HUMAN PERFORMANCE, PHYSIOLOGICAL RHYTHMS, AND CIRCADIAN TIME RELATIONS

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Chapter I

INTRODUCTION

Rhythmic fluctuations in biological phenomena have been recognized and studied for many years. Research on biological rhythms has been reviewed by a number of authors (Aschoff, 1963, 1965; Brown et al., 1970; Bünning, 1967; Cloudsley-Thompson, 1961; Colquhoun, 1971, 1972; Conroy and Mills, 1970; Foman, 1961; Halberg, 1969a; Harker, 1964; Kleitman, 1963; Luce, 1970; Menzel, 1962; Sollberger, 1965; and Wilkins, 1968). Particular attention had been directed towards those cycles with a period of about 24 hours, called circadian (Halberg, 1966) rhythms. Investigations of longer (ultradian) and shorter (infradian) cycles are steadily increasing. Much of this research has been aimed at identifying and quantifying the many biological rhythms. Recently the development of computerized programs for data analysis has greatly aided the study of rhythms (Halberg, 1966).

The circadian rhythms that have developed by evolution can be considered a prerequisite for health and efficiency (Aschoff, 1964). Knowledge of these rhythms can be used for practical purposes. The cycle in body temperature is considered by physicians when evaluating the importance of a fever. The need to monitor the circadian rhythm of blood pressure to recognize the early symptoms of hypertension has recently been promoted (Bartter, 1974). The importance of considering circadian rhythms in applying drug therapy has been dis-

cussed by Reinberg and Halberg (1971). Other medical applications have been reviewed by Luce (1970).

The advent of jet travel has made the problem of "jet lag" evident to many people. Disruption of performance and physiological cycles occurs after east-west flights that cross several time zones but not after north-south flights (Aschoff, 1969).

Ray et al. (1961) feel that performance changes can be expected for shift workers with variations in their work-rest schedules because of the interrelationships between physiological and psychological factors. Wilkins (1968) describes the increase in fatigue and decision time and the decrease in proficiency after a shift in sleep-activity schedules. The individual rates of recovery varied from one to five days and appeared to be correlated to the speed with which the physiological rhythms adjusted to the new activity pattern.

Work on a night shift results in continual altering of normal circadian rhythms. Even on a permanent night shift (as opposed to a rotating shift), the worker will have disruption of his cycles due to his desire to be awake during the day when he is not working so he can be with his family and engage in other activities. Disruption of circadian rhythms has been postulated as the major cause of many of the shift workers' complaints about sleep loss, digestive disturbances, and other health problems as a result of night work. Thus it would be advantageous to screen applicants for their ability to tolerate shift work and perform at an acceptable level.

Trumbull (1965) feels that selection of individuals and scheduling of work-rest cycles will result in performance maintenance or enhancement. Therefore he points out the need "for preparing periodicity maps which show the various phasic aspects of man in perspective or relationship." Most studies with that objective, however, have been conducted under strictly regulated laboratory conditions. Any phase relationships that could be used for screening shift workers would have to be present under daily living conditions. Therefore in this study physiological parameters were selected that would be most likely to show circadian rhythms in the general population and which could be easily measured (so as to avoid subject resentment) for monitoring while they conducted their normal life. Physical and mental performance measurements made at the same time could be compared to the physiological rhythms to determine if any relationships existed.

Alluisi and Chiles (1967) found that circadian rhythms evident in physiological parameters may also be shown in performance. Monitoring of performance level to determine the time of optimum efficiency or to ascertain that this efficiency has not been altered could have many applications. Unfortunately, performance testing in a manner that will be accurate is generally time consuming, exhausting to the subject, and requires expensive equipment. Thus if easily measured physiological parameters could be used as an indicator of performance levels, a more practical solution to the problem of worker screening would result.

In man, heart rate, body temperature, and the urinary excretion of electrolytes and adrenal steroids are the most commonly used indicators of circadian rhythms (Frazier et al., 1968). While self-measurements of body temperature, heart rate, blood pressure, etc. can be made to determine circadian cycles (Halberg et al., 1972), these require some training and practice and, more importantly, careful following of procedures (e.g., not ingesting hot or cold substances prior to taking oral temperature). Many people would find the time required to take these measurements annoying and might have a tendency to forget to take them. In addition to the problem already mentioned for temperature measurements, heart rate and blood pressure could easily be altered by excitement, etc., to give a noisy indication of any underlying or associated rhythms in performance.

Stanbury and Thompson (1951) have found that certain circadian rhythms of urinary excretion are relatively independent of changes in posture, activity, and environmental temperature. Similar patterns for a given individual were present whether eating or fasting. These results correspond with those of earlier workers that have been summarized by Wesson (1964). In an extended experiment, electrolyte excretion patterns of several subjects were examined for intervals up to two years (Stanbury and Thompson, 1951). Although absolute levels of excretion varied widely, the subjects continued to display the same pattern of change. This has been verified over a 21-month span by Sothern et al. (1974). Thus once the basic urinary

excretion patterns for an individual had been determined, they would not have to be frequently remeasured.

The object of this study was to determine which of several easily measurable physiological functions would be useful for screening workers for potential assignment to shift work. The detailed evaluation of these performance indicators was not part of this research.

Use of suitable urinary parameters would probably provide the best indication of an individual's circadian state for application to shift work. Samples can easily be taken by the individual at home as well as work. They can then be processed at a central laboratory. Several urinary parameters were selected, therefore, to be analyzed and compared to the results of performance testing. Body temperature and heart rate were also measured since they have already been linked to some types of performance. Physical performance was measured using a treadmill. Psychomotor performance was measured with a compensatory tracking task or with a target task. Mental performance was also evaluated with self-administered math tests. Except for testing involving specific equipment, the subjects performed the type of self-measurements that shift workers could make on themselves.

Chapter II

LITERATURE REVIEW

The study of biological rhythms requires the collection of data as a function of time. These time series were originally examined as plots of the observations made against time. This approach revealed many rhythms and can still be useful. But if there are some irregularities in the plots, "eyeball" interpretations can become very subjective. Electronic computers have greatly aided the analysis of rhythmic data. Evaluation of the changes with respect to time may be made in a consistent manner and then tested for significance using statistical procedures. This development has been compared to the use of the microscope (Halberg, 1966). The time series plots of original data give a macroscopic view of the rhythm while the computer gives a microscopic view.

Rhythm parameters may be estimated by fitting a periodic function to the time series using the method of least squares (Halberg et al., 1965). As shown in Figure 1, cosine curves with any desired period (e.g., 24 hours) can be fitted to the data to obtain point-and-interval estimates of the rhythm's mesor (M), amplitude (A or C), and acrophase (ϕ). This method of rhythmometric analysis also derives a P-value from a test of the null hypothesis (zero amplitude). The P-value is the probability of obtaining the estimated parameters (ϕ, M, A) if the proposed sinusoidal rhythm of the stated period was

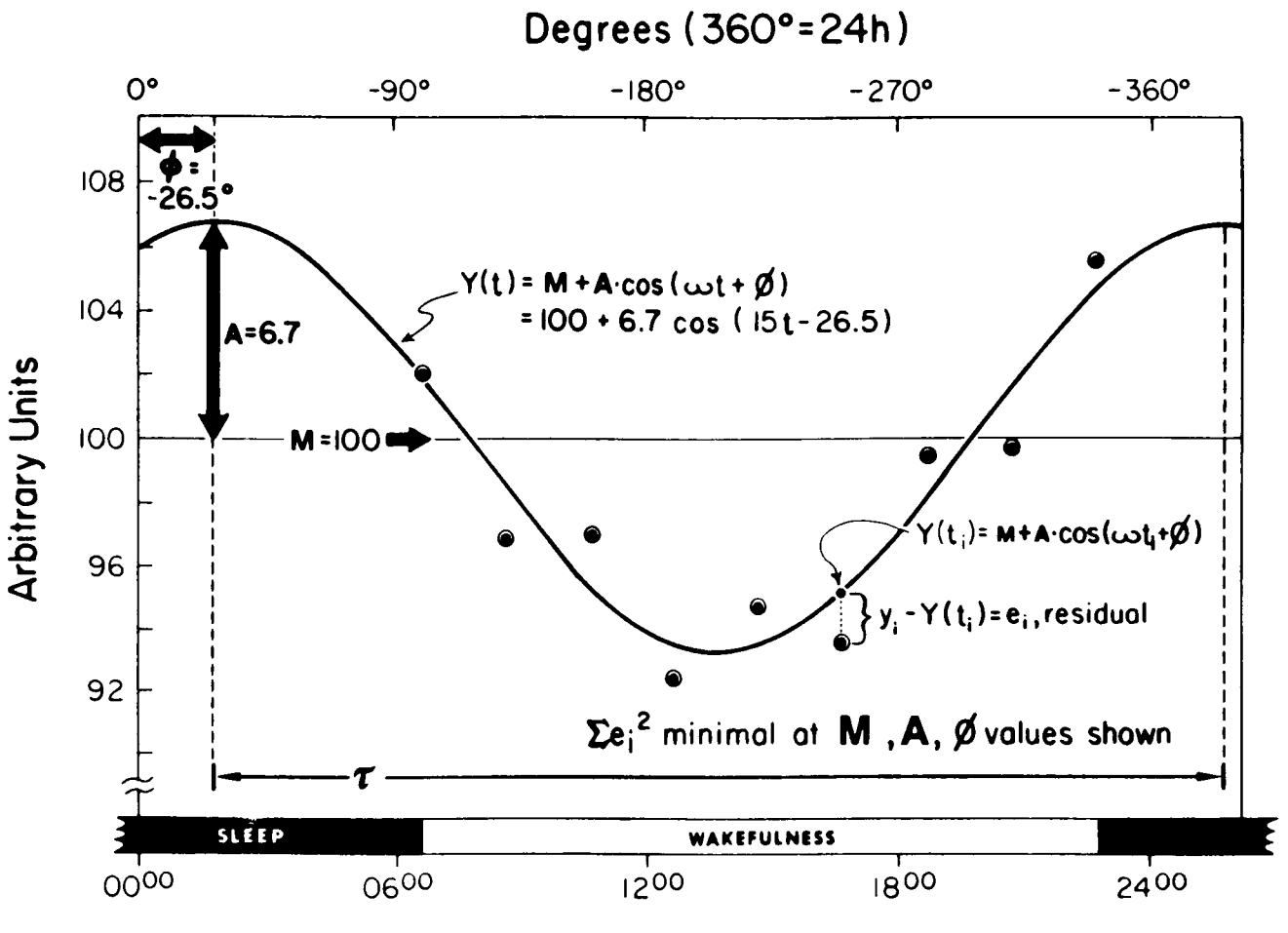

Figure 1. Estimating rhythm parameters by least-squares fitting of cosine function for single series of observations or of mean values. Abstract example with 24-hour cosine function, Y (t) [continuous curve], fitted to 2-hourly data. Yi [◉], obtained during wakefulness span. τ = period = 1/frequency = 1 cycle per 24 hrs. = 1/24 cycle per hour; M = mesor; A = amplitude; ω = angular velocity = $360°/\tau$, here = 360°/24 hrs. = 15°/hour; t = time (hours after 00^{00}). [Courtesy of F. Halberg, Director, Chronobiology Laboratories, University of Minnesota]

non-existant. If this P-value is sufficiently small (e.g., 0.05), one can consider the cosine model as an adequate description of the rhythm.

The word "mesor" was coined by Halberg (Kanabrocki et al., 1973) to indicate the average value (formerly called level) of the rhythm. This value equals the overall arithmetic mean only when data are evenly spaced and cover an integral number of cycles. The amplitude is half the crest-trough difference of the fitted cosine curve. Since the curve is fitted to all of the data, the cosine does not necessarily include the maximum and minimum values of the raw data. The acrophase is the lag in time of the crest of the fitted cosine curve from the selected reference point. Figure 2 illustrates the three types of acrophases based on differences in the reference point. A computative acrophase (ϕ) uses local midnight as the phase reference. An external acrophase (φ) is based on some point in the environmental cycle, such as the mid-point of the dark span. When the reference point is the acrophase of another rhythm with the same period as the cycle being described, the acrophase is called an internal acrophase (Φ).

The amplitude and acrophase values imputed from several time series (e.g., representing several individuals or several time series from one individual) by the method of least squares may be summarized by a mean cosinor (Halberg et al., 1972, 1967). This procedure is illustrated in Figure 3. If a rhythm in all series is synchronized by external factors to a similar extent, the several cosine curves will be similar in phase as shown in Abstract Example 2. Each in-

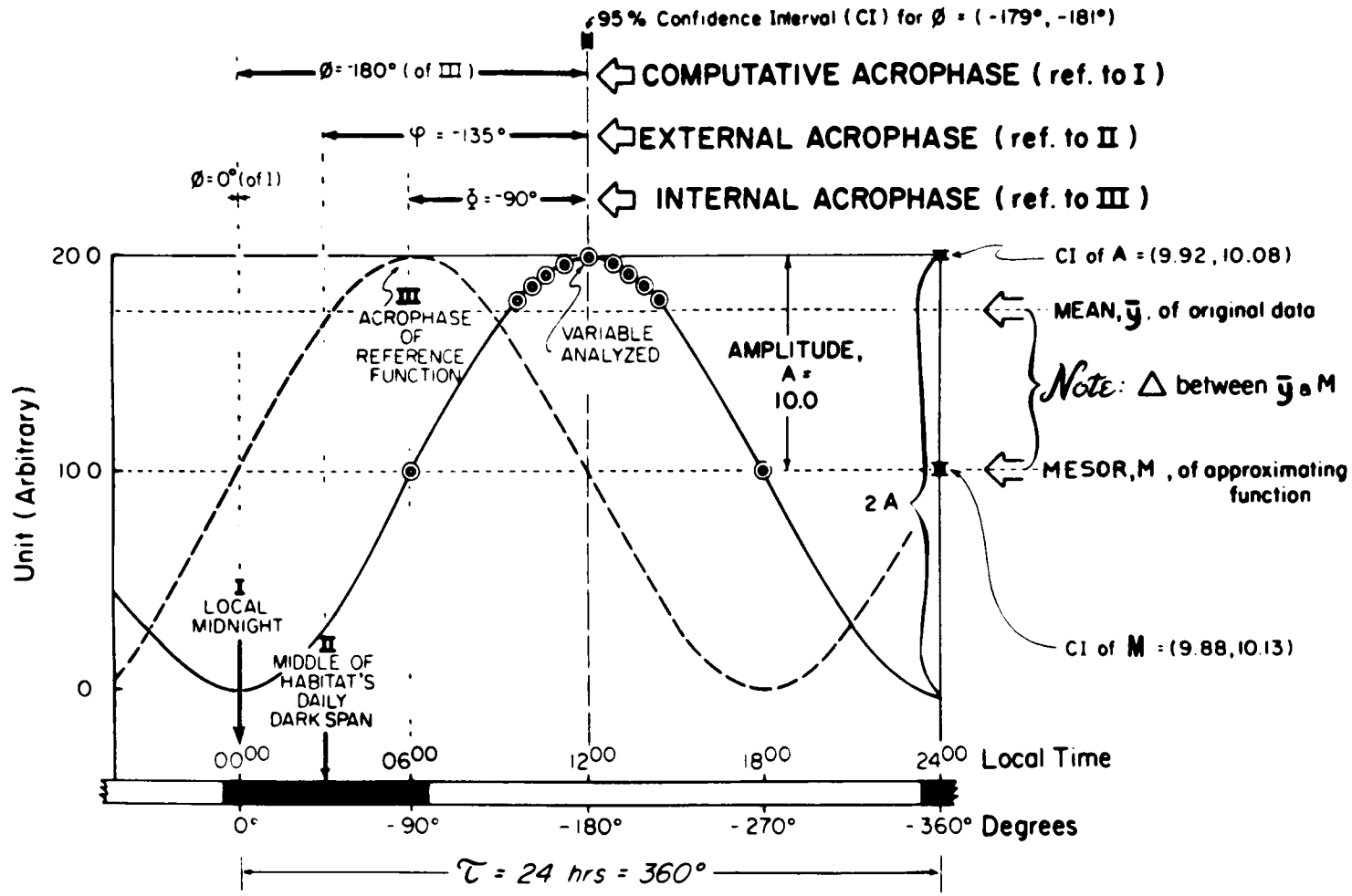

Figure 2. Scheme of quantitative rhythm characteristics. [Courtesy of F. Halberg, Director, Chronobiology Laboratories, University of Minnesota]

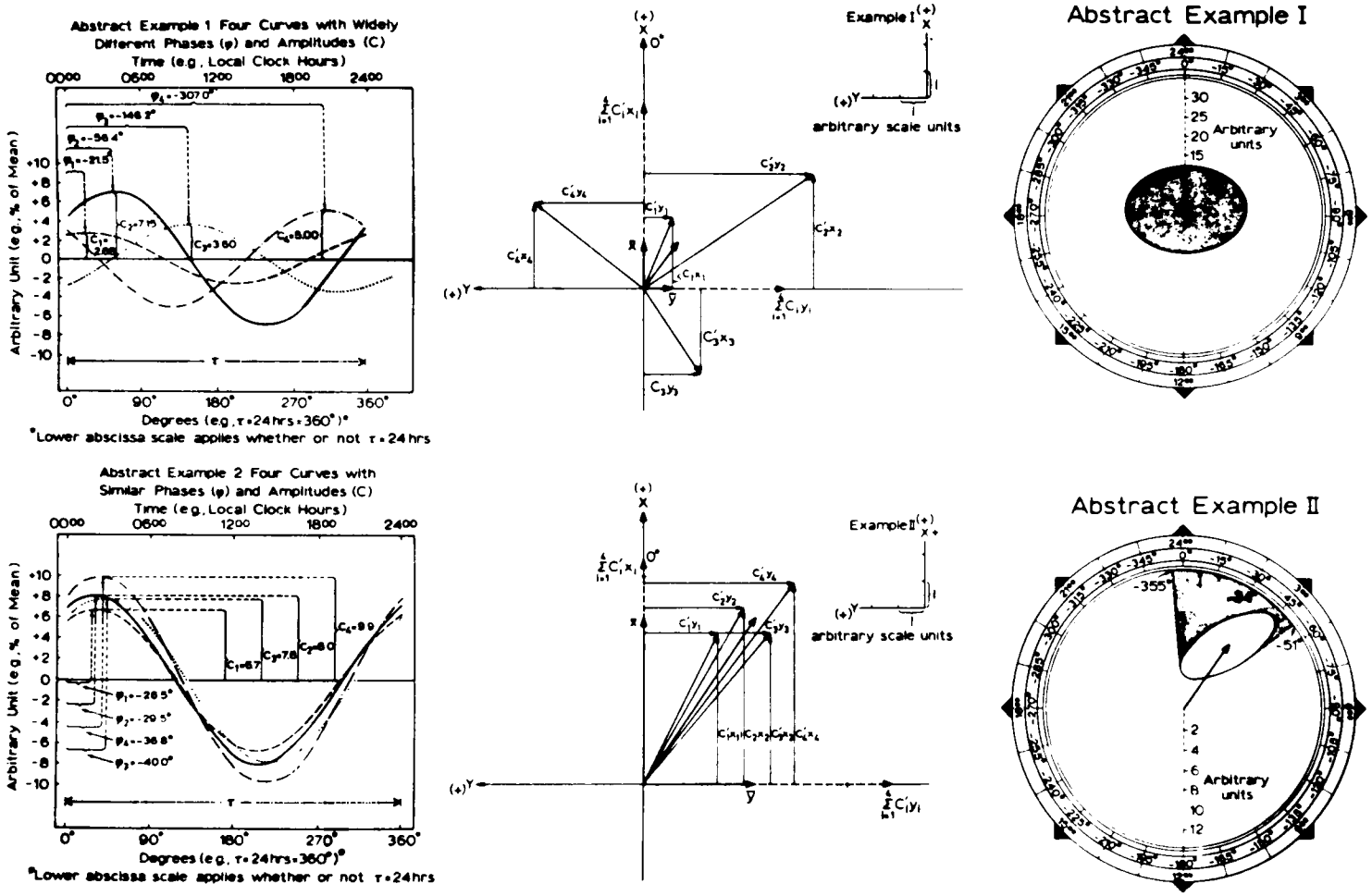

Figure 3. Abstract illustrations of cosinor procedure. [Courtesy of F. Halberg, Chronobiology Laboratories, University of Minnesota]

dividual rhythm can be represented by a vector whose length indicates the amplitude and whose direction indicates the acrophase. These can be combined to yield a single average vector. Hence, the origin of the term "cosinor" from cosine and vector.

