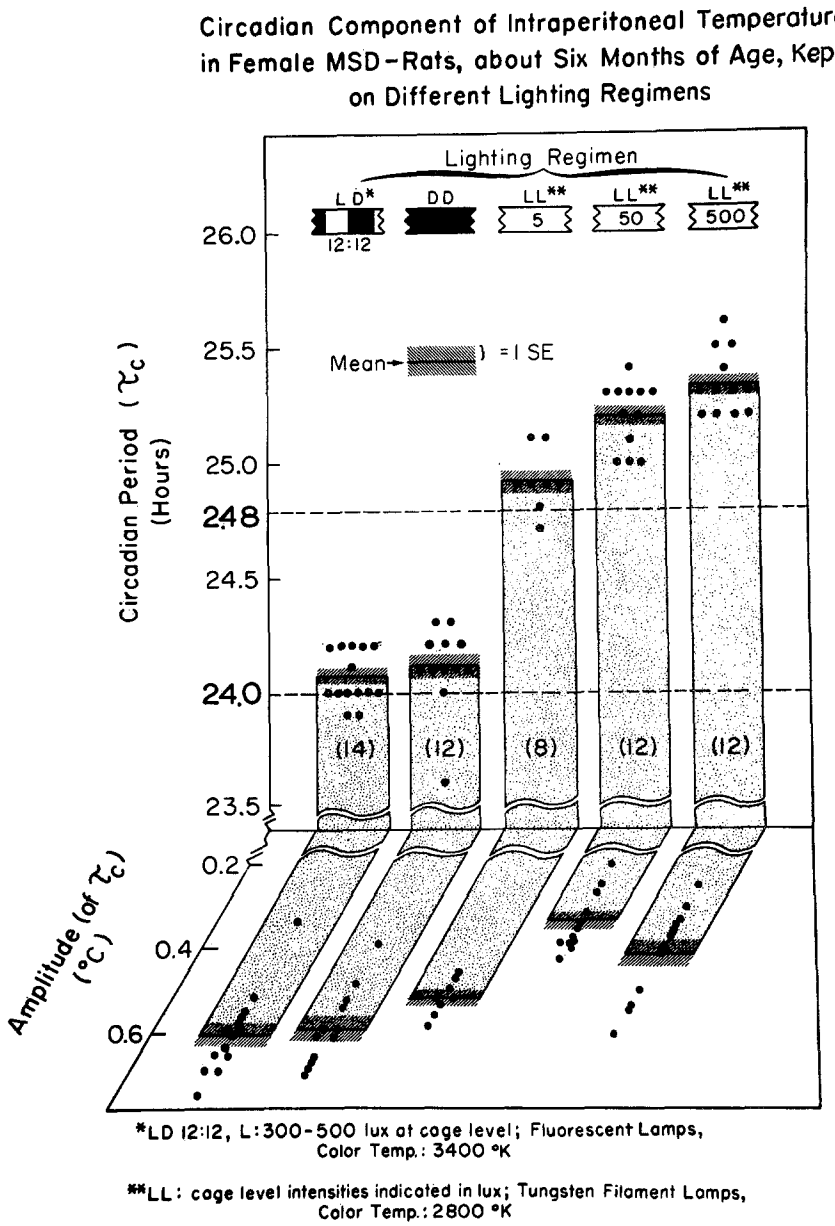
B. PHASE-SHIFTING BY ADVANCE OR DELAY OF $LD_{12:12}$ REGIMEN

Stage II of the test sequence calls for a determination of the rate at which circadian rhythms resynchronize following a phase-shift of the lighting regimen. A number of studies have revealed that the rate of rhythm resynchronization following a 90° (6-h) shift in an $LD_{12:12}$ lighting regimen depends on whether the change in regimen involved an advance ($+90°\Delta\phi S$, accomplished by a single 6-h span of light or darkness) or a delay ($-90°\Delta\phi S$, accomplished by a single 18-h span of light or darkness). Figure 15, derived from daily group cosinor analyses, indicates that the circadian temperature rhythm resynchronizes more rapidly (within 5 days) following a 6-h delaying shift of the lighting regimen than it does following a 6-h advancing shift (9 or more days required). In view of such results and of time limitations on the 21-day Biosatellite mission (including the possibility of an early recall), it was decided that Stage II should involve a 90° delaying shift of the regimen. More rapid resynchronization at this stage would allow more time for a study of desynchronized rhythms (Stage III).