The average vector is projected onto a polar graph (right side of Figure 3). The pole is zero on the amplitude scale. Acrophase is shown on one of the circular scales in degrees, with 360° equal to the cycle length (e.g., 24 hours). Clock hours are indicated on the outermost of the three perimeter rings. If desired, the inner ring can be partly darkened to indicate a fixed light-dark cycle. The end of the average vector is surrounded by an ellipse enclosing its 95% confidence region. Radial lines drawn through points tangent to the leading and trailing edges of the error ellipse indicate the 95% confidence limits of the acrophase of the average vector calculated from the individual acrophases. The intersections of the error ellipse with the vector and its extension enclose the 95% confidence interval of the averaged amplitude.

If the cosine curves fitted to the different series are out of phase (Abstract Example 1, Figure 3), the average vector is fairly short compared to the individual vectors and the pole is overlapped by the error ellipse. This situation could arise, for example, if 24-hour rhythms in the several series (individuals) are not similarly synchronized. Problems can also arise when the amplitudes of rhythms in the different series vary greatly in value. The pole is then usually overlapped by the error ellipse due to the large size of the

confidence interval for the amplitude. A mean "relative cosinor" (Halberg et al., 1967) can be used to adjust the data prior to obtaining the cosinors in this situation. The values of the individual amplitudes are first converted to a percentage of the individual mesors before the cosinor is calculated. Another way to summarize data from several individuals or from several time series from the same one is with the single cosinor. The data are pooled for analysis by the least squares procedure as though they were from a single time series for one person. If there was no synchronization of the different time series to the same external factors, the null hypothesis (zero amplitude) would be accepted.

Statistically significant cosinors may be obtained when an internal acrophase is used rather than a computative one as shown in Figure 2. For instance, when time series from either of two variables are summarized with a cosinor based on the computative acrophases, no group synchronized rhythm is apparent. But if one variable is referred to the other and a statistically significant cosinor is obtained, a relationship between the rhythms of the two variables is indicated. Further testing would be needed to show that this is a consistent relationship.

Following reversal of the sleep-wakefulness cycle, resynchronization of different circadian rhythms may occur at different rates. Martel et al. (1962) studied the excretion of water, sodium, potassium, creatinine, and ketogenic steroids before and after reversal of day-night activities and during the return to the original schedule.

The excretion patterns of these variables required four to eight days to adjust after phase shifting. Except for creatinine, the number of days necessary for a particular variable's rhythm to completely shift was the same for both changes in activity. Creatinine patterns required six days for reversal but only four days for return to the original schedule. The sodium rhythm showed the fastest rate of change with complete return to control values in four days while the potassium cycle was still reversing. Both had reached control levels after six days. The excretion of ketogenic steroids did not return to the original pattern until eight days after a change in day-night activity.

Urinary rhythms for subjects living a 21-hour day in the arctic were studied by Simpson et al. (1970). Rhythmometric analyses of the data at 24.2 hours (the average of the individual's best fitting periods) and 21 hours were conducted. The usual "single-component" circadian rhythm for 17-hydroxycorticosteroids, potassium, water, chloride, and the ratio of sodium to chloride showed two components with average periods of 21.0 and 24.2 hours. Sodium and pH excretion failed to show group synchronized rhythms statistically significant below the 5% level. However, for the urinary pH data, the 21-hour period described a rhythm at the 7% level but there was no statistically significant component ($P > 0.1$) found at 24.2 hours. The environmental effect upon urinary pH was accepted as the major synchronizer. Computations were made to compare the exogenous 21-hour period with the presumably endogenous 24.2-hour component.

Simpson et al. made these comparisons with all measured variables although in the two cases just mentioned (sodium and pH) rhythm description was not feasible with statistical significance below the 5% level.

Selected pairs of amplitudes from the least squares fits were used to compute the circadian amplitude ratio (CAR). The amplitude for the 21.0-hour period representing the social "input" was divided into the 24.2-hour amplitude. The latter value, deviating from the solar 24.0-hour and the lunar 24.8-hour periods, corresponds largely to the presumed organismic contribution. The relative contributions of environment and organism to circadian behavior on the abnormal schedule were approximated by the CAR. When the CAR was less than one, environment dominated and vice versa. Potassium and 17-hydroxycorticosteroids rhythms had apparently largely intrinsic spectral components. The social routine appeared most important to circadian changes in urine pH. The CAR ratios for the other variables were close to unity. Simpson et al. postulate that CAR tests may be dedeloped to determine adjustability of individuals to unusual schedules. Persons who strongly respond to social synchronizers, indicated by low CAR values, may adjust faster to the phase shifting required of shift workers, pilots, etc.

Daily acrophases for subjects living on a 21-hour day for three weeks were plotted by Simpson and Halberg (1972). At the beginning of each week, the acrophases for urinary excretion of 17-hydroxycorticosteroids, potassium, sodium, and water showed a

progressive advance. However there was not sufficient adjustment to correspond to a 12-hour schedule. During the last four "days" of the week, the acrophases showed a delay, often fairly rapid, before starting a gradual advance again. These results were interpreted as showing the interaction of the 21-hour activity routine and the near 24-hour endogenous adrenocortical cycle. When they were close to one another, the acrophases advanced slowly. A delay occurred when the two cycles were out of phase. Loss of performance ability while living on a 21-hour routine was described by Simpson (1972). Simpson and Halberg (1972) point out the need for minimizing such decrement and advocate research on the use of drugs to reduce or avoid such performance changes.

Simpson and Lobban (1967) found acclimation to a 21-hour day differed among individuals. Two subjects showed much more persistent 24-hour rhythms than the others. Although the rhythms altered at different rates for all subjects, for some subjects all rhythms seemed to adjust faster than for others. If this apparent ease of rhythm adjustment is consistent for most people, the number of rhythms that would have to be measured in a screening project would be greatly reduced.

There may be variability among individuals with regard to the ease with which their internal rhythms may become desynchronized. This could possibly be related to their ability to adjust to shift work. Activity, body temperature, and electrolyte excretion were measured during isolation on a total of fifty subjects by Aschoff

et al. (1967). In thirty-six subjects, these variables remained internally synchronized during the entire experiment during which they were exposed to constant conditions. A 1:2 ratio of the activity rhythms to the other rhythms was established in five subjects. Complete desynchronization occurred in nine subjects. Two of these subjects became desynchronized immediately while the others did not show "divergence" until after nine to twenty-three days of confinement.

No disruption in circadian rhythms have been found following north-south flights which do not cross time zones. Polarity has been shown in the phase shifting resulting from east-to-west versus west-to east flights over several time zones. Oral temperature rhythms adjusted faster after an east-to-west flight (causing a delay) than after a west-to-east flight (Halberg, 1968). The same polarity was shown by urinary excretion of 17-hydroxycortocosteroids, 17-keto-steroids, calcium, and chloride (Halberg et al., 1970a). However more desynchronization between the rhythms occurred during the delay than during the advance. Hauty and Adams (1965) reported that three to five days were required for adjustment of body temperature after a flight from Oklahoma to Tokyo but only one day was needed after the return flight. They attributed the faster adjustment to the fact that the subjects were in familiar surroundings. The studies of Haus et al. (1968), however, with flights in both directions from the subjects' home showed no benefits from any such psychological

effects. Physiologic readjustment always occurred faster after east-to-west flights as compared to west-to-east.

Polarity has been shown in other organisms besides man. Studies (Chiba et al., 1974) on phase shifting in the night active flour beetle, Tribolium confusum, have shown that response to a 90° delay in lighting regimen was faster than to a 90° advance. Similar polarity has been shown in rats (Halberg et al., 1971). In mice, adjustment of the rectal temperature rhythm to a 180° delay occurred faster with prolongation of the light span than with lengthening of the dark span (Haus and Halberg, 1969). Plants may also show such differences with phase shifting. The rhythmic pinnule movements of Albizzia julibrissin were studied by Koukkari et al. (1973). After a 4 hour change in the lighting regimen, phase shifting occurred in 1 or 2 days, faster than has been reported for insects, birds, and mammals. Advancing the cycle resulted in a faster shift of the movement rhythm than a delay.

The early work of Simpson and Galbraith (1905) showing circadian rhythms in axillary temperature of monkeys was reanalyzed using cosinor procedures by Halberg (1969b). The daily cosinor summaries of circadian acrophase clearly showed the effects of phase shifting. The animals adjusted more rapidly to a shift of 180° than to an advance of only 90°. This polarity, quantified by the cosinor procedures, was not readily apparent from macroscopic examination of the transient rhythm alteration. This example demonstrated the need for quantitative analysis of rhythmic data.

Sharp (1960) reported reversal of water, sodium, and potassium excretion in 6-9 days after inversion of the activity cycle. He pointed out the importance of strict light control. All previous studies without such control did not achieve reversal of rhythms. He indicated that the strong light stimulus at a normal waking hour which night workers receive when going home from work may hinder or prevent reversal of their normal rhythms. As additional evidence of the importance of light, Sharp cited the conclusion of Joslings (1940) that the time of sunrise controls the excretory rhythm because he found peak urine excretion occurred at the same local time every day on an around-the-world boat cruise.

The influence of light is further supported by the work of Lobban and Tredre (1964) who measured excretory patterns of water, sodium, potassium, and chloride in blind subjects. Those with light/dark sensation had excretory patterns identical to people with normal sight. Subjects that were totally blind had disturbances both in phase and amplitude. Potassium excretion was closest to normal while the excretion of water was completely reversed. Sighted persons can also show desynchronization of rhythms due to changes in light stimulus. Spitzbergen has minimal day-night light synchronization because it is inside the Arctic Circle. Night workers there have peak excretion of potassium when they are active rather than during the normal "daytime". Since water excretion is influenced more by light, it is out of phase with potassium in the summer. During the winter they are in phase (Lobban, 1963).

Blind patients who were divided on the basis of how long they had been without sight were studied by Remler (1948). Four of the eight subjects termed as "early-blind" had been totally blind from birth. The sixty-seven "late-blind" patients had been totally blind at least 5 months but no longer than thirteen years. Nine subjects with normal sight were included as controls. Macroscopic studies of circadian rhythms in rectal temperature, blood pressure, heart rate, and urinary excretion (volume and specific gravity) were made using 24-hour profiles. The data from all "early-blind" subjects were evaluated as displaying normal rhythms. It was considered virtually certain that at least one of these subjects had never received a light stimulus via the optic nerve. Altered rhythms, mostly inverted ones were described in half of the "late-blind" subjects. Unaltered physiologic rhythms were interpreted from the profiles of the other half. Remler concluded that circadian rhythms, having endogenous origins, can merely be "directed" by environmental factors and that rhythm alteration can result from severe trauma such as the loss of the eyes. Halberg (1968) points out that such results can be expected with circadian desynchronization.

Additional work is needed to determine what effect light has on the desynchronization of rhythms in night workers. Sharp (1960) used blindfolds and darkened tents to achieve reversal of the potassium cycle. Perhaps similar measures should be used by night workers to help them adjust faster to new activity patterns and minimize the tendency to return to normal on off-days.

Potassium excretion rhythms have the strongest resistance of the electrolyte cycles to external influence (Vagnucci et al., 1969). While its pattern of excretion is generally very similar to those of sodium and chloride (Wesson, 1964), desynchronization can be caused by extreme maneuvers such as abnormal (21 or 27 hour) period lengths (Lewis and Lobban, 1957) and reversal of day-night activities (Sharp, 1960, and Martel et al. 1962). Disruption of a worker's potassium cycle therefore, could indicate a great change in his life style and a probable simultaneous alteration in performance.

Sodium, creatinine, and phosphate rhythms might serve as indicators of physical performance. When Srivastava et al. (1967) measured creatinine excretion, they found that the 24-hour output was elevated on days with exercise. The average hourly output was greater during exercise at the higher work rate than at the lower work rate. Decreased sodium excretion during exercise with return to prework levels afterwards has been reported. Simultaneous measurement of potassium excretion during 45 minutes of work showed variable changes (Castenfors, 1967) while increased potassium excretion occurred during a 6-12 hour cross-country ski race (Castenfors et al., 1967). In neither study did potassium excretion during recovery return to prework levels. Kleitman (1925) found decreased phosphate excretion during exercise followed by an increase above normal during recovery. Heaton and Hodgkinson (1963), however, found no change in the excretion of sodium, potassium, creatinine, and phosphate after moderate exercise. In addition they found no

effect of meals on the excretion of these compounds. Schottstaedt et al. (1955) reported that variation in meals was not associated with major changes in the excretion of water, sodium, potassium, and creatinine.

The consistency of urinary circadian rhythms on a monthly basis was studied by Sothern et al. (1974) during 21 months for a single individual. Samples were pooled by a procedure that assigned each one to a predetermined subspan of the 24-hour span. The analyses of these samples thus represented an idealized day for each month. The subject did not undergo any prolonged unusual or strenuous dietary or physical conditions. He generally followed a schedule of arising about 8 AM and retiring around midnight. Peak excretion times for the variables in this study are summarized in Table 1. Magnesium, inorganic phosphorus, 17-ketogenic steriods, epinephrine, norepinephrine, and the ratio of sodium to potassium had statistically significant rhythms for all months they were measured. Significant rhythms were found most months for volume, sodium, potassium, creatinine, hydroxyproline, and the ratio of norepinephrine to the sum of norepinephrine plus epinephrine. Chloride and, to a lesser extent, calcium showed irregularity some months. Highly irregular within-day variations were found for chloride and hydrocortisone. Analysis of serial individual samples would be required to monitor the circadian rhythms of these more irregular variables. Clearly demonstrable circadian rhythms using pooled samples allowed them to be monitored with only five or six chemical determinations per variable

Table 1. Summary of peak excretion times for the urinary variables analyzed by Sothern et al. (1974).

Variable	Amplitude (95% C.I.)	Acrophase ϕ (95% C.I.)
Volume (ml/hr)	4.4 (2.4 , 6.4)	- 2° (-340, - 34)
Sodium (mEq/hr)	0.99 (0.58, 1.40)	-337° (-316, -353)
Potassium (mEq/hr)	0.93 (0.71, 1.15)	-202° (-183, -223)
Sodium/Potassium	1.18 (0.92, 1.44)	- 17° (- 6, - 28)
Calcium (mM/hr)	0.06 (0.03, 0.08)	-158° (-139, -200)
Chloride (mEq/hr)	0.25	-215°
Magnesium (mEq/hr)	0.07 (0.06, 0.08)	- 15° (-360, - 31)
Inorg. Phos. (mg/hr)	13.00 (10.5, 15.4)	-327° (-314, -341)
Creatinine (mg/hr)	5.2 (3.5, 6.8)	-314° (-284, -351)
Uric Acid (mg/hr)	3.0 (1.1, 4.8)	- 1° (-341, - 45)
Hydroxyproline (mg/hr)	0.30 (0.20, 0.41)	-360° (-338, - 18)
Hydrocrotisone (µg/hr)	0.37 (0.09, 0.65)	-172° (-128, -228)
17-KGS (mg/hr)	0.12 (0.10, 0.14)	-201° (-185, -223)
Epinephrine (µg/hr)	0.20 (0.17, 0.23)	-219° (-195, -242)
Norepinephrine (µg/hr)	0.47 (0.39, 0.56)	-222° (-198, -242)
NE/(NE + E)	0.04 (0.03, 0.05)	- 39° (- 15, - 66)

17-KGS: 17-ketogenic steroids
Inorg. Phos.: Inorganic phosphate

instead of as many as 197. This would obviously be a savings both in time as well as money for long term monitoring.

Proper nutrition may be necessary to maintain performance. Night shift workers may be particularly apt to eat inadequate meals due to increased digestive disturbances (Wyatt and Marriott, 1953). Diet quality would influence the excretion of urea and uric acid since they are the end products of the breakdown of purines and proteins, respectively (Harrow and Mazur, 1966). Decreasing urea nitrogen levels, for instance, would indicate a decrease in dietary protein. An increase in these parameters with no corresponding change in diet could indicate cellular catabolism and signal the need for finding the cause of such a breakdown. This might occur, for example, in a worker engaged in heavy physical labor who is not taking in enough calories or the proper balance of amino acids to prevent muscular destruction.

In addition to being an indicator of nutritional state, it has been postulated by Hale et al. (1968) that high urea output may be related to sleep loss. Phosphate excretion is also supposed to be sensitive to sleep changes (Wesson, 1964). Such an indicator would be useful in evaluating the effects of shift changes since sleep loss is a common complaint. It could also be useful in detecting performance decrement as a result of late night activities for workers in critical skills where maximum performance is always required (e.g., an air traffic controller).