C. RESYNCHRONIZATION OF FREE-RUNNING RHYTHMS PRIOR TO REENTRY

Immediately following reentry of the Biosatellite, 6 of the recovered rats were to be sacrificed for biochemical studies (Figure 4) whereas 2 rats were to be studied further

by telemetry. During preparatory work, circadian rhythms were found in many of the physiologic and chemical variables chosen for investigation at killing. Hence our first wish, had we unlimited facilities, might have been to study several groups of satellite rats, each group to be investigated at a different timepoint after recovery, say a first one as soon as possible after recovery and others at 4, 8, 12, 16, 20 and 24 h thereafter. But even in such a design the effects of lapse of time from cessation of 'zero-G' effects



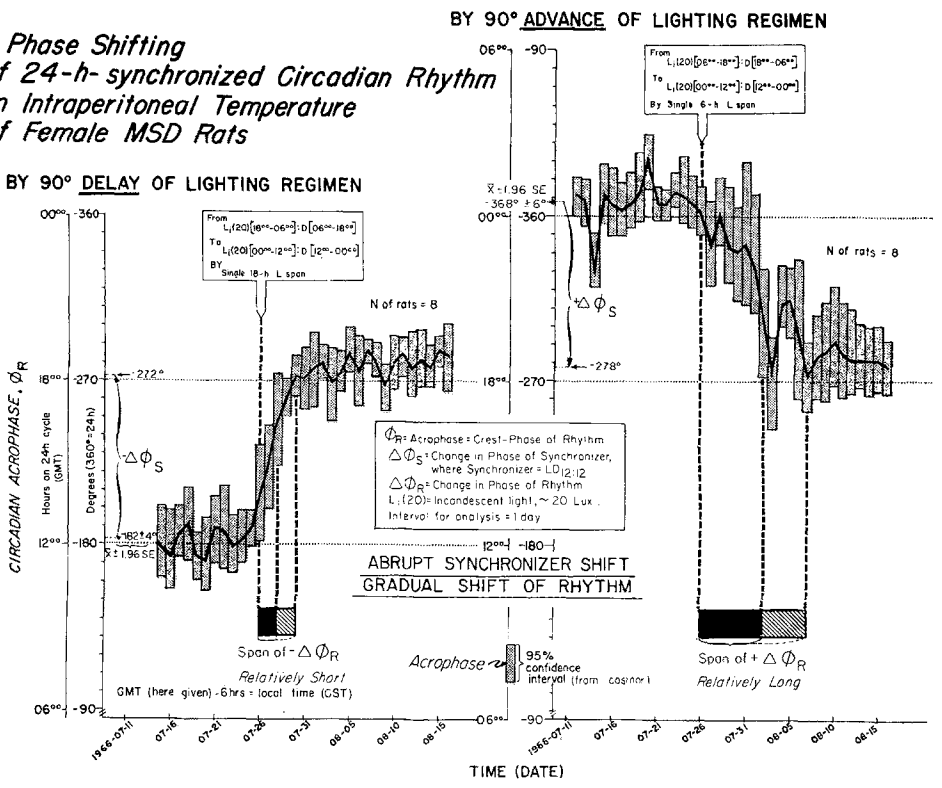


Fig. 15. Rhythm adjustment in mature female inbred Minnesota Sprague-Dawley rats is faster following a delay of the synchronizing lighting regimen that following an advance (Halberg *et al.*, 1967a).

Fig. 14. Objectively quantified endpoints from the generally applicable least squares spectra estimate the period, τ, and amplitude, C, of synchronized and desynchronized circadian temperature rhythms in inbred Minnesota Sprague-Dawley rats. Light intensities given are those measured on unobstructed floor in a covered cage. Rats are sometimes exposed to lower intensities than indicated, i.e., when sitting under the filled food hopper in the cage covers.

The lengthening in τ of temperature rhythm with increasing light intensity in dark-active animals corresponds to Aschoff's rule (Aschoff, 1969) derived from studies of gross motor activity. At a light intensity of about 5 lx, the circadian body temperature period of 6 of the 8 MSD rats studied on this regimen (hourly data covering about 3 weeks per animal) is extremely close to the lunar period of 24.8 h. On the basis of other data, Frank A. Brown, Jr. (1965a, b) has suggested that circadian rhythms in mammals are subject to period-lengthening from lunar influences. Terrestrial studies therefore seem desirable to evaluate such influences at several specified levels of light intensity. Furthermore, if in the U.S. Apollo program some experimental animals could be sent to the Moon, it would be interesting to explore possible differences in characteristics of the circadian intraperitoneal temperature rhythm of MSD rats kept in *LL* of 5 lx intensity on the Moon and on Earth.

Apart from extending Aschoff's rule regarding the change of the circadian τ, to a function other than body activity, i.e., to be intraperitoneal temperature of rats, with data subjected to frequency analyses, the effects of light intensity upon the C of rhythm also are clearly demonstrated.

would have been complicated by those of any and all rhythmic changes within the animal investigated. With only 6 animals available for immediate biochemical study, it seemed preferable to kill them all as soon as possible after recovery and thus to handle as best we could the lapse of time from reentry. However, for controls it was decided to start about 12 h prior to the anticipated time of recovery, with the 4-hourly killing of groups of control rats kept on earth so scheduled as to bracket the 24-h span centered around the time of killing of the animals recovered from space.

The comparison of controls with recovered rats might have been rendered more difficult by any inter-animal desynchronization of circadian rhythms that will occur under the *LL* regimen in Stage III of the test sequence. In addition, it seemed possible that rhythms in some variables might gradually diminish in amplitude during this stage.

Such considerations indicated the desirability of resynchronizing the rats to an $LD_{12:12}$ regimen prior to reentry; this was planned in such a fashion that a 12-h dark

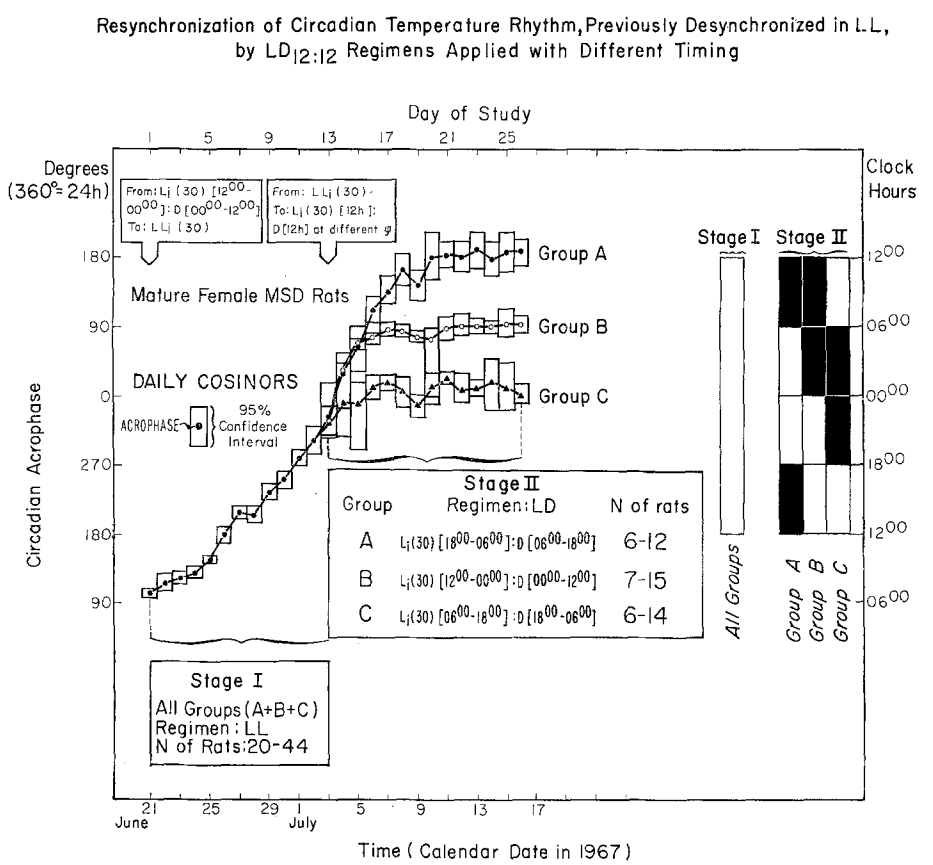


Fig. 16. Validation of command *C* to be carried out as a so-called 'Capture of circadian rhythms' – in order to synchronize rhythms in preparation for biochemical study – in keeping with DECISION C of the Biosatellite operations plan.

span would coincide with reentry and the associated loss of power in the cabin. In order to define more clearly the circadian system phase at reentry, it was important to know how quickly free-running rhythms could be resynchronized. The answer, obtained from the study of intraperitoneal temperatures summarized in Figure 16, was that this depends on the timing of the $LD_{12:12}$ regimen in relation to the rhythm's acrophase.

Resynchronization occurs rapidly if the *LD* regimen is in its 'usual' relation to the free-running circadian rhythm on the day of application; i.e., the middle of the *D* span is approximately coincident with the acrophase of the temperature rhythm (Group C). On the other hand, 5 to 7 days may be required if the *LD* regimen is far removed from its usual phase in relation to the body temperature rhythm on that day (Group A).

These results indicate that an $LD_{12:12}$ regimen with proper timing could be re-instituted as late as 1 or 2 days prior to the planned reentry of the Biosatellite. The timing of this regimen would have to be based upon quasicurrent analysis of information on circadian rhythms telemetered from rats in the satellite. (Maynard *et al.*, 1969.)