Kleitman (1949) stated that social influences are important in the establishment and maintenance of human circadian rhythms. Studies conducted in underground bunkers showed that several people living together generally maintained the same activity pattern. If the group schedule was too different from a member's individual period, he established his own activity pattern and became desynchronized from the others (Aschoff, 1969). This could cause obvious problems in a crew situation and seriously affect the group performance.

Alluisi et al. (1964) studied different work-rest schedules for simulated aircraft crew performance. They were investigating the schedules that would provide maximum efficiency with a minimum number of crew members. The circadian rhythms in performance efficiency and physiological parameters (heart rate and temperature) were well marked. Similar trends were found in the performance of confined subjects and an unconfined control group. The confined subjects worked twelve hours daily for fifteen days while the controls worked four hours per day for five consecutive days over a six week period. Alluisi et al. concluded from these trends that subjects could be selected with suitable motivation and abilities to perform adequately on a 4-hrs work and 2-hrs rest schedule for at least 15 days. Screening subjects for compatible circadian rhythms would also help to reduce the possibility of group desynchronization.

Freeman and Hovland (1934) reviewed studies dealing with circadian variations in performance. They tabulated previous studies of sensory, muscular, and mental performance according to the four

"types" of circadian curves reported (continuous rise, continuous fall, morning rise with afternoon fall, and morning fall with afternoon rise). Although there were conflicting results reported, Freeman and Hovland concluded that sensory and motor performance were generally superior in the afternoon. There was no agreement on when optimum mental performance occurred. They noted that while most researchers assumed work output was a function of some "basic physiological rhythm", there had been little actual research on the correlation. Only a few papers were reviewed which considered both physiological processes and performance and these generally referred to the similarity in curves from separate studies.

One of the most comprehensive studies on mental performance from the standpoint of sample size was conducted by Bjerner et al. (1955). They showed a circadian variation in the mental performance of employees of a gasworks. Three-man teams worked eight hour shifts beginning at 6 AM, 2 PM, and 10 PM and the shifts changed on a weekly basis. The errors in recording instrument readings and calculating gas production and consumption by addition and subtraction were tabulated from 175,000 entries over a 19 year period. The peak number of errors occurred at 3 AM with a lesser peak at 3 PM. Except for the greater fluctuation at night, the curves from 6 AM to 6 PM and 6PM to 6 AM were very similar. When the data were examined on a yearly basis, the bimodal peaks in errors were still present although some fluctuated an hour earlier or later from the overall averages of 3 AM or 3 PM. When errors during the first three nights of night shift

were compared with those of the last three, no differences in the curves were evident. This was still true when the work week was reduced from 56 to 48 hours and the workers were given a free day between the first three and the last three nights. Similar comparisons for day shifts or for total errors by day of week were not made.

Bjerner et al. (1955) cited Browne's findings (1949) of a similar peak at 3 AM in the time taken by telephone operators to respond to incoming phone calls. This peak was present even with 80 calls per hour, therefore the increased reaction time could not be blamed on the operators dozing.

Lehman (1958) felt that probably all functions regulated or strongly influenced by the autonomic nervous system would exhibit cycles if the proper measurements were used. The net effect of these rhythmic fluctuations is a 24 hour cycle of physiological and psychological readiness for performance. The curve shows a characteristic minimum at 3 AM, a slow rise to the highest values from 9-10 AM, a slight dip at noon, followed by a less definite rise, and then the final decline. Adjustment of assembly line work to alter output to match this curve has resulted in increased production of 5-10% and a concurrent decrease in fatigue. Lehman felt that the ideal performance curve shown by Bjerner et al. (1955) in their gas meter study is rarely duplicated due to factors such as social influence, fatigue, and sample size.

Frazier et al. (1968) reiterate the paucity of relevant studies on human performance cycles corresponding to physiological functions.

They studied the variability in vigilance performance of three subjects living for 14 days in a small chamber under rigidly controlled conditions. The tasks used consisted of systems monitoring, visual reaction time, auditory monitoring, and imbalance matching. The data were examined for rhythms using a least-squares spectral analysis program with 10-110 hour and 20-30 hour spectral windows. With the wide spectral window, they found rhythmicity outside the circadian range at frequencies that were sometimes the primary source of behavioral variation. However restriction of the spectral window to 20-30 hours showed a 24-hour period was also present in those cases. When the data were analyzed for the weeks separately, a shift in the periods was evident for some subjects. This was examined further by determining the best fitting periods for three day incremental blocks of data. The plotted results showed cyclic peaks in period length about every 3-4 days with the longest period at the end of the first week. The rigid activity schedule and controlled conditions make it unlikely that the physical environment was the cause of these variations.

Frazier et al. concluded that the psychological effect of confinement in the test chamber may be a source of the variation. The subjects were able to communicate with the support staff and a technician entered the chamber when necessary to maintain the equipment. It was soon suspected that one of the subjects was dislodging monitoring electrodes to obtain social interaction. The other subjects admitted feeling gratified when they had the opportunity to

communicate with the staff. The changes in the periods of heart rate and response to the systems monitoring task were similar. These results suggest that alterations in circadian rhythmicity can be caused by socio-emotional factors even when the physical environment is very uniform and that these changes can occur both at behavioral and somatic levels. Therefore the authors postulated that performance changes generally attributed solely to length of vigil effects may also be strongly influenced by circadian variation in vigilance performance. This would explain conflicting results obtained in studies on the effect of vigil length. If the variations found in period length are characteristic of vigilance performance, the practice of testing subjects at the same time of day to remove circadian rhythmicity as a source of variation in experiments could result in false conclusions being drawn.

The relationship between body temperature and the capacity for work using a bicycle ergometer was studied by Asmussen and Boje (1945). They measured rectal temperature and the time required for 35 or 450 turns of the pedals corresponding to a 100 meter sprint and a 1500 meter run, respectively. They found that performance time was decreased when the body temperature had been increased by previous exercise, hot baths or radio diathermy. Measurements of muscle temperature showed a rapid increase at the start of the work which leveled off in 10-15 minutes. The increase in rectal temperature was slower with about 30 minutes required to reach a lower level. When the length of the preliminary exercise was varied from 0-50

minutes, it was found that the increase in muscle temperature in the first 10-15 minutes corresponded with the greatest improvement in performance. That the local muscle temperature was more important than increased rectal temperature was also shown in a second experiment. The maximum push exerted by the calf and arm muscles were compared before and after exercising on the bicycle ergometer. After exercise the calf muscle temperature was increased and performance had improved. Exercise had no effect on the temperature or performance of the arm muscle even though the rectal temperature had increased.

Kleitman and Jackson (1950) measured body temperature and performance (color naming, reaction time, or operation of a Link Trainer) of nine subjects under different military duty schedules. Color naming correlated with body temperature at all times. This was considered the most routine and uninteresting task. The reaction time and scores on the Link Trainer did not correspond to body temperature changes at night as well as they did during the daytime. Values for both were not as low at night as they expected. This may have been due to social pressure for maximum group effort or to the physiological stimulation from increased coffee consumption. The subjects were divided into two groups based on the level of body temperature. Those with higher body temperature (who consumed three times as much coffee) had higher scores on the Link Trainer. This did not extend to the other tests as the subject with lowest body temperature and poorest Link Trainer scores had the best score for color naming. Thus tests used to rank (or screen) subjects must be carefully selected.

It appears that performance decrement is a function of the disruption of circadian cycles. In their studies on east-west flights, Hauty and Adams (1965) found that performance deterioration accompanies the desynchronization of physiological rhythms that occur following a flight over more that 4 time zones. Performance is restored with resynchronization of the physiological cycles. Lewis and Lobban (1957) and Hauty (1963) have shown that inability of physiological rhythms to adjust to abnormal work/rest schedules resulted in impaired performance.

Changes in the body temperature cycle accompany reversal of activity patterns. Van Loon (1963) obtained a "flattened" curve intermediate between a normal body temperature curve and a completely inverted one for night shift workers with incomplete reversal of day-night activity. British shift workers studied by Reid (1961) showed considerable variation in their ability to adapt body temperature to night work and in the rate of adjustment. Inversion of body temperature cycle took 1-3 days for 37% while 38% showed no inversion. In a Netherlands study, Murrell (1965) reported that most men inverted in 1-3 days but would revert to their original cycle with only one day off.

Colquhoun et al. (1968) found six nights were required for body temperature to adjust to a night watch. Until adjustment occurred performance dropped with the nightly fall in body temperature. Teleky (1943) indicated that time for inversion varies with the individual, but occurs faster if physical activities are involved.

Shift work has physiological effects, social and domestic effects, along with detrimental effects on performance and an increase in accidents. The most important physiological effects are: 1) sleep loss, 2) digestive disturbances, and 3) other health-related problems. These may well be the result of disruption and desynchronization of some physiological rhythms. Lewis and Lobban (1957) found that inability to adapt physiologically to abnormal routines resulted in performance decrement. In addition, they also reported that the subjects did not generally feel as well as they did under normal routines.

Generally there is agreement in the literature that shift workers suffer sleep loss. Mott et al. (1965) reported a Scandinavian study where shift workers averaged 1 hour less sleep per day than day workers. De La Mare and Walker (1968) reported that permanent day workers averaged 7.7 hours of sleep per day and that permanent night workers averaged 7.1 hours of sleep. On the other hand Tune (1968) reported that (based on small samples) he found no differences between shift workers and day workers in the amount of sleep. He stated, however, that the quality of sleep may have been poor for the shift workers. Tune (1969) stated that shift workers build up a sleep debt that must eventually be paid. This sleep loss is due to the poor quality of sleep. Brown (1957) reported that one-half of a sample of 41 men working rotating shifts complained of sleep loss. A study by Mott et al. (1965) reported that over half of night shift workers have five times as many sleeping disorders as day shift workers. These problems were sleep disorders due to fatigue,

the sleep being "less refreshing", nervousness, and body discomfort.

Walker (1966) interviewed 110 shift workers from plants that had changed from a weekly rotation of shifts to a more rapid system of rotation that required a man to work all three shifts each week. The majority felt that it was easier to sleep under the rapid rotation system because of the two 24-hour breaks that occurred each week between the shift changes. On the otherhand, De La Mare and Shimmin (1964) compared permanent night workers with shift workers who rotated at two week intervals and found that 2/3 of the former felt that their regular hours of work made sleep easier.

Murrell (1965) suggested that it is the disruption of normal diurnal rhythms that causes problems with sleeping. Many of the body functions normally reach peak activity in the afternoon. "When a man transfers from day shift to night shift, he is having to work at a period when his body wants to rest, and when he attempts to rest his body is at its most active." This is the traditional explanation for loss of sleep.

Weitzman et al. (1968) recorded the sleep patterns for one week of normal night-time sleep followed by two weeks of reversal of the sleep-waking cycle. They found a significant and persistent alteration in sleep patterns after reversal and a small increase in waking time. The subjects reported feeling uncomfortable for about a week after the inversion and had decreased appetite for several days. Of the five subjects, none showed reversal of the circadian

cycle in body temperature during the first week of the altered sleep-waking cycle and only one did during the second week. A reversal was shown by the urinary volume rhythm and also by the urine creatinine rhythm. The urinary cycle of 17-hydroxycorticosteroids was altered, showing a tendency toward reversal during the second week, and plasma levels were also disrupted.

Extinction of the old rhythm is probably the most important factor in adapting to new work/rest schedules (Alluisi, 1969). Staying up long enough to be in a low-alertness condition during the first sleep period of the new schedule will probably enhance adjustment of the rhythms.

Another problem resulting from shift work that is probably related to disruption of circadian cycles is digestive disturbance. Complaints from shift workers of problems with appetite, digestion, and elimination range from 5% to 49% (Faulkner, 1970). Disruption of the normal pattern of meals is compounded by their poor nutritional value for many shift workers (Wyatt and Marriott, 1953). Aanonsen (1959) found that workers who had switched from shift work to day work for health reasons had 2-1/2 times more neuroses, nervous disorders, and peptic ulcers than workers who had never been on shift work. Other diseases of the digestive tract were twice as prevalent in these workers. While on shift work, 90% suffered from sleep disturbances. A survey of Dutch workers by Dirken (1966) failed to confirm that complaints of gastrointestinal disorders were more common among shift workers. It is difficult to accurately assess the in-

cidence of these problems since workers suffering from them are likely to transfer to a day job. The capacity to follow shift regimes varies strongly with the individual and decreases with age (Sollberger, 1965).

Wyatt and Marriott (1953) studied the records and interviewed the workers of three factories to analyze the factors involved in night work and shift changes and to indicate their probable effects. When the output rates were averaged, the day shift had a slightly higher rate than the night shift. Comparison of weekly output rates in factories with shift changes fortnightly or monthly showed that output on the day shift increased from the first week to the last. In addition, the average output of the first week on the day shift was below that of the last week on the night shift. This reduction in initial output of the day shift was attributed by them to a reduced working capacity induced by the night shift being carried forward to the following week. However, when the individual records of workers were studied about one-third worked as well or better on the night shift. A possible carry-over effect of the night shift was also postulated because of the increased number of absences during the first week of the day shift. No definite relationship between shift and accident rate was apparent (local conditions pecular to the factory were considered to be more influential).

The workers were questioned by Wyatt and Marriott to determine the subjective effects of shift work. The day shift was the preferred shift of the majority of the workers. They cited as reasons for their preference the opportunity for recreation and home life in the

evenings (41%), an increased feeling of well-being and more normal and restful sleep (33%), and better appetite, digestion, and regular meal times (20%). The few who liked or were impartial toward the night shift were apparently influenced primarily by the higher pay rate. Changes in meal times were generally more disturbing than changes in sleeping times. Immediate adjustment to new meal times when going from night to day work was reported by 61% of the workers and in one to six days by most of the remainder. When changing from day to night work, only 37% reported no effects. Another 39% were adapted after six days. Most of the remainder (almost 25%) did not become completely adapted before the shift changed again. The upset in digestion caused by changing meal times was evidenced by the large number (43%) of workers who took patent medicines every day or several times a week. Similar complaints about the effects of the night shift on the amount and quality of sleep were reported by the workers. In addition to these physiological changes, night work was blamed for psychological problems. The disruptions in daily or weekly routines, decreased participation in recreational activities, and conflicts in marital relationships all produced serious effects.

This survey of Wyatt and Marriott (1953) did disclose wide variation in the susceptibility of individuals to these effects of night work and shift changes. They noted that some men were unable to adapt to night work even after long experience with changing shifts and were therefore unsuited for shift work. This indicates a need

for selection of workers particularly suited for night work and the desirability of close medical supervision afterwards.

Chapter III

MATERIALS AND METHODS

General Procedures for All Experiments

Psychomotor and/or physical performance were measured in three separate studies. The first two experiments conducted in April, 1970, used four subjects on a psychomotor task and five on a physical loading task. All nine subjects were students enrolled in biology classes at Texas Tech University. Their ages ranged from 20-24 and all were in apparent good health. During the third experiment in June, four subjects (17-19 years old) alternated between a physical loading and a psychomotor task. One subject was a Texas Tech student whereas the other three were in senior high school. Psychomotor performance was measured with a tracking task in the spring whereas in the summer a target task was used. A treadmill was always used for physical loading. Subjects received a complete physical examination before any treadmill studies.

The general nature of each experiment was explained to the subjects. They received written instructions (Appendix A) regarding methods and times of measurements. These procedures were also explained orally to the subjects. An opportunity was provided for them to ask questions regarding all aspects of the experiments. The subjects in the spring experiments were paid on an hourly basis for doing the performance tests and for taking the physiological measurements. The summer subjects received a lump sum for the entire ex-

periment. While no "incentive" rewards were used for any of the experiments, an attempt was made to obtain fullest cooperation of the subjects by explaining the need for care when taking self measurements.

The same physiological measurements were made on all subjects. Each individual recorded his oral temperature, measured his heart rate, took a urine specimen, and worked a multiplication test at intervals throughout the day beginning as soon as he awakened and ending at bedtime. While subjects were requested to take these measurements at 3-4 hour intervals, they were not required to take them at specific times nor to take a certain number each day. Each subject also kept a daily log of his activities and diet. Samples of the forms used by the subjects for records and measurements are shown in Appendix B.

The multiplication tests consisted of six problems, each requiring a four-digit number to be multiplied by a second four-digit number. This test was patterned after one used by Kleitman (1963) with the number of digits reduced to save time. A random number table (Steel and Torrie, 1960) was used to prepare the tests. After the first few sets of problems had been worked it was apparent that the number of zeros varied considerably between tests. Therefore zeros were omitted from the rest of the tests. When preparing the tests for the summer experiment the number "one" was also omitted so that all the test sets had the same number of multiplication steps. The subjects used a stopwatch to time themselves

during the test. They were instructed not to look at the watch until they had completed the test. Most subjects finished a test in six minutes or less. After the summer experiment was in progress, however, it was discovered that two subjects had such low math abilities that it took them 20-30 minutes to complete a test. Therefore during the work day it was necessary to stop the subjects after they had worked on the test for five minutes to prevent undue delay in the testing schedule. This was done with the hope of finding a way to compensate for the differences when analyzing the data. No reliable way of adjusting the data could be devised, however, so the summer math scores were discarded.

Urine specimens collected by the subjects were taken to the laboratory for initial processing at least once a day. The specimens were kept cold in an ice chest or refrigerator until brought in by the subjects. The volumes were measured and approximately 60 ml from each specimen was frozen for subsequent analyses. A total of 1081 urine specimens were collected: 360 from the spring treadmill experiment, 197 from the spring tracking runs, and 524 from the treadmill-target task in the summer. The eight parameters measured for all specimens were volume and the concentrations of sodium, potassium, urea-nitrogen, uric acid, creatinine, inorganic phosphate, and chloride.

Sodium and potassium were analyzed with a Corning model 227 integrating flame photometer. Specimens were routinely diluted 1:200 instead of 1:100 as indicated in the manufacturer's instructions

because too many samples measured off-scale otherwise. A few samples were rediluted as necessary to obtain readable values. With these exceptions, the operating instructions were followed exactly.

The urine was analyzed for urea-nitrogen, uric acid, creatinine, inorganic phosphate, and chloride with a Technicon single-channel AutoAnalyzer. The Technicon developed procedure used for analysis of each compound is shown in Table 2.

The majority of the specimens required a 1:2 dilution for the chloride analyses and a 1:20 dilution for the other four Technicon procedures to have values readable within the range of the standards. Any specimens falling outside of the standard values were appropriately rediluted and rerun. All analyses were conducted at the rate of sixty determinations per hour.