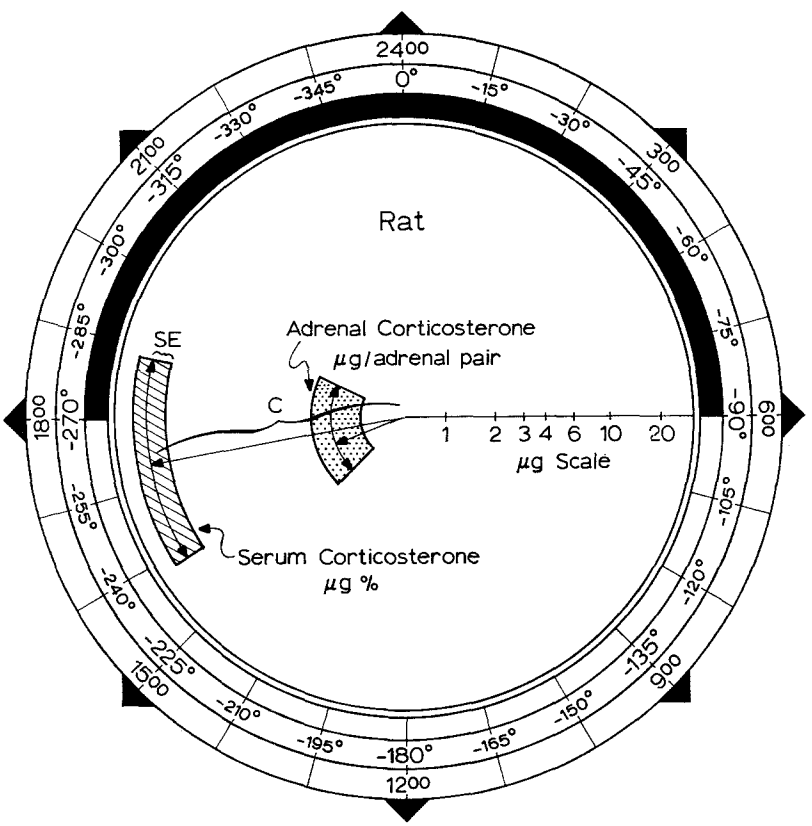


Fig. 17(a)

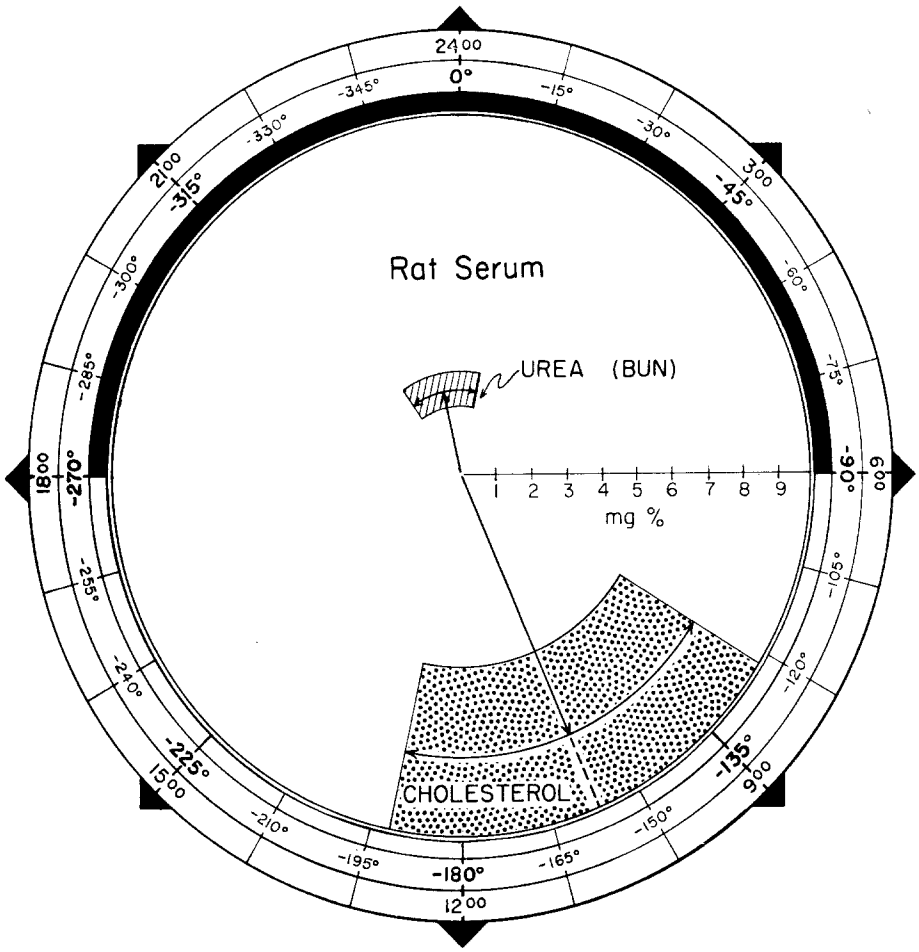


Fig. (17b).

D. BIOCHEMICAL RHYTHMS

Interpretation of results on circadian rhythmic biochemical variables in satellite and control rats at the time of recovery would require comparisons both in relation to telemetered data obtained prior to reentry and with respect to known circadian changes in those variables. This requirement can be appreciated if one considers the possibility that the external timing of circadian rhythms, even in relation to an $LD_{12:12}$ regimen, may differ between satellite rats and ground control rats. In such a case the direct comparison of mean biochemical values for satellite and control rats at the time of biosatellite recovery might be misleading.

For this reason a number of studies were made of circadian rhythms in biochemical variables in the rat – Figures 17 and 18. Table IV summarizes this information in terms of circadian level and amplitude as well as acrophase (with respective variability