Data Analysis

The performance studies were all designed as fixed model, randomized block factorial experiments and were evaluated using the University of California Biomedical Computer Program BMD10V for the analysis of covariance (Dixon, 1970). Since time and money were limited, detection of performance variation was considered most important. The further description of any rhythm in terms of period, acrophase, amplitude, etc. could then be determined in subsequent experiments.

Since the physiological measurements were taken by each subject individually, there was no need to set up a rigid schedule.

Table 2. Technicon auto analyzer methods used for the analyses of the urine specimens.

Compound Analyzed	Technicon Method Number
Urea nitrogen	N - 1c
Uric acid	N - 13b I/II
Creatinine	N - 11b
Inorganic phosphate	N - 4c I/II
Chloride	N - 5b I/II

Such a schedule might have altered the subjects' usual social and work routines and made it impossible to determine if (or how accurately) these rhythms can be detected under the variable conditions of daily life. In addition, any physiological or psychological changes produced by such a regime might have biased the results of the performance tests.

Physiological and performance rhythms have been shown to be present in subjects tested under regimented conditions. Application of these findings to everyday work situations requires the detection of these rhythms in spite of the increased "noise" expected with the more random activity patterns of normal life. Unequal spacing in data collection can remove much of the inconvenience associated with the interruptions necessary for such measurements. The development of computer programs for the rhythm analysis of unequally spaced data make such studies of daily routines practical. Circadian rhythm analyses were conducted at the Chronobiology Laboratories, University of Minnesota using the previously described (pp. 6-12) programs based on fitting 24-hour cosine curves to the data.

A special computer program (Appendix C) was written during the course of this research to aid in the necessary adjustment of computed acrophases to obtain a cosinor based on an internal acrophase. The minimum angle on a polar graph (360° = 24 hr) that would include the subjects' acrophase vectors for any variable was calculated by the program as an indication of the spacing of the acrophases. For convenience, this angle was termed the acroangle (from acrophase

and angle). Acroangles were first calculated for each variable based on the computative acrophases (midnight = 0°). The acrophase of each variable was then taken in turn as the reference for the calculation of adjusted acrophases and the corresponding acroangles of the remaining variables. A table of all acroangles was then printed and indicators placed by those that were smaller than both the acroangle for computative acrophases and a designated maximum value. Plots of the individuals' acrophases adjusted to each reference were also made so that the relative placements could be examined. New computer cards were then punched by the program for use with the cosinor programs.

Small acroangles would be expected with synchronization of one rhythm with another or of both with an unknown synchronizer. Since amplitudes were not considered, a cosinor based on internal acrophases yielding a small acroangle was not necessarily statistically significant. If the amplitudes were too small or dissimilar, a test of the null hypothesis (zero amplitude) did not indicate a statistically significant rhythm. Of course, a significant cosinor was not conclusive proof of synchronization. It indicated that such a connection might be found with further testing.

Spring Psychomotor Experiment

Performance of four subjects was measured with a compensatory tracking task using a cathode ray tube. The equipment had previously been used in a series of vibration experiments by El-Nawawi (1971).

All operating procedures were identical except that these subjects never received any vibration. As the horizontal line moved randomly up and down, the subject attempted to keep the line in the center of the screen with a vertical control lever. A dashed line was painted across the center of the tube as a guide. The differences between the subject's response and the programmed deviations were recorded as an error score for a 45 second interval out of every minute. Error scores for 5 minute periods and for the total run were also calculated.

Tracking runs were scheduled at nine different times on two consecutive weekends (Figure 4). Subjects rotated in order until all had been tested at each time with the exception of one subject that could not attend two of the sessions. Two runs per day at approximately the same times of day were conducted during the week (Figure 5). Selection of times for testing subjects was influenced by their individual class schedules. Runs were scheduled originally for one hour periods but due to equipment problems and subject conflicts most runs averaged about 45 minutes in length. Oral temperature of each subject was measured before and after each run. Subjects conducted the self measurements previously described only on the nine days of the experiment.

Spring Treadmill Experiment

The maximum oxygen uptake of eleven potential subjects was measured using the treadmill and the nomogram of Workman and Armstrong (1964). The five subjects with the closest values were selected for the treadmill task. Each subject received a complete

Time	Date 4/18	4/19	4/25	4/26
800	--	6	8	7
930	6	7	9	8
1100	7	8	6	9
1230	8	9	7	6
1400	9	6	8	7
1530	6*	7	9	8
1700	7	8	6*	9
1830	8	9	7	6
2000	9	6	8	7

*Missed run, not made up

Figure 4. Weekend tracking test schedule for subjects 6-9, spring 1970.

	Date				
Time	4/20	4/21	4/22	4/23	4/24
830	9	9	9	9	9
930	8	8*	8	8	8
1030					
1130					
1230					
1330	6		6		6
1430	7	7	7	7	7*
1530	9	6			
1630		9	9	9	9
1730					
1830	8	8	8	8	8
1930	6	6	6		6
2030	7	7	7	7	7

*Missed run, not made up

Figure 5. Weekday tracking schedule for subjects 6-9, spring 1970.

physical examination before starting the experiment. Subjects started taking the self measurements described previously two days before the runs were started and continued them until all runs were completed.

During the testing the subjects walked on the treadmill for an hour at three different times of the day to obtain a total of two runs at each time (Figure 6). No subject was scheduled for more than one run per day. An average rest span of 15 minutes preceeded each treadmill run. While the subject was seated, oxygen consumption and heart rate were continuously recorded. The treadmill was level while the subject walked at 4 or 2 mph (starting with the higher speed) alternating at 5 minute intervals during the test span. At the end of the hour, the treadmill was stopped and a chair placed on it for the subject. Monitoring of heart rate and oxygen consumption continued for a 20-30 minute recovery span.

Physical loading was measured by continuously monitoring oxygen consumption and heart rate before, during, and after the treadmill run. The high speed treadmill (Warren E. Collins, Inc., Braintree, Mass.) is capable of speeds from 1-16 mph with a grade from 0-40°. A physiograph (Narco Bio-Systems, Inc., Houston, Tx.) recorded the heart rate and also converted it to a linear value of rate using a cardiotach. Oxygen consumption was measured with an oxygen consumption transducer and computer (models OCT-1000-40E and OCC-1000-40E, Technology Versatronics, Inc., Yellow Springs, Ohio). The instrument atuomatically reset to the baseline after every 1000 ml of oxygen

Time	Date 4/20	4/21	4/22	4/23	4/24	4/25	4/26
930				1		3	1
1130	2	5*	5	4	2	4	3
1330	3		3		1	1	
1530	4	2	4		5	2	5
1730	1	3	1	3			
1930	5	4		2	4	5	2

*missed-made up 4/27

Figure 6. Treadmill Loading Schedule for Subjects 1-5, Spring 1970.

consumed for ease of measurement. One channel of the physiograph served as a recorder for the oxygen meter. The manufacturers' instructions on use were followed exactly for all equipment.

This experiment was designed to have six 1-hour treadmill runs for each subject. As it was conducted at the same time as the tracking task described earlier, two other members of the Project THEMIS team served as operators. They were experienced with the equipment and procedures from other THEMIS experiments. During the course of the testing week, one subject missed one experimental run. He stayed on the self-measurements until the run was made up the first of the following week. Otherwise the operators reported no problems or difficulties.

When the data were later being transcribed from the physiograph paper, however, it was evident that the runs had not been made as scheduled. All data from one treadmill run had to be eliminated from the statistical analyses because the run was done entirely at 2 mph rather than cycling between 4 and 2 mph. The other runs were made with the proper alternation of speeds but several were less than 1 hour in length, the shortest being only 30 minutes long. All of the data were used in the analyses of covariance because the BMD10V program automatically adjusted the calculations when data were missing. Since average values per run were used for the rhythm analyses, only the first 30 minutes of data from each run were used in preparing the computer cards for cosinor analyses.

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Summer Treadmill-Psychomotor Experiment

Four subjects were used for a Project THEMIS experiment that alternated a treadmill task with a target task. This study was designed by Project THEMIS members to obtain information on human performance over extended work periods that would be relevant to projected U. S. Army plans for a 24 hour combat period. On the basis of tests administered by the Department of Psychology, Texas Tech University, two subjects were selected to represent "enlisted men" and two to represent "officers".

During the two week testing periods, subjects were housed in a university dormitory and fed a standard diet except for Sunday when they were allowed to go home during the day. The diet (Appendix D), designed by Dr. Margaret Kassouny of the Department of Food and Nutrition, Texas Tech University, consisted of a balanced ratio of fats, proteins, and carbohydrates in equal amounts at each of the four meal times. The quantities for each subject provided the 45-57 cal/kg body weight necessary to maintain their body weight during testing.

On the days when they were not being tested (except Sunday), the subjects were still under supervision. They generally watched television, read, or occupied themselves with other light activities. Arousal time on work days was always about 6:15 AM. The subjects went to bed about 10 PM on the 8 hour days and at about 1:30 AM after the 16 hour work days. Since they went to and from the dorm together, all subjects followed a similar sleep-activity pattern.

While these subjects followed a more controlled schedule than the spring subjects, it was not as rigid as that found in most studies.

A performance task originally designed by Fornwalt (1965) was administered by THEMIS members from the Department of Psychology, Texas Tech University. A rotating wheel was divided equally by four lines with a small portion of the wheel visible through an opening in the instrument panel. A vertical hair-line was painted on this window. The subject scored a hit by pushing the proper button when the two lines were superimposed. The panel consisted of two rows of five lights corresponding to ten buttons on the desk top beneath them. The lights came on one at a time in random order and stayed on until the corresponding button was punched for a hit. After the hit was made, a different light came on for the next hit. If a new light did not appear, the subject pushed a reset button to start the machine over again. Counters recorded the number of hits, attempts, wrong buttons pushed, and total punches. These were read by an observer on one minute intervals. Five minute totals and average values/minute were calculated. The subject worked on this task for 90 minutes at a time.

The treadmill task was the same in design as the one used in the spring except that fixed speeds for all subjects were not used. Instead the grade and speed necessary for each subject (Table 3) to utilize 3.75 or 6.25 Kcal/min were determined from the nomogram of Workman and Armstrong (1964) based on their maximum oxygen uptake.

Table 3. Treadmill speeds and grades required for subjects 10-13 to utilize 3.75 Kcal/min or 6.25 Kcal/min corresponding to light and moderate work loads.

Subject Number	Light Work (3.75 Kcal/min) Speed mph	Grade %	Moderate Work (6.25 Kcal/min) Speed mph	Grade %
10	2.0	0	3.5	0
11	4.0	0	4.0	6
12	2.0	0	4.0	0
13	2.0	0	4.5	0

These are the midpoints of the ranges for light and moderate work loads as defined by Christensen (1962).

Subjects worked on alternate days except for Sunday for a two-week span. They worked an eight-hour day for one week and a sixteen-hour day for the other. During the work day the subject alternated between the treadmill and target tasks on two-hour intervals. Thus in an eight-hour day he had two sessions with each task. The complete schedule is shown in Figure 7. All but one subject started the previously described self measurements one week before testing began. One of the original four subjects selected dropped out during this pre-test span and had to be replaced only three days before testing started. Self measurements continued until all testing was finished.

The summer work periods were divided into three shifts. Each shift was eight hours in length and consisted of two 4-hour periods following a meal. Each work period was composed of one session on the treadmill and one session on the psychomotor task. Thus each shift had two treadmill and two psychomotor runs in 8 hours.

Shift 1 represented an 8-hour work day. The 16-hour work days were divided into shift 2 for the first 8 hours and shift 3 for the second 8 hours. Analyses of these treadmill data were done using average values for 5 minute spans of the full 60 minute run with speed as a factor or of the 30 minutes of data for either moderate or low speed. The covariate was minutes elapsed from the beginning of the run. Subjects were used as blocks. Results show the effects of shift, day of week, and time of day. Shifts 1 and 2 are compared

	Date: Week 1					
Time Span	6/22	6/23	6/24	6/25	6/26	6/27
	T P	T P	T P	T P	T P	T P
0800 - 0945	11 12	13 10	11 12	13 10	11 12	13 10
0945 - 1000			R E S T			
1000 - 1145	12 11	10 13	12 11	10 13	12 11	10 13
1145 - 1215			M E A L			
1215 - 1400	11 12	13 10	11 12	13 10	11 12	13 10
1400 - 1415			R E S T			
1415 - 1600	12 11	10 13	11 12	10 13	12 11	10 13
1600 - 1630			M E A L			
1630 - 1815		13 10		13 10		13 10
1815 - 1830			R E S T			
1830 - 2015		10 13		10 13		10 13
2015 - 2045			M E A L			
2045 - 2230		13 10		13 10		13 10
2230 - 2245			R E S T			
2245 - 0030		10 13		10 13		10 13

Figure 7. Schedule of treadmill (T) and psychomotor (P) performance for subjects 10-13, summer 1970.

Date: Week 2

Time Span	6/29		6/30		7/1		7/2		7/3		7/4	
	T	P	T	P	T	P	T	P	T	P	T	P
0800 - 0945	11	12	13	10	11	12	13	10	11	12	13	10
0945 - 1000					R E S T							
1000 - 1145	12	11	10	13	12	11	10	13	12	11	10	13
1145 - 1215					M E A L							
1215 - 1400	11	12	13	10	11	12	13	10	11	12	13	10
1400 - 1415					R E S T							
1415 - 1600	12	11	10	13	12	11	10	13	12	11	10	13
1600 - 1630					M E A L							
1630 - 1815	11	12			11	12			11	12		
1815 - 1830					R E S T							
1830 - 2015	12	11			12	11			12	11		
2015 - 2045					M E A L							
2045 - 2230	11	12			11	12			11	12		
2230 - 2245					R E S T							
2245 - 0030	12	11			12	11			12	11		

Figure 7. Continued.

to give the effects of the work week, day of week, and time of day. The time of day effect was best shown using shifts 2 and 3 for analysis of covariance. Day of week and time of day effects for each individual shift were also determined.

The psychomotor data were analyzed in similar fashion to give the effects of shift, work week, day of week, and time of day. The psychomotor counters measured four factors; hits, attempts, wrong buttons, and total. The total included every punch of the proper button whether a hit or miss and the number of resets. It did not register if the wrong button was used. The attempts counter gave the number of punches of the proper button (or the sum of the hits and misses). The number of resets and misses were determined by subtraction.

Because of the large number of resets that had to be made, it was felt that the total number of hits possible for a subject to make might have been reduced. With four targets on a wheel revolving at 5 rpm, there was a potential for scoring a maximum of 20 hits/min. When a hit was made, the button-indicator light went out and there was a slight pause for the counters to register before the next light came on. The subject had slightly less than 3 seconds to check the light panel to determine which button to use, position his finger on that button, and look up at the window to watch for the pointer. Thus the time needed for him to realize that a light was not going to come on, to push the reset button, and to wait for the equipment to reset could have been long enough for him to lose an opportunity

to score a hit. In an attempt to remove this possible bias, the number of resets was subtracted from 20 (the maximum number of hits possible) to get a better approximation of a perfect score. The number of actual hits was divided into this figure to generate coded data designated as possible hits. Some subjects, however, quickly developed the habit of keeping their free hand on the reset button to reduce the time needed to reset the equipment. Thus the calculation of the possible hits [(20-resets)/hits] may not have been needed nor accurate.

For rhythm analysis, one would rather know the time of the best performance (i.e., lowest number of errors) rather than the time of worst performance which is obtained by using the actual totals. Some values, therefore, were inverted or otherwise coded to shift their maximums by 180° prior to chronobiological analysis. This would not be needed for conventional analysis by covariance.

Chapter IV

RESULTS AND DISCUSSION

Analysis of Covariance: Spring Psychomotor Experiment

The tracking runs of subjects 6-9 made on the five week days were scheduled as previously shown in Figure 5 to conform to a fixed model, randomized block factorial design. The two times of day selected were 6-8 hours apart which would correspond to approximately 1/4-1/3 of a 24-hour cycle. With only a few exceptions, these runs were made at the same time every day so a comparison could also be made between the days of the week. When the tracking scores were averaged prior to analysis of variance, it was evident that the average value per run of every subject increased by about 30-35 units starting Tuesday afternoon (April 21). Because it was such a large jump and it occurred at the same time for all subjects, an equipment malfunction or change in sensitivity was suspected.

An attempt was made using rhythm analysis to determine if this was a change in machine sensitivity for which a correction could be made. If a baseline shift had occurred (resulting in a higher mesor but the same period amplitude), an average value could be subtracted to produce coded error scores. An increase in the amplification (resulting in higher mesor and amplitude) could be corrected by using values that were percentages of the mean. However, the rhythm

analyses of the data before and after the change indicated that the baseline had been raised while the amplitude of the wave was decreased.

The cause of this change is unknown. At no time during the course of the experiment were any adjustments made to the equipment. Although the room was kept locked when not in use, a number of people had a key who might have entered for other purposes. Thus the possibility that the control settings were accidentally or purposely altered cannot be ruled out. Several statisticians were consulted as to possible ways to code or adjust the data to remove this apparent bias. None could see any way that this could be done without casting doubt on the validity of the results. Thus no attempt was made to conduct analyses of variance or covariance on the tracking task data. Resources were not available for this experiment to be repeated.

Analysis of Covariance: Summer Psychomotor Experiment

The results from the analysis of covariance of the summer psychomotor data are shown in Tables 4-9. Unfortunately, resets were significant for about half of the factors. It can not be assumed, therefore, that the resets were a random factor that would probably not have affected the results. The number of resets may have been influenced by the amount of use during a run and/or the length of time the machine was in operation. Regardless of the relationship, it is obvious that the equipment must be redesigned to eliminate the need for resetting before further experimental use. Some time of day effects were significant when resets were not significant sug-

Table 4. Effects of shift, day of week, and time of day for shifts 1, 2, and 3 on psychomotor performance of subjects 10-13 shown by the significance level from the analyses of covariance of the actual hits, misses, sum (actual hits plus misses), resets, possible hits ([20-resets]/actual hits), and wrong button.

Source of Variation	df	Wrong Button	Resets	Sum	Misses	Actual Hits	Possible Hits
Blocks	3	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005
Shift (S)	2	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005
Day (D)	2	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005
Time (T)	1	NS	<0.1	<0.005	<0.0005	NS	NS
S x D	4	<0.005	<0.0005	<0.0005	<0.0005	NS	NS
S x T	2	<0.01	NS	<0.001	NS	<0.0005	<0.0005
D x T	2	<0.025	NS	<0.0005	<0.0005	<0.01	NS
S x D x T	4	<0.0005	<0.0005	<0.0005	<0.0005	NS	NS
Covariate	1	<0.025	NS	<0.005	<0.0005	NS	NS
Error	1274						

Table 5. Effects of week, day of week, and time of day for shifts 1 and 2 on psychomotor performance of subjects 10-13 shown by the significance level from the analyses of covariance of the actual hits, misses, sum (actual hits plus misses), resets, possible hits ([20-resets]/actual hits), and wrong button.

Source of Variation	df	Wrong Button	Resets	Sum	Misses	Actual Hits	Possible Hits
Blocks	3	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005
Weeks (W)	1	<0.0005	<0.025	<0.005	NS	<0.0005	NS
Day (D)	2	<0.005	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005
Time (T)	1	<0.05	NS	<0.0005	<0.0005	NS	NS
W x D	2	<0.001	<0.005	<0.005	<0.0005	NS	NS
W x T	1	NS	NS	<0.0005	NS	<0.0005	<0.0005
D x T	2	<0.01	NS	NS	<0.1	<0.1	NS
W x D x T	2	<0.1	<0.01	<0.005	<0.0005	NS	NS
Covariate	1	NS	NS	<0.005	<0.005	NS	NS
Error	848						

Table 6 . Effects of day of week and time of day for shifts 2 and 3 on psychomotor performance of subjects 10-13 shown by the significance level from the analyses of covariance of the actual hits, misses, sum (actual hits plus misses), resets, possible hits ([20-resets]/actual hits), and wrong button.

Source of Variation	df	Wrong Button	Resets	Sum	Misses	Actual Hits	Possible Hits
Blocks	3	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005
Day (D)	2	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005
Time (T)	3	<0.0005	<0.0005	<0.0005	<0.0005	<0.01	<0.005
D x T	6	<0.0005	<0.0005	<0.0005	<0.0005	NS	NS
Covariate	1	<0.01	NS	NS	<0.01	NS	<0.1
Error	848						

Table 7. Effects of day of week and time of day for shift 1 on psychomotor performance of subjects 10-13 showm by the significance levels from the analyses of covariance of the actual hits, misses, sum (actual hits plus misses), resets, possible hits ([20-resets]/actual hits), and wrong button.

Source of Variation	df	Wrong Button	Resets	Sum	Misses	Actual Hits	Possible Hits
Blocks	3	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005
Day (D)	2	<0.0005	<0.0005	<0.001	<0.0005	<0.0005	<0.0005
Time (T)	1	<0.0005	NS	NS	<0.0005	<0.0005	<0.0005
D x T	2	NS	<0.025	NS	<0.005	<0.05	NS
Covariate	1	NS	NS	<0.0005	<0.001	NS	<0.05
Error	422						

Table 8. Effects of day of week and time of day for shift 2 on psychomotor performance of subjects 10-13 shown by the significance level from the analyses of covariance of the actual hits, misses, sum (actual hits plus misses), resets, possible hits ([20-resets]/actual hits), and wrong button.

Source of Variation	df	Wrong Button	Resets	Sum	Misses	Actual Hits	Possible Hits
Blocks	3	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005
Day (D)	2	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005
Time (T)	1	NS	NS	<0.0005	<0.0005	<0.005	<0.1
D x T	2	<0.005	NS	<0.005	<0.0005	NS	NS
Covariate	1	NS	NS	NS	<0.05	NS	<0.1
Error	422						

Table 9. Effects of day of week and time of day for shift 3 on psychomotor performance of subjects 10-13 shown by the significance level from the analyses of covariance of the actual hits, misses, sum (actual hits plus resets), resets, possible hits ([20-resets]/actual hits), and wrong button.

Source of Variation	df	Wrong Button	Resets	Sum	Misses	Actual Hits	Possible Hits
Blocks	3	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005
Day (D)	2	<0.001	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005
Time (T)	1	<0.025	NS	NS	<0.05	NS	NS
D x T	2	<0.0005	<0.0005	<0.0005	<0.0005	<0.025	<0.025
Covariate	1	<0.0005	<0.1	NS	<0.05	NS	NS
Error	422						

gesting a possible circadian rhythm. However the possibility that the data have been influenced by the number of resets is too great to reach a definite conclusion regarding the presence of circadian or other rhythmic variations.

The only data which might be usable is that for the number of errors. Since this would primarily be a perceptual error (associating the wrong button with the signal light), the resets might not have influenced the results. These data showed a significant day of week effect except for shift 1. A time of day effect was also present. It is interesting to note that the covariate (elapsed time from start of run) was not significant for the number of errors made during shift 1 or 2 but was for shift 3, the comparison of all shifts, and for shifts 2 and 3 combined. This would suggest that the rate of making errors was greater at night. Again, these results can be taken only as an indication of possible rhythmic changes because of the unknown influence of the resets.

Analysis of Covariance: Spring Treadmill Experiment

Each session on the treadmill for subjects 1-5 was made as nearly identical as possible with regard to routine and loading. Thus in the absence of effects from rhythms, for instance those with periods of about 24 hours (circadian) or about 7 days (circaseptan), the results of the analyses of covariance on heart rate, oxygen consumption, and oxygen pulse should all have been statistically non-significant. Because this investigation was primarily concerned with identifying the most likely indicators of performance, a level of

$P < 0.1$ was used in preparing the tables of results. Selection of this value for P was considered appropriate as it maximized the probability of recognizing an existing rhythm in any parameter. However, when the tables were completed, most of the significant results had $P \leq 0.05$. These results can be used in designing a larger experiment to specifically characterize the anticipated correlations or to show that they probably do not exist. Lack of significant results in this experiment, however, cannot be taken as proof that rhythms in a parameter or correlation between parameters do not exist. These might become evident with more frequent sampling over an appropriate time span.

The factors used in the analyses of covariance were the time of day to indicate possible circadian rhythms and the day of week to indicate possible circaseptan rhythms. Tables 10 to 12 reveal that the factors were usually significant. The covariate used for all analyses was the elapsed time from the beginning of the run. It was significant only for the oxygen consumption and oxygen pulse data at 2 mph. For the analyses using all of the data (combined speeds), speed was also a factor with time of day and day of week. LeBlanc (1957) has shown that up to a speed of 3.4 mph differences of 0.25 mph in walking rate can be detected by heart rate measurements.

The results from the analyses of covariance of the data on heart rate are shown in Table 10. Resting values were only significant for the block or "between subject" effect. Even with the selection of the subjects to have similar characteristics, variation occurred,

Table 10. Effects of day of week and time of day on heart rate of subjects 1-5 shown by the significance level from the analyses of covariance of the rest, work (WK), and work minus rest (W-R) values from their treadmill runs.

Source of Variation	df	Prework Rest	2 and 4 mph Combined*		4 mph Segment		2 mph Segment	
			WK	W-R	WK	W-R	WK	W-R
Blocks	4	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005
Day (D)	1	NS	<0.0005	<0.1	<0.0005	NS	<0.005	NS
Time (T)	2	NS	<0.0005	NS	<0.0005	NS	<0.0005	NS
D x T	2	NS	<0.1	<0.005	NS	<0.025	NS	<0.01
Covariate	1	--	NS	NS	NS	NS	NS	NS
Error df	-	19	287		142		140	

*Speed was a factor and was always significant. Interactions with it were never significant.

Table 11. Effects of day of week and time of day on oxygen consumption of subjects 1-5 shown by the significance level from the analyses of covariance of the rest, work (WK), and work minus rest (W-R) values from their treadmill runs.

Source of Variation	df	Prework Rest	2 and 4 mph Combined*		4 mph Segment		2 mph Segment	
			WK	W-R	WK	W-R	WK	W-R
Blocks	4	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005
Day (D)	1	<0.025	NS	<0.0005	NS	<0.001	NS	<0.001
Time (T)	2	NS	<0.0005	NS	<0.005	NS	<0.005	NS
D x T	2	NS	<0.025	<0.0005	NS	<0.025	<0.01	<0.0005
Covariate	1	--	NS	NS	NS	NS	<0.005	<0.025
Error df	-	19	287		142		140	

*Speed was a factor and was always significant. Interactions with it were never significant.

Table 12. Effects of day of week and time of day on oxygen pulse of subjects 1-5 shown by the significance level from the analyses of covariance of the rest, work (WK), and work minus rest (W-R) values from their treadmill runs.

Source of Variation	df	Prework Rest	2 and 4 mph Combined*		4 mph Segment		2 mph Segment	
			WK	W-R	WK	W-R	WK	W-R
Blocks	4	<0.05	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005
Day (D)	1	<0.025	<0.01	<0.0005	NS	<0.005	<0.005	<0.025
Time (T)	2	NS	<0.05	NS	NS	NS	NS	NS
D x T	2	NS	<0.01	<0.0005	NS	<0.025	<0.01	<0.001
Covariate	1	--	NS	NS	NS	NS	<0.05	<0.1
Error df	-	19	287		142		140	

*Speed was a factor and was always significant. Interactions with it were never significant.

therefore, blocking of the data was important. In all of the results for this experiment, blocks were significant, verifying the need for this statistical approach. When the entire run (combined speeds) or each separate speed was analyzed, the working values of heart rate showed significant differences for time of day and day of week effects. It must be kept in mind that an alternating work load was used. Therefore the results using data from only one speed can not be assumed to be valid for performance done at that speed without the alternating speed. When the increase in the heart rate above rest values (work minus rest or W-R) was analyzed, the time of day effect was not significant. A day of week effect was evident for the combined speeds at the 0.1 level but not for the separate speeds. A day of week-time of day (Day x Time) interaction was significant for all W-R values and the work values at two and four mph.

Table 11 shows the results for the oxygen consumption data. Resting values were signfiicant for a day of week effect but not for time of day. None of the work values showed a day of week effect but all of the W-R values were significant. A time of day effect was present for all of the work values and none of the W-R data. The "Day x Time" interaction was significant for all work and W-R values. The covariate was significant for the two and four mph data.

The oxygen pulse results are shown in Table 12. The time of day effect was significant only for the combined speeds work data. Resting values were significant for a day of week effect as were the work and W-R values for the combined speeds. The day of week effect

was also significant for work values at two mph and for all W-R data. Except for the four mph work data, all work and W-R values for the "day x time" interaction were significant. The covariate was significant for the analysis of both work and W-R at two mph.

Since the oxygen pulse consisted of the ratio of oxygen consumption to heart rate, it would not necessarily show significant results when either heart rate or oxygen consumption do and vice versa. If rhythms were present in both heart rate and oxygen consumption but the periods were closely in phase, the ratio would stay fairly constant and probably show little change. Thus the results would not necessarily be significant. The oxygen pulse would change if the periods were out of phase or if one parameter had a large enough variation in its amplitude even though the other one did not. The detection of a rhythm in the oxygen pulse cannot replace the analysis of the actual heart rate and oxygen consumption data. Since a large value for oxygen pulse could be considered an indication of more efficient performance, characterization of such a rhythm could have some practical applications. There could possibly be less strain on the heart and/or a smaller oxygen debt created with exertion at the peak of the oxygen pulse rhythm as compared to other times.

Analysis of Covariance: Summer Treadmill Experiment

The data on heart rate, oxygen consumption and oxygen pulse from the treadmill runs were analyzed in several different ways by analysis of covariance, to emphasize different factors. The results

of these analyses are shown in Tables 45-62 in Appendix E. Elapsed time from the start of the run was always used as the covariate. This was significant except for a few instances. Blocks were generally highly significant but in one case the significance level was only 0.1. Work load was used as a factor when appropriate and was always highly significant. Except for a few indicated cases, possible interactions involving work load were not significant and were therefore not included in the tables.

Comparisons and contrasts between the three physiological variables and the analysis of covariance factors could be more readily made when the table entries were rearranged to group the results for a particular factor together. Analyses of resting values of heart rate, oxygen consumption, and oxygen pulse (Table 13) showed a statistically significant effect of shift only for oxygen consumption. Work values for the entire run or for the separate work loads for all three parameters showed significant differences between shifts. The work minus rest (W-R) data for oxygen consumption from the combined loads and from the moderate work load were not significant. All other W-R values showed a significant shift effect. Since shift 3 was at a later time, differences were not unexpected.

Therefore just shifts 1 and 2 were compared (Table 13). These analyses gave an indication of differences between the two work weeks for muscular performance at the same time of day. Resting values for both oxygen consumption and oxygen pulse showed significant differences between shifts 1 and 2. Work values for all three variables

Table 13. Comparison of the effect of shift on heart rate, oxygen consumption, and oxygen pulse of subjects 10-13 from the analyses of covariance of all three shifts or of shifts 1 and 2 for rest, work (WK) and work minus rest (W-R) values from their treadmill runs. Complete results are shown on Tables 45 to 50 in Appendix E.

Physiological Variable	Prework Rest	Significance Level for Shift from Analyses of Covariance					
		Combined Light and Moderate Work		Moderate Work Segment		Light Work Segment	
		WK	W-R	WK	W-R	WK	W-R
Shifts 1, 2, and 3							
Heart Rate	NS	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005
Oxygen Consumption	<0.09	<0.0005	NS	<0.1	NS	<0.0005	<0.05
Oxygen Pulse	NS	<0.0005	<0.0005	<0.01	<0.0005	<0.0005	<0.0005
Shifts 1 and 2							
Heart Rate	NS	<0.005	<0.0005	<0.01	<0.0005	<0.1	<0.0005
Oxygen Consumption	<0.04	<0.0005	NS	<0.1	NS	<0.0005	NS
Oxygen Pulse	<0.07	<0.0005	NS	<0.005	NS	<0.0005	NS

were significant for the entire run and each separate work load. The only W-R values that were significant were for heart rate.

In the analysis of covariance of data from all three shifts and from shifts 1 and 2 the day of week (Table 14) and time of day (Table 15) effects were also included. The heart rate values showed significant day of week effect only for the light work load or for light and moderate work combined. A significant day of week effect was shown for all rest, work, and W-R values for oxygen consumption. Oxygen pulse values were significant except when comparing the rest values for shifts 1 and 2. Examination of the time of day results revealed significant differences for most values of the three variables. Exceptions were the W-R heart rate at the light load for just the comparison of shifts 1 and 2 and the rest values for oxygen consumption and oxygen pulse for both comparisons.

To further examine the time of day effect, the data from shifts 2 and 3 were combined for analysis of covariance. Each shift contained treadmill runs at two different times of day. Therefore by combining them the time of day effect can be analyzed for an interval covering four different times of day. By increasing the number of times of day utilized and thereby increasing the degrees of freedom, the effect of time of day can be analyzed more precisely. Heart rate was the only variable showing a significant time of day effect for rest values (Table 16). All work and W-R data for heart rate, oxygen consumption, and oxygen pulse were significant. The analysis of covariance also showed day of week effects present for all the oxygen consumption

Table 14. Comparison of the effect of day of week on heart rate, oxygen consumption, and oxygen pulse of subjects 10-13 using the significance level from the analyses of covariance of all three shifts or of shifts 1 and 2 for rest, work (WK) and work minus rest (W-R) values from their treadmill runs. Complete results are shown on Tables 45-50 in Appendix E.

Physiological Variable	Significance Level for Day of Week from Analyses of Covariance						
	Prework Rest	Combined Light and Moderate Work		Moderate Work Segment		Light Work Segment	
		WK	W-R	WK	W-R	WK	W-R
Shifts 1, 2, and 3							
Heart Rate	NS	<0.025	NS	NS	NS	<0.025	NS
Oxygen Consumption	<0.003	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005
Oxygen Pulse	<0.01	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.025
Shifts 1 and 2							
Heart Rate	NS	<0.005	NS	NS	NS	<0.0005	NS
Oxygen Consumption	<0.06	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005
Oxygen Pulse	NS	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005

Table 15. Comparison of the effect of time of day on heart rate, oxygen consumption, and oxygen pulse of subjects 10-13 using the significance level from the analyses of covariance of all three shifts or of shifts 1 and 2 for rest, work (WK) and work minus rest (W-R) values from their treadmill runs. Complete results are shown on Tables 45-50 in Appendix E.

Physiological Variable	Significance Level for Time of Day from Analyses of Covariance						
	Prework Rest	Combined Light and Moderate Work		Moderate Work Segment		Light Work Segment	
		WK	W-R	WK	W-R	WK	W-R
	Shifts 1, 2, and 3						
Heart Rate	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.005	<0.0005
Oxygen Consumption	NS	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005
Oxygen Pulse	NS	<0.0005	<0.0005	<0.025	<0.0005	<0.0005	<0.0005
	Shifts 1 and 2						
Heart Rate	<0.0005	<0.025	<0.0005	<0.05	<0.0005	NS	<0.0005
Oxygen Consumption	NS	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005
Oxygen Pulse	NS	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005

Table 16. Comparison of the effects of day of week and time of day on heart rate, oxygen consumption, and oxygen pulse of subjects 10-13 using the significance level from the analyses of covariance of shifts 2 and 3 for the rest, work (WK), and work minus rest (W-R) values from their treadmill runs. Complete results are given in Tables 51-53 in Appendix E.

Physiological Variable	Significance Level from Analyses of Covariance of Shifts 2 and 3						
	Prework Rest	Combined Light and Moderate Work		Moderate Work Segment		Light Work Segment	
		WK	W-R	WK	W-R	WK	W-R
	Day of Week						
Heart Rate	NS	NS	<0.025	NS	NS	NS	<0.05
Oxygen Consumption	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005
Oxygen Pulse	<0.001	<0.0005	NS	<0.0005	NS	<0.0005	NS
	Time of Day						
Heart Rate	<0.001	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005
Oxygen Consumption	NS	<0.0005	<0.0005	<0.025	<0.005	<0.0005	<0.0005
Oxygen Pulse	NS	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005

data. The only significant heart rate values were for W-R for the light load and for the combined loads.

Each shift was analyzed separately by analysis of covariance for day of week and time of day effects. When the results were compared (Tables 17 and 18), some trends were apparent. Except for the rest values from shift 2, oxygen consumption showed a significant day effect for all rest, work, and W-R values. Heart rate, however, had a significant day of week effect only for work and W-R values of shift 1 and for W-R of shift 2. The day of week factor was significant for all work values of oxygen pulse. Rest values were significant only for shift 3 and W-R only for shift 1. Thus while heart rate and oxygen pulse showed some evidence of differences between days, oxygen consumption variation had a larger and therefore more obvious effect.