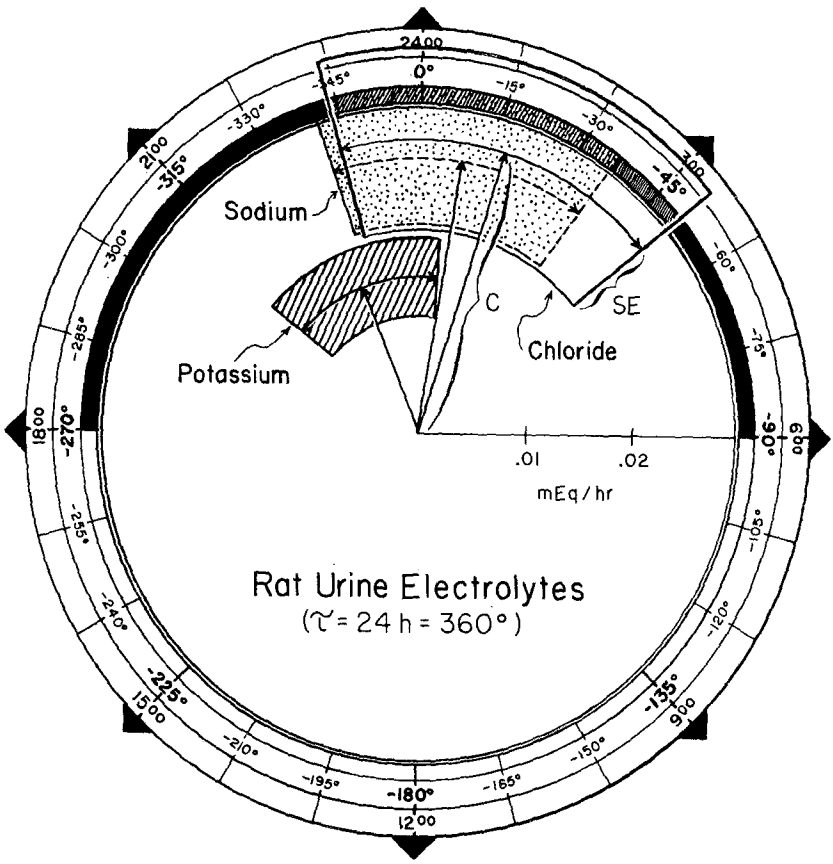


Fig. 17(c)

Fig. 17a-c. Cosinors of some biochemical functions in rat adrenal, serum and urine.

measures), based on the methods of data analysis described in Section 2E. Also included are results from data on intraperitoneal temperature, gross body movement and feedings, obtained by methods outlined in Section 2B.

This information as well as that from concomitant control animals was to be used in the evaluation of results from the Biosatellite rats. Indeed the question whether rhythms in biochemical variables are affected by space flight is of at least equal importance to those concerning 'biophysical' functions, such as temperature, or behavior, such as activity and feeding. Eventually, chemosensors should facilitate such studies on earth as well as in flight (Reynolds, 1968); however, until such time as these sensors become available, it is worthwhile attempting to obtain as much information as possible from a single biochemical sample, qualified by rhythmometry based on multiple biophysical and/or behavioral samples from the same individual. This approach is particularly promising when, as in the case of the test design worked

out for the Biosatellite, some physiological functions are already being monitored longitudinally on the same animals.

Against such background information on variables such as telemetered i.p. temperature, among others, one could compare a range of biochemical values from recovered animals with a circadian-temperature-rhythm-qualified range of usual values in terrestrial controls. Moreover, such a range of usual values is indeed a reasonable reference standard when it is based not only on prior experience, but is revalidated by a complete transverse profile obtained on terrestrial controls during the same 24-h span using the particular biochemical methods and conditions of observation specified for the animals recovered from space*.

5. Discussion

Although the study of rat biorhythms in earth orbit has not been realized under the Biosatellite program, some of the preparations described in this report may yet contribute meaningfully in several areas:

(1) Possible future studies of biorhythms in extraterrestrial space; (Pittendrigh, 1967; Halberg, 1962; Halberg *et al.*, 1970);

(2) Space medicine (Halberg, 1962);

(3) Aviation medicine (Halberg, 1969);

(4) Labor practices, including shift work (Menzel, 1962; Reinberg and Ghata, 1964;

(5) General medical observation, diagnosis and treatment (Halberg *et al.*, 1967b; Menzel, 1962; Halberg and Reinberg, 1967).

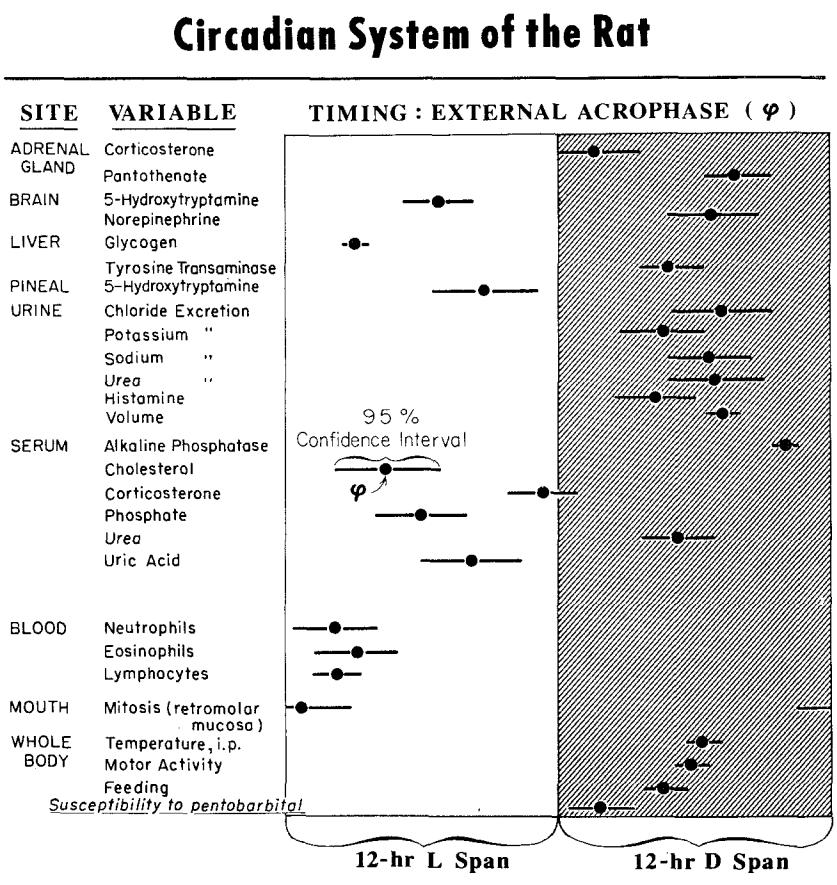
Any future 'Skylab' series of biologic investigations in earth-orbit may include studies of biorhythms. The methods and findings described herein could be directly applied to such studies.

The scheduling of astronaut activities during extended space flight may benefit from careful consideration of biorhythms. Methods of data analysis briefly summarized in Section 2E were applied to information on heart rate in astronauts and cosmonauts during Gemini and Vostok missions (Halberg *et al.*, 1970). Results indicate that

* The many variables here investigated document of course internal time relations that have to be scrutinized in any study dealing with rhythms. Whether families of control parameters in human beings co-vary systematically along a uniformly and/or physiologically demarcated time scale is a topic of concern in need of investigation. Covariance may be assessed along the conventional uniformly demarcated time-scale as an internal circadian acrophase (Halberg, 1969). Alternatively, multivariable data also may be interpreted for man on an episodal basis – be this basis demarcated by episodes representing physiologic events such as spontaneous arising time, by external social ones, such as the schedule of interactions with colleagues at work, regulated by clock, or quite often, by a complex of physiologic, psychologic and sociologic pertinent events.

Episodal biologic time may relate occurrences with different frequencies, such as the number of heart beats (that may serve as an episodal marker) between two consecutive respirations or the latter as they may change in relation to events of the sleep-wakefulness or menstrual cycles. The mathematical basis of such episodal time and its biologic pertinence may well be scrutinized in the light of firm information on laboratory animals, conveniently subjected to a multivariable approach by the methods presented in this paper. That biologic modulations at different frequencies are more primitive and less labile in the rat as compared to man constitutes a testable working hypothesis.

statistically significant circadian rhythms persist during at least 2 weeks in earth-orbit. For a number of reasons, the data available do not allow definitive statements about possible changes in the characteristics of this rhythm during space flight. Nevertheless, the results do not contradict the position that circadian rhythms, which characterize most if not all bodily functions (including performance), are innate features of man and will accompany him wherever he goes. This possibility should be considered in planning the work-rest schedules of astronauts, especially during prolonged missions. The gradual diminution of periodic input from the earthly environment, if not its complete absence, may lead to eventual impairment of human performance and health because of biorhythm disturbances. For this reason, it appears advisable that the monitoring of medical information from astronauts include attention to characteristics of circadian and other biorhythms with different frequencies. As longer space



Original data from Chronobiology Laboratories, University of Minnesota, except for those on liver tyrosine transaminase (J. Axelrod), blood leukocytes (J. Pauly and L. Scheving), brain 5-hydroxytryptamine, norepinephrine, and susceptibility to pentobarbital (L. Scheving), and on urinary volume and histamine (C. Wilson)

Fig. 18. Timing of several presumably 24-h-synchronized circadian rhythms in the rat – derived from fitting, by least squares, 24-h cosine functions to original data (see also Haus *et al.*, 1969).

flights are contemplated, circannual (about-yearly) and other low-frequency rhythms already demonstrated and quantified for mammals, including man, also gain in interest (Halberg *et al.*, 1965; Haus and Halberg, 1970).

The effects of regimen shifts on quantitative features of circadian rhythms observed in rats during the studies described herein (Figure 15), have also been found in men adjusting after jet flights across time zones (Figure 19). In man, as in the rat, a de-