When the results are compared for time of day effect, heart rate rest values were significant for all three shifts but rest oxygen consumption was significant only for shift 1. The work and W-R data for shifts 1 and 2 are all significant for oxygen consumption but the results are not consistent for heart rate. However, with regard to shift 3, all work and W-R heart rate values were significant and those for oxygen consumption generally were not. Rest values for oxygen pulse were never significant. The work and W-R values were usually significant for all three shifts. These data suggest that during muscular work oxygen consumption was more variable during the period from 0800 to 1600 whereas heart rate showed greater differences from 1600 to 2400 when working a 16-hour day.

Table 17. Comparison of effect of day of week on heart rate, oxygen consumption, and oxygen pulse of subjects 10-13 using the significance level from the analyses of covariance of shift 1, 2, or 3 for rest, work (WK), and work minus rest (W-R) values from their treadmill runs. Complete results are given in Tables 54-62 in Appendix E.

Physiological Variable	Significance Level for Day of Week from Analyses of Covariance						
	Prework Rest	Combined Light and Moderate Work		Moderate Work Segment		Light Work Segment	
		WK	W-R	WK	W-R	WK	W-R
Shift 1							
Heart Rate	NS	<0.0005	<0.01	<0.01	<0.025	<0.005	<0.1
Oxygen Consumption	<0.1	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005
Oxygen Pulse	NS	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005
Shift 2							
Heart Rate	NS	NS	<0.0005	NS	<0.025	NS	<0.005
Oxygen Consumption	NS	<0.0005	<0.001	<0.0005	<0.05	<0.0005	<0.001
Oxygen Pulse	NS	<0.0005	NS	<0.001	NS	<0.001	NS
Shift 3							
Heart Rate	NS	NS	NS	NS	NS	NS	NS
Oxygen Consumption	<0.03	<0.0005	<0.0005	<0.0005	<0.001	<0.0005	<0.01
Oxygen Pulse	<0.05	<0.0005	NS	<0.0005	NS	<0.0005	NS

Table 18. Comparison of effect of time of day on heart rate, oxygen consumption and oxygen pulse of subjects 10-13 using the significance level from the analyses of covariance of shift 1, 2, or 3 for rest, work (WK), and work minus rest (W-R) values from their treadmill runs. Complete results are given in Tables 54-62 in Appendix E.

Physiological Variable	Significance Level for Time of Day from Analyses of Covariance						
	Prework Rest	Combined Light and Moderate Work		Moderate Work Segment		Light Work Segment	
		WK	W-R	WK	W-R	WK	W-R
				Shift 1			
Heart Rate	<0.006	<0.0005	<0.05	<0.005	NS	<0.05	<0.025
Oxygen Consumption	<0.07	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005
Oxygen Pulse	NS	<0.0005	<0.025	<0.01	NS	<0.0005	<0.01
				Shift 2			
Heart Rate	<0.01	NS	<0.0005	NS	<0.0005	NS	<0.0005
Oxygen Consumption	NS	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005
Oxygen Pulse	NS	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005
				Shift 3			
Heart Rate	<0.0005	<0.0005	<0.0005	<0.0005	<0.005	<0.0005	<0.0005
Oxygen Consumption	NS	NS	<0.05	NS	NS	NS	<0.025
Oxygen Pulse	NS	<0.1	<0.0005	NS	<0.01	NS	<0.0005

All these data show that heart rate, oxygen consumption, and oxygen pulse could be influenced by the time of day and day of week. The latter factor emphasizes the danger in assuming that rhythmic effects on experimental results can be eliminated by always scheduling tests at the same time of the day for a particular subject. If different treatments had been applied on different days, the present results would have been used to prove that one was superior. Of course, the converse could happen, an effect of the treatment might be negated by circaseptan variation and the treatment incorrectly assumed to be ineffective.

Rhythmometric Analysis

Using the least-squares method of Halberg et al. (1965), a cosine curve was fitted to each individuals' time series. Examples of the results of these analyses are shown in Tables 19 and 20 for oral temperature and resting heart rate, respectively. When tabulating the results of fitting cosine curves or of cosinor summaries, if the P value is greater than 0.05, the 95% confidence intervals or standard errors for amplitude and acrophase are customarily not given. The imputations of the rhythm parameters (ϕ, M, A) were then used to prepare a mean cosinor (Halberg et al., 1972, 1967) for each variable. The cosinors referenced to midnight that were statistically significant for physiological variables measured before and during performance testing are shown in Table 21. The measurements of oral temperature, resting heart rate, and the excretion of potassium, sodium, and water

Table 19. Results from fitting 24-hour cosine curve to oral temperature (°F) data obtained during performance testing for subjects 1-13.

	P	Mesor		Amplitude		Acrophase	
		M	SE	A	SE	ϕ	(95% C.I.)
1	<0.009	97.93	0.07	0.66	0.11	-261°	(-247,-275)
2	<0.009	98.63	0.07	0.61	0.10	-284°	(-266,-302)
3	<0.009	97.63	0.09	0.56	0.13	-257°	(-232,-282)
4	<0.009	98.20	0.05	0.80	0.07	-266°	(-256,-276)
5	<0.009	97.93	0.08	0.43	0.13	-244°	(-216,-271)
6	<0.009	98.48	0.02	0.17	0.03	-296°	(-271,-321)
7	<0.009	98.20	0.07	0.56	0.10	-267°	(-247,-267)
8	<0.026	98.56	0.02	0.11	0.04	-284°	(-245,-323)
9	<0.009	98.32	0.06	0.43	0.09	-254°	(-232,-276)
10	<0.009	97.71	0.04	0.20	0.06	-354°	(-306,- 24)
11	<0.120	97.78	0.06	0.16		-357°	
12	<0.011	97.69	0.06	0.22	0.07	-296°	(-249,-343)
13	<0.009	97.97	0.06	0.36	0.07	-303°	(-272,-334)

ϕ reference = local midnight; τ = 24 hrs. = 360°; 15° = 1 hr.

Table 20. Results of fitting 24-hour cosine curve to heart rate (beats/minute) data obtained during performance testing for subjects 1-13.

	P	Mesor		Amplitude		Acrophase	
		M	SE	A	SE	ϕ	(95% C.I.)
1	<0.01	71.82	1.57	11.36	2.53	-240°	(-222,-259)
2	<0.01	65.16	1.20	8.54	1.75	-286°	(-264,-308)
3	<0.01	58.39	0.73	5.14	1.13	-235°	(-215,-255)
4	<0.01	81.48	0.98	10.27	1.12	-288°	(-272,-304)
5	<0.01	73.29	1.17	7.42	1.94	-264°	(-242,-286)
6	<0.10	76.01	0.78	2.41		-259°	
7	<0.01	64.49	1.36	6.93	1.96	-261°	(-231,-292)
8	<0.01	77.36	0.55	2.86	0.87	-233°	(-206,-259)
9	<0.03	74.49	2.11	6.97	2.82	-270°	(-255,-316)
10	<0.01	58.56	0.74	4.94	0.92	-317°	(-292,-343)
11	<0.13	70.73	0.89	2.11		-340°	
12	<0.78	58.79	0.86	0.67		-338°	
13	<0.01	73.47	1.32	6.10	1.61	-315°	(-274,-356)

ϕ reference = local midnight; τ = 24 hrs. = 360°; 15° = 1 hr.

Table 21. Mean cosinors showing group synchronized rhythms in physiological variables of subjects 1-5 and 10-13 before (B) and for subjects 1-13 during (D) performance testing.

Variable		P	Mesor		Amplitude		Acrophase[a]	
			M	(95% C.I.)	A	(95% C.I.)	ϕ	(95% C.I.)
Oral Temp	B	<0.007	98.11	(97.34, 98.87)	0.42	(0.19, 0.65)	-277°	(-246,-312)
(°F)	D	<0.001	98.07	(97.40, 98.75)	0.36	(0.23, 0.50)	-273°	(-258,-302)
Rest Heart Rate	B	<0.001	67.59	(58.18, 81.00)	6.57	(3.82, 9.31)	-277°	(-247,-313)
(b/m)	D	<0.001	69.54	(54.45, 84.63)	5.12	(2.92, 7.32)	-273°	(-248,-305)
Urinary:								
Volume	B	<0.018	55.67	(4.08,107.27)	21.81	(1.38,42.34)[b]	-256°	(-187,-323)
(ml/hr)	D	<0.014	78.30	(3.37,153.24)	24.36	(2.11,46.23)[b]	-243°	(-186,-309)
Potassium	B	<0.001	2.66	(0.06, 5.25)	52.44	(32.68,72.27)[b]	-216°	(-156,-238)
(mEq/hr)	D	<0.001	3.50	(1.46, 5.53)	40.01	(26.55,53.48)[b]	-203°	(-174,-234)
Sodium	B	<0.023	10.66	(2.35, 18.98)	26.30	(0.32,52.27)[b]	-246°	(-153,-283)
(mEq/hr)	D	<0.036	10.89	(4.34, 17.45)	16.75	(-1.24,34.74)[b]	-240°	(-178,-336)
Chloride (mg/hr)	D	<0.006	91.77	(33.50,150.05)	31.49	(13.05,49.94)[b]	-206°	(-172,-262)
Uric Acid	B	<0.049	28.97	(12.76, 45.19)	20.26	(-3.45,43.97)[b]	-238°	(-122,-294)
(mg/hr)	D	<0.07	33.28		13.66		-203°	

[a] ϕ reference = local midnight; 360° = 24 hr.

[b] Amplitude expressed as % of mean

had significant group synchronized cosinors before and during the performance testing. The cosinors for the excretion of chloride and uric acid did not show consistent results for data obtained before and during performance testing. This was probably a result of changes in amplitude. The amplitude for chloride excretion increased during performance testing while that for uric acid excretion decreased.

Table 22 shows the physiological variables with statistically significant cosinors when referenced to midsleep instead of midnight. Since Halberg et al. (1969b) showed better synchronization between different groups of subjects when midsleep instead of local midnight was used as the phase reference, an increasing number of acrophases reported in the literature have been referenced to midsleep. The acrophases for group synchronized rhythms found in this research were very similar to those cited for oral temperature (Table 23), resting heart rate (Table 24), urine volume (Table 25), and the excretion of potassium (Table 26), sodium (Table 27), and chloride (Table 28).

No group synchronized rhythms were found for the excretion of urea nitrogen, uric acid, creatinine, and inorganic phosphate when mean cosinors were prepared using the data from all subjects. Since the subjects for the summer treadmill-psychomotor experiment had been on a standardized diet, mean cosinors (Table 29) for these four variables were prepared using the data from only subjects 10-13. Statistically significant cosinors were obtained for urea nitrogen, uric acid, and creatinine excretion but not for urinary inorganic phosphate.

Table 22. Mean relative cosinors for physiological variables of subjects 1-13 referenced to midsleep.

Variable	P	Mesor		Amplitude*		Acrophase	
		M	(95% C.I.)	A	(95% C.I.)	ϕ	(95% C.I.)
Oral Temp (°F)	<0.001	98.1	(97.8 ,98.4)	0.36	(0.21, 0.51)	-218°	(-202,-251)
Rest HR (b/m)	<0.004	69.5	(68.0 ,71.0)	6.18	(2.19,10.17)	-204°	(-167,-254)
Urine Volume (ml/hr)	<0.012	78.3	(74.9 ,81.7)	25.45	(6.07,44.84)	-193°	(-135,-251)
Urine K (mEq/hr)	<0.001	3.51	(2.96, 4.06)	39.41	(22.66,52.15)	-153°	(-123,-185)
Urine Na (mEq/hr)	<0.041	10.89	(9.89,11.88)	17.81	(0.77,34.85)	-197°	(-124,-283)
Urine Chloride (mg/hr)	<0.004	91.95	(89.0 ,94.5)	30.95	(11.28,50.61)	-161°	(-120,-212)

*Amplitude expressed as % of mesor for all variables except temperature.

Table 23. Summary of circadian rhythm of oral temperature.

Acrophase Φ (95 % C.I.)	Source
-213° (-205, -221)	Kanabrocki et al., 1973
-196° (-187, -208)	"
-265° (-249, -281)$^{\phi}$	Günther et al., 1974
-256° (-248, -265)$^{\phi}$	Halberg et al., 1974
-200° (-193, -206)	Halberg et al., 1969b
-229° (-199, -258)	"
-197° (-178, -216)	"
-200° (-179, -221)	"
-203° (-191, -209)	"
-205° (-191, -219)	"
-197° (-177, -217)	"
-204° (-184, -224)	"
-205° (-189, -221)	"
-195° (-155, -235)	"
-199° (-181, -220)	"
-221° (-199, -345)	"
-246° (-234, -258)	"
-185° (-174, -196)	"
-183° (-135, -219)	"
-225° (-207, -268)	"
-219° (-204, -236)	"
-231° (-210, -256)	"
-222° (-205, -238)	"
-194° (-171, -221)	"
-157° (-125, -202)	"
-215° (-198, -232)	"
-205° (-150, -255)	"

$^{\phi}$reference = midnight local time instead of midsleep

Table 24. Summary of circadian rhythm of resting heart rate.

Acrophase Φ (95 % C.I.)	Source
-190° (-169, -211)	Kanabrocki et al., 1973
-215° (-197, -230)	"
-282° (-272, -292)	Halberg, 1969a
-206° (-160, -236)	"
-228° (-198, -263)	"
-225° (-195, -291)	"
-196° (-169, -227)	"
-210° (-187, -273)	Halberg et al., 1969b
-219° (-203, -236)	"
-210° (-179, - 22)	Halberg et al., 1970b
-173° (-142, - 20)	"
-195° (-167, -244)	"
-242° (-220, -262)$^{\phi}$	Halberg et al., 1974

ϕ reference = local midnight instead of midsleep

Table 25. Summary of circadian rhythm in urine volume.

Acrophase ϕ (95 % C.I.)	Source
-151° (-118, -186)	Haus and Halberg, 1966
-212° (-175, -327)	Simpson et al., 1970
-177° (-152, -318)$^{\Phi}$	Kanabrocki et al., 1973
-254° (-224, -266)	Günther et al., 1974

Φ reference = midsleep instead of midnight

Table 26. Peak excretion times reported for urinary potassium.

Acrophase Φ (95 % C.I.) or Time of Peak	Source
-172° (-156, -205)	Kanabrocki et al., 1973
-204° (-163, -307)$^{\Phi}$	Simpson et al., 1970
-219° (-187, -246)$^{\Phi}$	"
-197° (-177, -219)$^{\Phi}$	"
-215° (-181, -252)$^{\Phi}$	Reinberg et al., 1967
-227° (-200, -265)$^{\Phi}$	"
-200° (-184, -220)$^{\Phi}$	Günther et al., 1974
-235° (-192, -284)$^{\Phi}$	Lakatua et al., 1974
-171° (-156, -186)	Halberg et al., 1969b
-161° (- 98, -202)	"
-157° (-130, -181)	"
-117° (- 95, -171)	"
-165° (- 94, -242)	"
-152° (-145, -160)	"
-172° (-135, -210)	"
-145° (-126, -165)	"
-124° (-104, -143)	"
-114° (- 93, -135)	"
-182°	"
-126° (-107, -145)	"
-129° (- 96, -161)	"
-159° (-146, -172)	"
-140° (-104, -158)	"
-151° (- 87, -189)	"
-149° (-125, -215)	"
-156° (-126, -189)	"
-158° (-132, -177)	"
-155° (-139, -174)	"

Table 26. Continued.

Acrophase Φ (95 % C.I.) or Time of Peak	Source
-150° (-137, -174)	Halberg et al., 1969b
-140° (-123, -167)	"
-150° (-117, -187)	"
-174° (-154, -231)	"
-123° (- 92, -143)	"
-133° (-102, -138)	"
1200 or before	Wesson, 1964
1200	Stanbury and Thomson, 1951
1000 - 1300	Martel et al., 1962
1700 - 2100	Folk, 1961
1200 - 2000	Buchsbaum and Harris, 1971
1500 - 1800	Bartter et al., 1962
1800 - 2100	"
1000 - 1300	Sharp, 1960

ϕ reference = midnight local time instead of midsleep

Table 27. Peak excretion times reported for urinary sodium.

Acrophase ϕ (95 % C.I.) or Time of Peak	Source
-182° (-158, -204)$^{\Phi}$	Kanabrocki et al., 1973
-200° (-135, -250)$^{\Phi}$	Halberg et al., 1969a
-186° (-169, -244)$^{\Phi}$	"
-151° (-119, -183)	Haus and Halberg, 1966
-228° (-146, -279)	Simpson et al., 1970
-241° (-223, -257)	Günther et al., 1974
-278° (-200, -334)	Lakatua et al., 1974
1800 - 2100	Bartter et al., 1962
2000	Buchsbaum and Harris, 1971
1200 or before	Wesson, 1964
1200	Stanbury and Thomson, 1951
1000 - 1300	Martel et al., 1962
1000 - 1300	Sharp, 1960

Φ reference = midsleep instead of midnight

Table 28. Peak excretion times reported for urinary chloride.

Acrophase ϕ (95 % C.I.) or Time of Peak	Source
-161° (-141, -186)$^{\Phi}$	Kanabrocki et al., 1973
-206° (-176, -239)	Simpson et al., 1970
-214° (-198, -232)	"
-206° (-173, -257)	"
1200 or before	Wesson, 1964
1200	Stanbury and Thomson, 1951
1000 - 1300	Sharp, 1960

Φ reference = midsleep instead of midnight

Table 29. Mean relative cosinors for urea nitrogen, uric acid, and creatinine excretion of subjects 10-13 referenced to midsleep.

Variable	P	Mesor		Amplitude*		Acrophase	
		M	(95% C.I.)	A	(95% C.I.)	ϕ	(95% C.I.)
Urea N (mg/hr)	<0.012	483.4	(478.77,488.05)	23.18	(12.01,34.35)	-201°	(-111,-254)
Uric Acid (mg/hr)	<0.049	23.64	(22.06, 25.22)	23.14	(0.30,45.99)	-199°	(-100,-272)
Creatinine (mg/hr)	<0.012	68.04	(64.43, 71.65)	19.69	(0.72,38.66)	-203°	(-113,-259)

*Amplitude expressed as % of mesor

The detection of a circadian rhythm in urea nitrogen, uric acid, and creatinine only during the work period may have been facilitated by the imposition of a standardized diet and regular meal times. After the experiment was over, the food intake sheets kept by the subject for the prework week (with no dietary restrictions) were analyzed by Dr. Margaret Kassouny of the Department of Food and Nutrition at Texas Tech University. The results of her analysis are shown in Table 30. It is evident that the subjects did not have a very adequate diet. Note that only one subject had an adequate protein intake. As described earlier, the standard diet given the subjects during the work weeks was completely balanced.