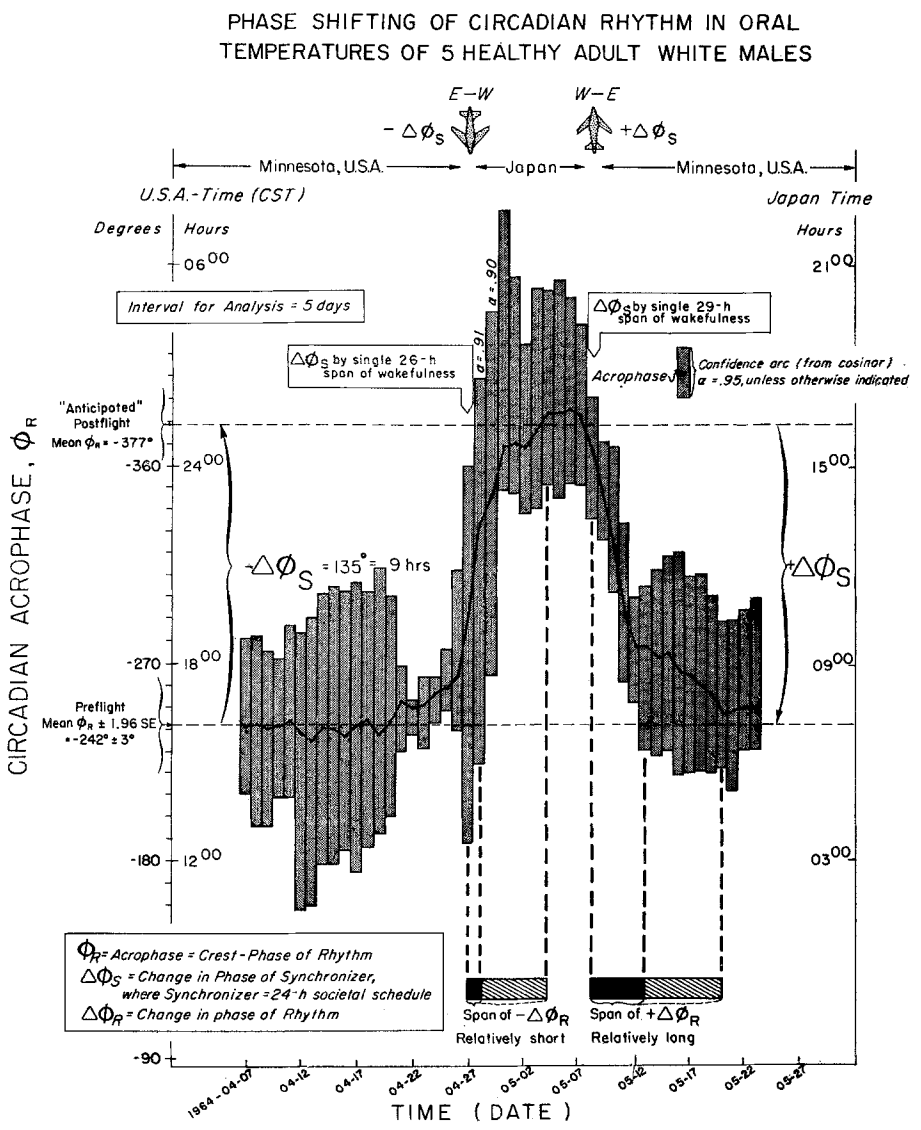


Fig. 19. Rhythm adjustment following a flight from east to west, involving social synchronizer delay, seems to be faster than that following a flight from west to east involving synchronizer advance – even though rhythm advance is associated with return to familiar home setting (see also Haus *et al.*, 1968).

TABLE IV

Aspects of the circadian system of adult rats
Determined in the Chronobiology Laboratories, University of Minnesota

Site	Kind	Units	Level, C_0 $C_0 \pm SE'$	Amplitude, C $C \pm SE''$	Acrophase, ϕ (0.95 confidence arc)
Whole body	I.p. temperature	°C	37.9 ±0.1	0.57 ±0.02	−184° (−174 to −194)
	Gross body movement				−177° (−167 to −188)
	Feeding activity				−159° (−146 to −173)
Serum	Alkaline phosphatase	KAU	46.0 ±0.5	10.7 ±0.7	−239° (−231 to −247)
	Cholesterol	mg%	93.1 ±1.7	7.69 ±2.41	−337° (−302 to − 12)
	Corticosterone	μg%	39.0 ±3.0	20.8 ±4.2	− 80° (− 57 to −103)
	Phosphate	mg%	6.77 ±0.46	0.46 ±0.12	−360° (−331 to − 30)
	Urea Nitrogen	mg%	20.1 ±0.4	2.39 ±0.49	−168° (−145 to −191)
	Uric acid	mg%	1.66 ±0.02	0.10 ±0.03	− 33° (−359 to − 67)
Urine	Chloride	mEq/h	$0.095_5 \pm 0.006_1$	$0.027_2 \pm 0.008_4$	−197° (−164 to −231)
	Potassium	mEq/h	$0.040_5 \pm 0.002_7$	$0.014_4 \pm 0.003_7$	−130° (−130 to −186)
	Sodium	mEq/h	$0.076_0 \pm 0.004_7$	$0.025_5 \pm 0.006_4$	−189° (−161 to −216)
	Urea nitrogen	mg/h	8.51 ±0.42	1.99 ±0.57	−193° (−162 to −225)
Adrenals	Corticosterone	μg/100 mg	2.61 ±0.21	1.40 ±0.30	−115° (− 90 to −139)
Liver	Glycogen	%	3.66 ±0.08	1.81 ±0.12	−314° (−307 to −322)

ϕ reference = middle of daily light span, 360° = 24 h. For serum, see Haus *et al.* (1969).