Since the excretion rates of urea nitrogen and uric acid are influenced by the intake of proteins and nucleoproteins respectively (Harrow and Mazur, 1966), the increase in mesor for these variables during the work weeks was expected. The amplitude was also increased. These factors would probably account for the detection of the rhythms during the work weeks.

Although creatinine is ultimately formed from amino acids, its excretion is independent of nitrogen intake (Harrow and Mazur, 1966). During the work weeks, the creatinine mesor was only slightly lower than the prework value (probably not a significant amount) and the amplitude was greater. The increase in amplitude and therefore the finding of a group synchronized rhythm may have been a result of exercising at regular intervals as observed by Srivastava et al. (1967) who showed that creatinine excretion increases during exercise. The

Table 30. Evaluation of caloric and nutriental intake of subjects 10-13 during the prework week with unrestricted diet.

	Subject			
	10	11	12	13
Calories	high	low	marginal	marginal
Protein	marginal	marginal	marginal	adequate
Vitamin A	marginal	low	low	marginal
Vitamin C	adequate	low	low	marginal
B-complex	marginal	marginal	marginal	marginal
Calcium	marginal	low	adequate	adequate
Iron	marginal	marginal	marginal	marginal

reduced level they found after exercise was still greater than the pre-exercise values. They also reported that excretion increased in proportion to the work load.

In addition to the diet, the activities of subjects 10-13 were also more regimented than those of subjects 1-9, e.g., meals and performance tests were always given at the same times. The subjects were housed together which would have aided social synchronization. They generally arose between 0600 and 0630 to go as a group to breakfast and then on to the laboratory. The subjects were not, however, required to retire at a certain time so they did have some control over part of their activity pattern. On nontesting days, each subject was also able to regulate the amount of his activity with the exceptions of meal times and avoidance of strenuous sports. Standardization of meals and/or activity was probably the main cause of finding a group synchronized rhythm for excretion of urea nitrogen, uric acid, and creatinine in these subjects during performance testing.

Of the peak excretion times reported in the literature for these variables (Tables 1 and 31), only creatinine excretion showed similar times. The acrophase value for creatinine excretion given by Kanabrocki et al. (1973) falls within the 95% confidence interval of subjects 10-13. The acrophases of subjects 10-13 for urea nitrogen and uric acid excretion were earlier than those cited in the literature. Perhaps another reference, such as timing of meals, would be better for describing group rhythms for these variables.

Table 31. Peak excretion times reported for urinary urea nitrogen, creatinine, and inorganic phosphate.

Variable	Acrophase ϕ (95 % C.I.) or Time of Peak	Source
Urea N	-283° (-258, -297)$^{\Phi}$	Kanabrocki et al., 1973
	1300	Gerritzen, 1940
Creatinine	-165° $^{\Phi}$	Kanabrocki et al., 1973
	1900 - 2200	Martel et al., 1962
	1700 - 2200	Addis et al., 1951
	1200 - 2000	Buchsbaum and Harris, 1971
Inorganic Phosphate	2000	Buchsbaum and Harris, 1971
	2400	Wesson, 1964

Φ reference = midsleep instead of midnight

The performance variables that had statistically significant ($P < 0.1$) group synchronized cosinors are shown in Table 32. The acrophase for the duration of the multiplication test indicates the longer times for taking the tests or the time of poorer performance. Optimum speed of performance for subjects 1-9 occurred twelve hours from that time or at -279° (about 1830). During the weeks when treadmill and psychomotor performance were measured for subjects 10-13, they were given an addition test designed by Dr. Jerry Ramsey, Department of Industrial Engineering, Texas Tech University. The percent of correct solutions showed a group synchronized cosinor. The acrophase for this variable was -46° or about 0300. This value is somewhat misleading, however, since examination of the raw data showed that although the subjects were more accurate later at night, they also were working fewer problems. In addition, some subjects were not very enthusiastic about taking this test, so there may have been an effect of boredom or fatigue that increased during the day. With no measurements taken at night, "depressed" performance would make the cosine curve indicate better performance at night.

The cosinor for average oxygen consumption per sixty minutes on the treadmill for subjects 10-13 was not significant below the 5% level when referenced to midnight. When midsleep was the reference, however, a group synchronized rhythm was found with $P < 0.013$. The acrophase for work oxygen consumption referenced to midnight was at -67° (about 0430) indicating the time when work oxygen consumption would be greater. Therefore the optimum per-

Table 32. Group synchronized cosinors found for mental and physical performance.

Variable	P	Mesor M	Mesor (95% C.I.)	Amplitude A	Amplitude (95% C.I.)	Acrophase ϕ	Acrophase (95% C.I.)
Math Duration[a] (minutes)	<0.003	4.41	(1.83, 6.99)	0.33	(0.17, 0.50)	-79°	(-1, -97)
Addition: % Right[b]	<0.005	73.22	(32.08, 114.37)	6.05	(4.72, 7.37)	-46°	(-321, -105)
Oxygen Consumption[b] (ave. ml/min from 60 min run)	<0.067	1223.5		42.32		-67°	
Oxygen Consumption[c] (ave. ml/min from 60 min run)	<0.013	1233.6	(1223.8 , 1243.4)	5.18	(2.54, 7.82)	-26°	(-345, -62)

[a]Data from subjects 1-9 only; ϕ reference = local midnight; τ = 24 hrs. = 360°.

[b]Data from subjects 10-13 only; ϕ reference = local midnight; τ = 24 hrs. = 360°.

[c]Data from subjects 10-13 only; Φ reference = midsleep instead of midnight.

formance (or maximum work for least oxygen consumption) would be in the late afternoon. The acrophase for maximum oxygen consumption is very likely related to body temperature. When the body temperature is low, some oxygen would be utilized in the production of metabolic heat to increase muscle temperature. During treadmill work the temperature of the contracting muscles of the legs increases until it is about 1°C above the core temperature. Non-contracting muscles of the arm, however, remain about 1°C below core temperature (Amussen and Boje, 1945). Increasing muscle temperature shifts the oxygen dissociation curve to the right facilitating release of oxygen to the muscle tissue (Barcroft and King, 1909). At the lungs the lower core temperature would allow the curve to shift back to the left indicating increased oxygen uptake. Of course the Bohr effect due to CO_2 would also be present.

No group synchronized cosinor for work oxygen consumption was found when the data from subjects 1-5 were pooled with that from subjects 10-13. This might have been due to differences in scheduling between the two groups, one run per day versus zero to four per day. In addition subjects 10-13 underwent a considerable phase shift from the prework to work week. As shown in Table 33 for resting heart rate and oral temperature, there was no overlapping of the confidence intervals for data obtained before and during performance testing. The prework values are comparable to those obtained for subjects 1-5 during the week of treadmill testing. This phase shift was most likely the result of the late bed time and the increase in physical

Table 33. Circadian rhythms of subjects 10-13 for temperature and resting heart rate before (B) and during (D) performance testing as determined by the single[a] cosinor procedure.

Variable		P	Mesor		Amplitude		Acrophase[b]	
			M	(95% C.I.)	A	(95% C.I.)	ϕ	(95% C.I.)
Oral Temp	B	<0.009	98.15	(98.05,98.25)	0.31	(0.15, 0.47)	-267°	(-240,-294)
(°F)	D	<0.009	97.80	(97.74,97.86)	0.22	(0.16, 0.28)	-320°	(-296,-344)
Rest HR	B	<0.009	66.57	(63,94,69.19)	8.64	(4.84,12.44)	-273°	(-249,-297)
(b/m)	D	<0.009	65.62	(64.31,66.93)	3.51	(1.90, 5.12)	-332°	(-299,-365)

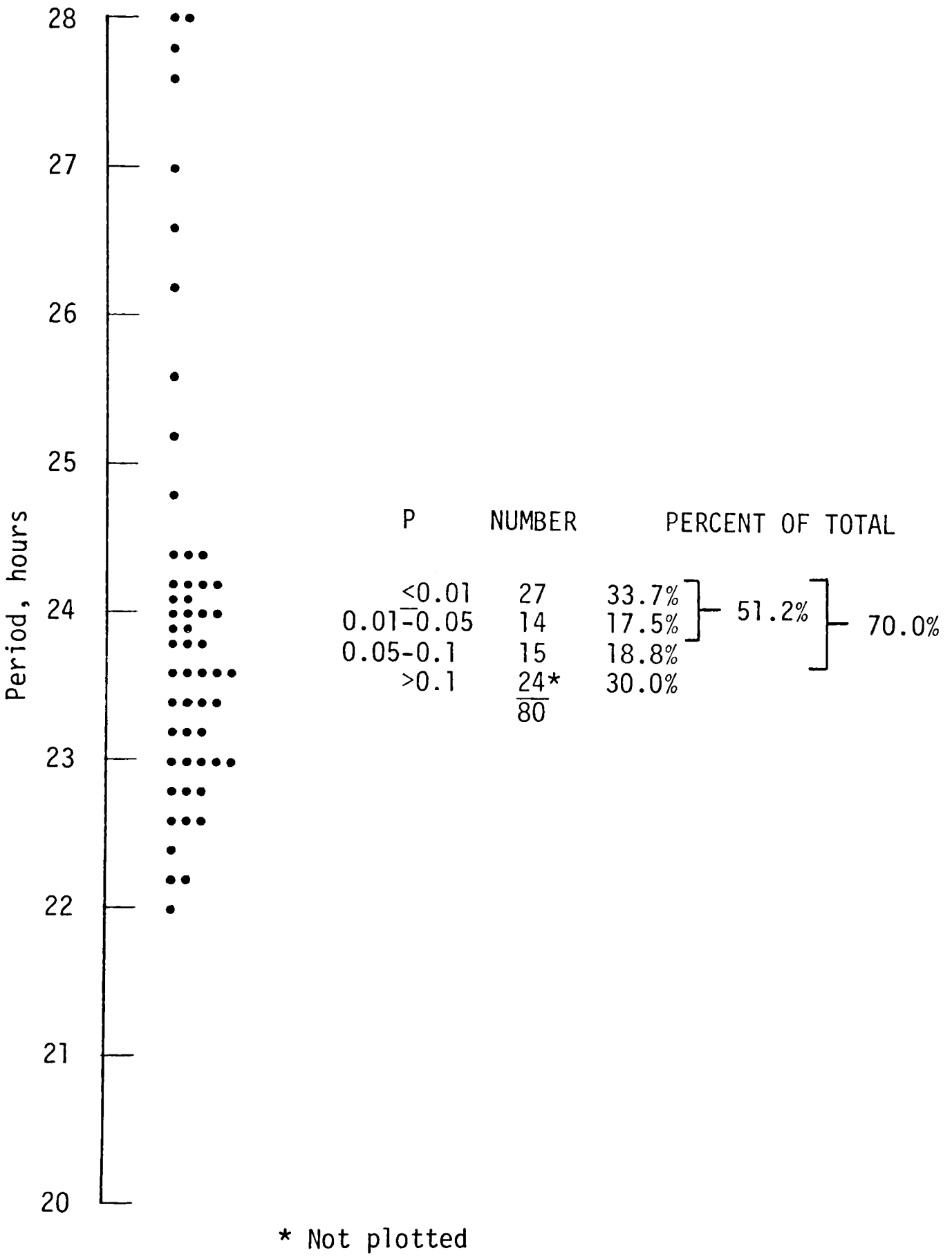
[a]Original data points from all subjects pooled for least squares analysis.

[b]τ = 24 hr. = 360°, 1 hr. = 15°, ϕ reference = local midnight

activity on a regular basis, especially in the evening. This shows how circadian rhythms may be altered by a change in the daily routine.

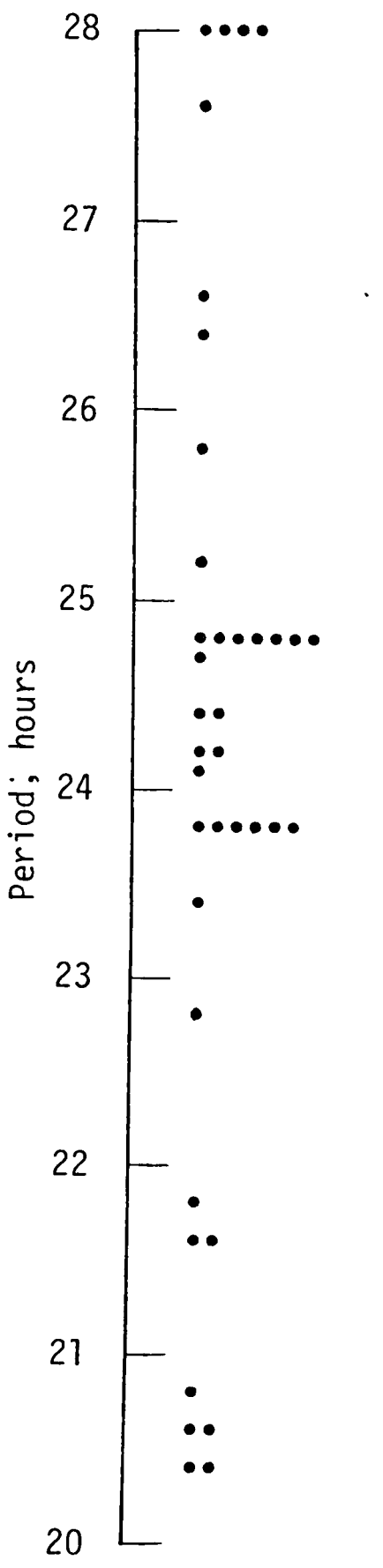
The mean cosinors for all of the above work were based on fitting the data with a cosine curve having a period of 24 hours. When the data were analyzed by the least squares program to obtain the rhythm parameters, in addition to giving the values when compared to a 24-hour period, the program also determined the period between 20 and 28 hours which best fitted the data. This may not be exactly 24 hours and, in fact, was not for most of the variables for each subject. The distribution of the periods that were significant are shown in Figures 8-10. Most fell in the range from 22.5 to 24.5 hours. It must be noted for those periods close to 24 hours that the period "best fitting" the data was not necessarily better or even significantly different from a period at 24 hours. It merely means that the residual sum of squares was smallest when the data were compared to a cosine curve having that period. The output from the cosinor program did not provide the required variances to determine if significant differences between periods exist. It is interesting to note that of the 80 variables analyzed, over 50% were significant at the 0.05 level or smaller and over 70% were significant at the 0.1 level or less. This could not be considered a "normal" distribution.

No definitive reason can be given for the differences in length of the best fitting periods among the variables for the same subject as shown in Tables 34-36. The obvious external synchronizers were the solar day of 24 hours and the social day. As can be seen from the



* Not plotted

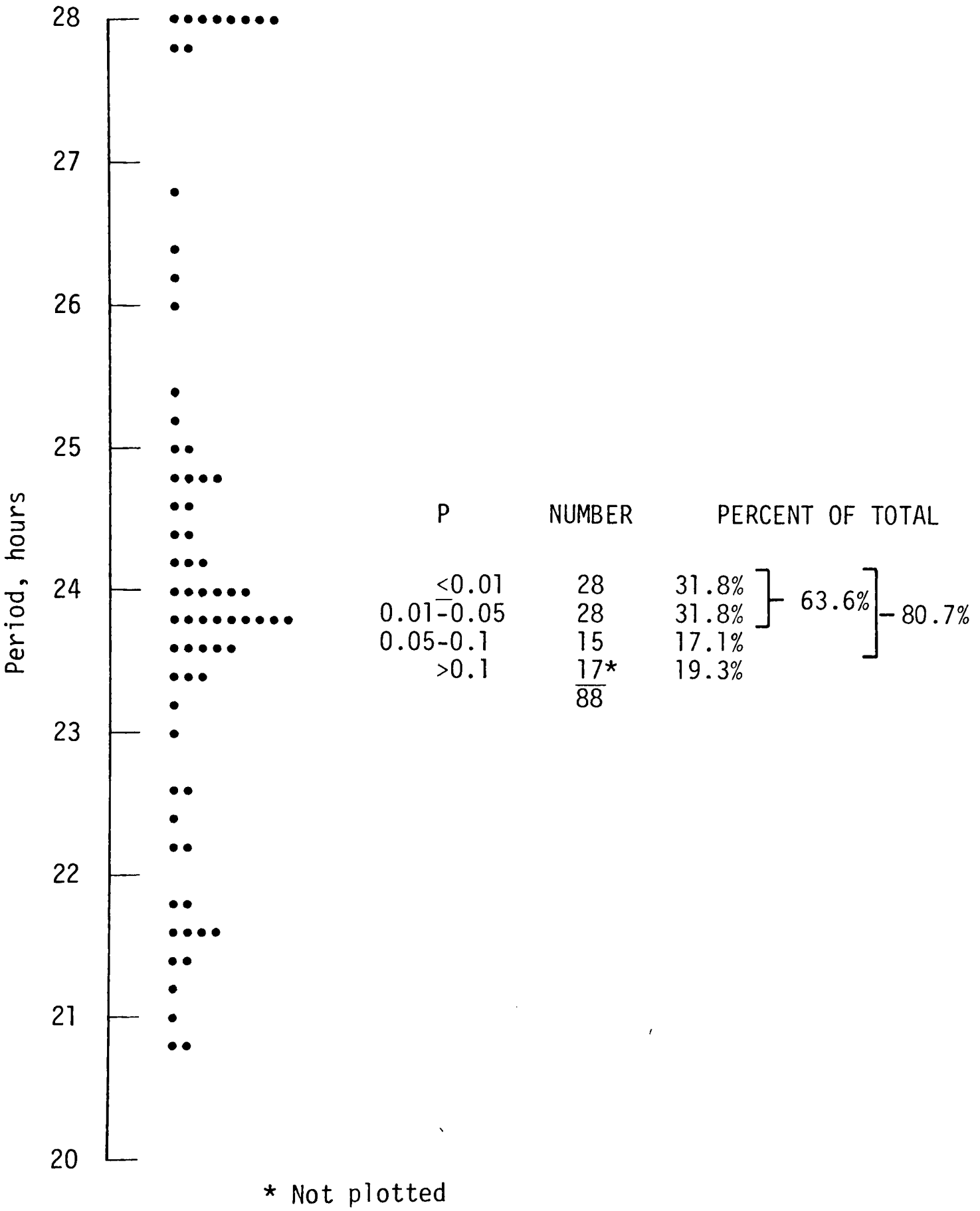
Figure 8. Distribution of significant ($P < 0.1$) best-fitting periods and levels of significance for variables of subjects 1-5.