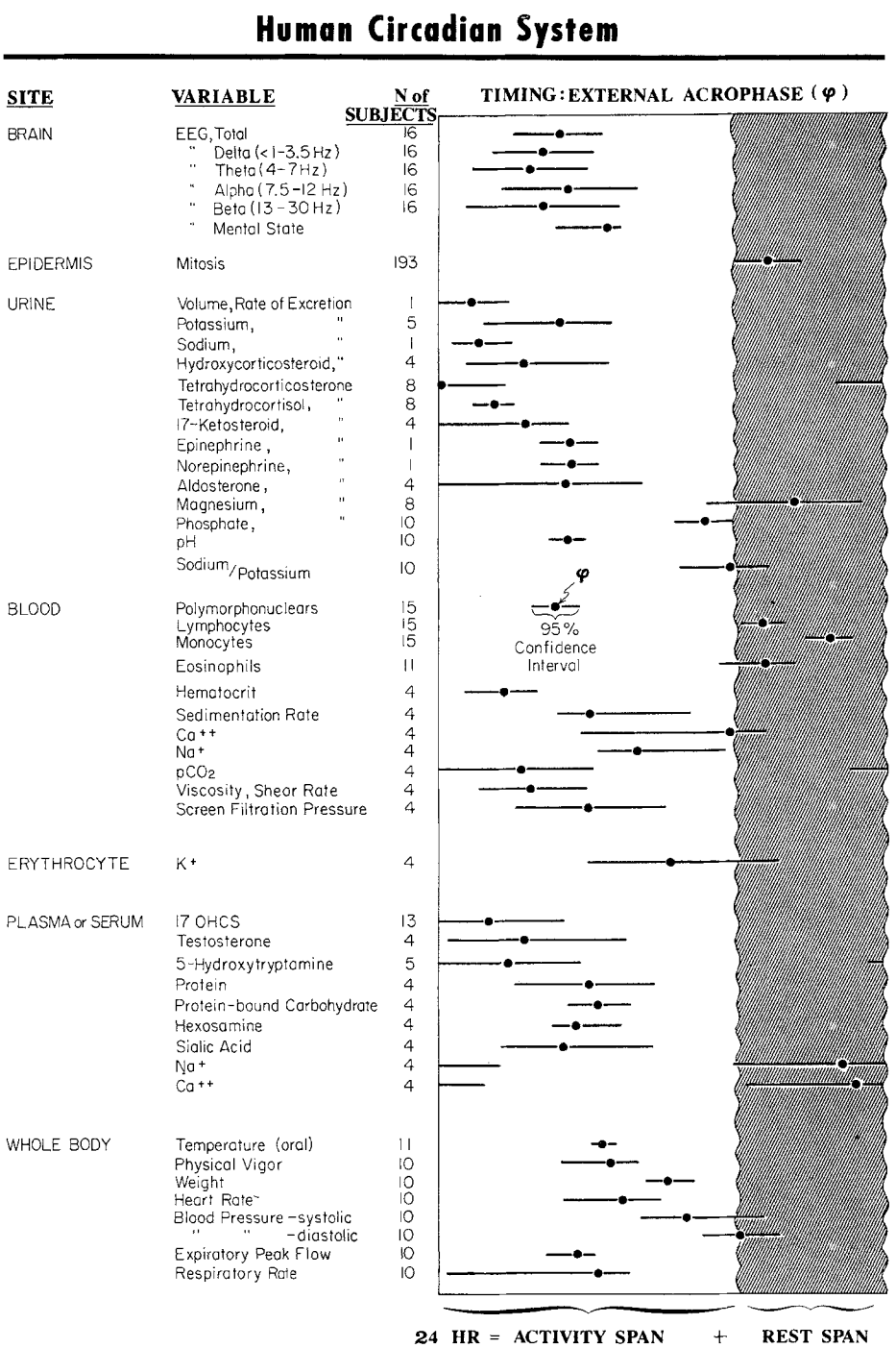


Fig. 20. Circadian acrophase chart showing timing in relation to light span, on top. The internal acrophase, ϕ, in relation to the rest-activity cycle, may roughly be approximated by reference to the bottom scale. For original data and/or references see Halberg *et al.*, 1969.

laying change in schedule (east-to-west flight) results in a faster adjustment (resynchronization) of circadian rhythms than does an *advancing* change (west-to-east flight). The fact that several days are required for adjusting circadian rhythms to a new schedule, with the amount of time depending *inter alia* at least superficially on the direction of travel, should be considered in planning the activities of 'fast' travelers, be they pilots, (Klein *et al.*, 1970) diplomats, businessmen, athletes, tourists or others (Siegel *et al.*, 1969).

Similar considerations apply to laborers required to change work shifts. Available evidence indicates that work performance will probably suffer for a few days after such a shift (Halberg *et al.*, 1969). The fact that many performance measures exhibit circadian rhythms suggests that there probably will be optimal time spans for performing any critical task.

Finally, the ability to quantify characteristics of circadian rhythms in man, using analytic methods such as those developed during preparations under the Biosatellite program, should play an increasingly important role in medicine. As an example, Figure 20 depicts the timing of a wide variety of rhythmic variables in healthy human beings: clearly, different functions reach their maximum at different times. The use of such 'maps' along with information about level and amplitude of the respective rhythms promises to become a valuable aid in diagnosis and treatment of a number of pathologic states (Halberg, 1968; Conroy and Mills, 1970).

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