P	NUMBER	PERCENT OF TOTAL		
≤0.01	15	28.85%	50.0%	73.1%
0.01-0.05	11	21.15%		
0.05-0.1	12	23.10%		
>0.1	14*	26.90%		
	52			

* Not plotted

Figure 9. Distribution of significant ($P < 0.1$) best-fitting periods and levels of significance for variables of subjects 6-9.



* Not plotted

Figure 10. Distribution of significant ($P < 0.1$) best-fitting periods and levels of significance for variables of subjects 10-13.

Table 34. Periods between 20-28 hours best fitting the data of subjects 1-5 for oral temperature, rest heart rate, mathematics scores, treadmill performance, and urinary variables.

Variable	Subjects 1	2	3	4	5
Oral Temp.	24.8*	24.0	23.4*	23.8*	23.6*
Rest HR	24.1*	23.9*	24.1*	24.4*	23.9*
Opt. Addn.	NS	NS	NS	26.2*	23.8*
Opt. Mult.	25.2	27.6	23.0	25.6	NS
Math. Dur.	24.0	23.8*	26.6*	28.0	22.8
HR 30M	NS	NS	23.6	24.2*	NS
OC 30M	NS	23.4	NS	23.6*	23.0
OP 30M	23.2	NS	28.0	23.2	22.8
Volume	24.0	23.4*	23.2*	NS	23.0*
Potassium	23.6*	22.6*	24.2*	NS	23.2*
Sodium	24.4*	23.0*	24.2*	28.0	NS
Chloride	24.4*	23.0*	24.2*	28.0	NS
Urea N	23.4*	22.0	NS	27.0	NS
Uric Acid	23.6*	22.2	24.0	NS	NS
Creatinine	22.6	22.6	NS	27.8	NS
In. Phos.	NS	22.8	NS	NS	NS

Opt. Addn.: Optimum addition
Opt. Mult.: Optimum multiplication
Math. Dur.: Duration of math test
In. Phos.: Inorganic phosphate
HR: Heart rate
OC: Oxygen consumption
OP: Oxygen pulse
30M: First 30 min. of work

*Significant 24-hr. period for data.

Table 35. Periods between 20-28 hours best fitting the data of subjects 6-9 for oral temperature, rest heart rate, mathematics scores, and urinary variables.

Variable	Subjects 6	7	8	9
Oral Temp.	23.8*	24.2*	22.8*	24.2*
Rest HR	24.1*	23.8*	23.8*	24.4*
Opt. Addn.	NS	NS	NS	20.6
Opt. Mult.	24.8	26.6	NS	NS
Math Dur.	NS	26.4	23.8*	NS
Volume	24.8	25.2	20.8	21.8
Potassium	24.6*	24.8*	23.8*	21.6
Sodium	28.0	24.4*	23.4*	28.0
Chloride	24.8*	24.8*	20.4*	21.6
Urea N	28.0	NS	20.4	NS
Uric Acid	28.0	NS	20.6	NS
Creatinine	NS	24.8	25.8	NS
In. Phos.	23.8*	NS	27.6	24.8*

Opt. Addn.: Optimum addition
Opt. Mult.: Optimum multiplication
Math Dur.: Duration of math test
In. Phos.: Inorganic phosphate
*Significant 24-hr. period for data.

Table 36. Periods between 20-28 hours best fitting the data of subjects 10-13 for oral temperature, rest heart rate, addition scores, treadmill performance and urinary variables.

Variable	Subject 10	11	12	13	Variable	Subject 10	11	12	13
Oral Temp.	24.8*	26.4	23.8*	24.2*	OP 30M	23.4	21.0	24.0	28.0
Rest HR	23.8*	21.8	NS	23.4*	OP 60M	23.4	20.8	24.0	28.0
Ad # WK	22.6	26.2	NS	24.8*	OP W-R	23.6*	24.8	27.8	NS
Ad # Rt	22.6	28.0	28.0	25.0	Volume	NS	21.4*	24.4	23.8*
Ad % Rt	24.0	28.0	27.8	25.2	Potassium	NS	21.8	24.0	23.6
HR 30M	23.6*	22.2	NS	NS	Sodium	NS	24.2*	24.0	23.8*
HR 60M	23.6*	22.4	26.8	NS	Chloride	NS	24.6*	24.2*	23.2*
HR W-R	23.8*	25.4	23.0	NS	Urea N	22.2	21.6	23.8	23.6*
OC 30M	NS	21.4	NS	28.0	Uric Acid	24.6	21.6	23.8*	23.8*
OC 60M	26.0	21.2	NS	28.0	Creatinine	25.0	21.6	NS	23.8*
OC W-R	NS	21.6	20.8	28.0	In. Phos.	24.8	24.4*	NS	24.0

Ad # WK: Number of addition problems worked
Ad # Rt: Number of correct addition problems
Ad % Rt: Percent of addition problems correct
HR: Heart rate
OC: Oxygen consumption
OP: Oxygen pulse
30M: First 30 min. of work
60M: 60 min. of work
W-R: Work minus rest
*Significant 24-hour period for data

indicated periods on these tables, all but one within 1/2 hour of a 24.0-hour period were also significant at 24.0 hours. Some with a best fitting period over 2 hours different from 24.0 hours were also significant at 24 hours. The number, spacing, and values of the data points allowed a closer least squares fit to a period slightly different from 24 hours. Additional data might well have shown 24 hours as the best fitting period.

Such spacing of the data may also explain the periods significant at other than 24 hours and not at 24 hours. Another explanation is that the rhythms were affected by secondary synchronizers or modulators that were not in phase with the primary synchronizer. Social interaction, meal spacing, changes in sleep habits, etc. could have produced a beat effect with the 24 hour period that appeared as a single non-24 hour period (Halberg et al., 1960). This would involve the interaction of two circadian components. Similar effects might be produced by the interaction of a circadian component with a weekly or other non-circadian one (Naylor, 1958).

The variability in the length of the best fitting periods for the variables in this study is very similar to that shown by Frazier et al. (1968) in their study of rhythmicity in vigilance performance. The spectral analysis procedure they used showed that even when a 24-hour period was the primary source of variation, lesser peaks often occurred additionally at 21-22 hours and/or at 26-28 hours. Sometimes the primary period was in one of these two regions instead of at 24 hours. Thus the possibility exists that some of the var-

iables in this study which have a best fitting period not at 24 hours but which also have a significant cosine fit at 24 hours would show similar multiple peaks with their method of spectral analysis. The finding in a separate study of similar patterns in the frequencies that were sources of variation in their work suggest that these differences in periods may not simply have been a result of experimental procedures or scheduling. Additional investigation is needed to determine if these findings are characteristic for most rhythms and if so, what is the reason for them. Since multiple frequencies could not be determined from plotting of raw data or use of analysis of covariance, re-analysis of previously collected data using spectral techniques would be very important.

Investigation of Internal Acrophases

Using midsleep as a reference instead of midnight is an example of an internal acrophase instead of a computative one. Halberg et al. (1969b) showed that the use of such a reference yielded very similar results when computing the acrophases for several human physiological variables measured by different observers on different continents at different times. They suggested the use of the body temperature rhythm as an internal time reference. The acrophases of other stable rhythms could also be used as internal references.

The use of an internal reference point rather than midnight may yield more significant group synchronized cosinors. Although the importance of the rhythm's amplitude in these computations cannot

be ignored, the imputed acrophases of the individuals must be in close proximity if the cosinor is to be statistically significant. Thus the acroangle program was written in the course of this research as a rapid screening technique to determine which variables yielded the smallest grouping of acrophases and were, therefore, potential internal references. For the variables with promising acroangles, new cosinor cards were prepared using acrophases adjusted to a new reference instead of midnight. Cosinors were then computed using these adjusted acrophases. Of the cosinors thus determined, some were statistically significant with $P < 0.1$ when referred to an internal acrophase instead of midnight. Cosinors with a P value between 0.1 and 0.05 were included to reduce the probability of not finding two rhythms that were related (Type II error). In future testing involving phase shifting to determine if the rhythms are indeed related and how this could be applied to screening procedures, it would be more important to avoid making a Type I error (rejection of the null hypothesis when it is true).

For subjects 1-5, the statistically significant cosinors referred to internal acrophases are shown grouped by variable in Table 37 and grouped by reference in Table 38. The acrophases for oral temperature and resting heart rate referred to midsleep corresponded to those cited in the literature (Tables 8 and 9). If the acrophase for math duration referred to midsleep was adjusted by 180° to indicate the time of faster multiplication, the value obtained, -201°, would be very close to the acrophase for body temperature. Kleitman (1963)

Table 37. Circadian rhythms of subjects 1-5 with internal phase references.

Variable	Φ Reference	P	Acrophase* Φ (95% C.I.)
Oral Temp.	Midsleep	<0.006	-204° (-173, -220)
Rest HR	Oral Temp.	<0.008	-359° (-319, - 50)
"	Midsleep	<0.006	-202° (-160, -250)
Opt. Addn.	OP 30M	<0.06	-188°
Math Dur.	Oral Temp.	<0.005	-171° (- 20, -182)
"	Midsleep	<0.06	- 21°
OC 30M	OP 30M	<0.06	-335°
"	HR 30M	<0.09	- 51°
OP 30M	Opt. Addn.	<0.02	-136° (-103, -263)
"	HR 30M	<0.07	-138°

Opt. Addn.: Optimum addition performance
Math Dur.: Duration of math test
HR: Heart rate
OC: Oxygen consumption
OP: Oxygen pulse
30M: First 30 min. of work

*τ = 24 hr. = 360°, 1 hr. = 15°, Φ reference = indicated parameter

Table 38. Internal phase references for circadian rhythms of subjects 1-5.

Φ Reference	Variable	p	Acrophase* Φ (95% C.I.)
Oral Temp.	Rest HR	<0.008	-359° (-319, - 50)
"	Math Dur.	<0.005	-171° (- 20, -182)
Midsleep	Oral Temp.	<0.006	-204° (-173, -220)
"	Rest HR	<0.006	-202° (-160, -250)
"	Math Dur.	<0.06	- 21°
Opt. Addn.	OP 30M	<0.02	-136° (-103, -263)
HR 30M	OP 30M	<0.07	-138°
"	OC 30M	<0.09	- 51°
OP 30M	OC 30M	<0.06	-335°
"	Opt. Addn.	<0.06	-188°

Opt. Addn.: Optimum addition performance
Math Dur.: Duration of math test
HR: Heart rate
OC: Oxygen consumption
OP: Oxygen pulse
30M: First 30 min. of work

*τ = 24 hr. = 360°, 1 hr. = 15°, Φ reference = indicated parameter

found a similar relationship for his multiplication test which was used as a basis for the test used in this research. This relationship was further shown in this experiment by the statistically significant cosinor for math duration when oral temperature was the reference. A significant cosinor referred to oral temperature was also obtained for resting heart rate. This supports the work of Alluisi et al. (1964) which showed a close similarity between the cyclic patterns in the graphs of axillary temperature and heart rate. These data were also collected as self-measurements.

The score for the additions involved in the multiplication test was adjusted by subtracting the error from a constant. This yielded an optimum addition score that was largest when performance was at its best. A cosinor for optimum addition significant at the 6% level was obtained when oxygen pulse was used as the reference. The reverse was also significant: optimum addition as the reference for the oxygen pulse cosinor. This situation (two variables serving as reference for each other) would not always occur, however, due to the differences in amplitudes.

The cosinors of subjects 6-9 that were significant when referred to an internal acrophase are shown grouped by variable in Table 39 and grouped by reference in Table 40. Midsleep was a reference for significant cosinors for resting heart rate and optimum addition only. Cosinors for optimum addition were also significant when referred to resting heart rate, math duration, and the excretion of potassium or uric acid. The optimum addition acrophase was the reference of sig-

Table 39. Circadian rhythms of subjects 6-9 with internal phase reference.

Variable	Φ Reference	P	Acrophase* Φ (95% C.I.)
Rest HR	Midsleep	<0.038	-209° (- 45, -219)
Opt. Addn.	Midsleep	<0.036	-119° (-168, -353)
"	Rest HR	<0.029	-270° (-141, -320)
"	Math Dur.	<0.039	-101° (- 1, -174)
"	Potassium	<0.005	-343° (-245, - 54)
"	Uric Acid	<0.075	-250°
Volume	Opt. Addn.	<0.044	-101° (- 40, -213)
Potassium	Math Dur.	<0.068	-117°
Uric Acid	Opt. Addn.	<0.022	- 89° (- 49, -200)
Creatinine	Rest HR	<0.092	-323°

Opt. Addn.: Optimum addition performance

Math Dur.: Duration of math test

HR: Heart rate

*τ = 24 hr. = 360°, 1 hr. - 15°, Φ reference = indicated parameters

Table 40. Internal phase references for circadian rhythms of subjects 6-9.

Φ Reference	Variable	P	Acrophase* Φ (95% C.I.)
Midsleep	Rest HR	<0.038	-209° (- 45, -219)
"	Opt. Addn.	<0.036	-119° (-168, -353)
Rest HR	Opt. Addn.	<0.029	-270° (-147, -320)
"	Creatinine	<0.092	-323°
Opt. Addn.	Volume	<0.044	-101° (- 40, -213)
"	Uric Acid	<0.022	- 89° (- 49, -200)
Math Dur.	Opt. Addn.	<0.039	-101° (- 1, -174)
"	Potassium	<0.068	-117°
Potassium	Opt. Addn.	<0.005	-342° (-245, - 54)
Uric Acid	Opt. Addn.	<0.075	-250°

Opt. Addn.: Optimum addition performance
Math Dur.: Duration of math test
HR: Heart rate

* τ = 24 hr. = 360°, 1 hr. = 15°, Φ reference = indicated parameter

nificant cosinors for urine volume and uric acid excretion. Math duration was the reference for a significant potassium excretion cosinor. Resting heart rate was the reference for a significant creatine excretion cosinor.

Statistically significant cosinors for subjects 10-13 when referred to internal acrophases are shown grouped by variable in Table 41 and grouped by reference in Table 42. Possible relationships between oral temperature, resting heart rate, and scores from the addition test were indicated also for subjects 10-13 as they were for subjects 6-9. Furthermore, the number of correct addition problems was the reference for statistically significant cosinors for the excretion of water, urea nitrogen, and creatinine.

The 60 minute average values for work heart rate could be referred to all of the urinary variables measured except inorganic phosphate to obtain a significant cosinor. Inorganic phosphate served as a reference for a significant cosinor for both work minus rest (W-R) heart rate and oxygen consumption. The values for W-R oxygen consumption could also be referenced to oral temperature, resting heart rate, and the percent of correct addition problems. Work oxygen consumption had significant cosinors when referred to resting heart rate and W-R heart rate.

The only significant cosinor obtained with oral temperature as the reference was for the work minus rest (W-R) values of oxygen consumption. There was no apparent correlation with resting heart rate or math ability as in the spring treadmill experiment. Since

Table 41. Circadian rhythms of subjects 10-13 with internal phase references.

Variable	Φ Reference	P	Acrophase* Φ (95% C.I.)
Oral Temp.	Midsleep	<0.004	-280° (-233, - 38)
	Ad % Rt.	<0.096	-283°
Ad # Rt.	Midsleep	<0.061	- 17°
	Rest HR	<0.081	- 71°
HR 30M	Chloride	<0.057	-127°
HR 60M	Rest HR	<0.09	- 10°
	Volume	<0.029	-115° (- 67, -233)
	Potassium	<0.061	-129°
	Sodium	<0.022	-104° (- 55, -216)
	Chloride	<0.026	-137° (-108, -265)
	Urea N	<0.043	-106° (- 47, -220)
	Uric Acid	<0.026	-108° (- 53, -218)
	Creatinine	<0.033	-106° (- 54, -222)
HR W-R	In. Phos.	<0.069	- 11°
OC 30M	Rest HR	<0.024	- 85° (-352, -156)
	HR W-R	<0.035	-101° (-384, -202)
	OC 60M	<0.045	-355° (-334, -118)
	OC W-R	<0.029	-331° (-271, - 68)
	OP 30M	<0.05	- 3° (-265, - 81)
	OP 60M	<0.04	-355° (-265, - 51)
	OP W-R	<0.055	-296°
OC 60M	Rest HR	<0.023	- 95° (-354, -155)
	HR W-R	<0.01	-110° (- 45, -198)
	OC 30M	<0.01	- 4° (-348, - 31)
	OC W-R	<0.004	-340° (-272, - 49)
	OP 30M	<0.008	- 6° (-315, - 72)
	OP 60M	<0.009	-356° (-307, - 62)
	OP W-R	<0.038	-305° (-229, - 26)
OC W-R	Oral Temp.	<0.017	- 23° (-309, - 96)
	Rest HR	<0.079	-115°
	Ad % Rt.	<0.011	- 43° (-315, -105)
	OC 30M	<0.014	-119° (- 33, -177)
	OC 60M	<0.012	- 20° (-309, - 90)
	In. Phos.	<0.061	-171°

Table 41. Continued.

Variable	Φ Reference	P	Acrophase* Φ (95% C.I.)
OP 30M	OC 30M	<0.085	- 2°
	OP 60M	<0.03	-353° (-186, -359)
OP 60M	OC 30M	<0.05	- 11°
	OC 60M	<0.086	- 7°
	OP 30M	<0.063	- 8°
OP W-R	HR W-R	<0.01	-174° (- 45, -195)
Volume	Ad # Rt.	<0.066	-178°
Sodium	Midsleep	<0.084	-214°
Chloride	HR 60M	<0.009	-206° (-157, -296)
Urea N	Midsleep	<0.012	-201° (-111, -254)
	Ad # Rt.	<0.005	-172° (- 66, -204)
Uric Acid	Midsleep	<0.049	-199° (-100, -272)
Creatinine	Ad # Rt.	<0.022	-173° (- 20, -220)

Ad # Rt.: Number of correct addition problems

Ad % Rt.: Percent of correct addition problems

HR: Heart rate

OC: Oxygen consumption

OP: Oxygen pulse

30M: First 30 min of work

60M: 60 min of work

W-R: Work minus rest values

*$_{\tau}$ = 24 hr. = 360°, 1 hr. = 15°, Φ reference = indicated parameter.

Table 42.- Internal phase references for circadian rhythms of subjects 10-13.

Φ Reference	Variable	P	Acrophase* Φ (95% C.I.)
Midsleep	Oral Temp.	<0.044	-280° (-233, - 38)
	Ad # Rt.	<0.061	- 17°
	Uric Acid	<0.049	-199° (-100, -272)
	Urea N	<0.012	-201° (-111, -254)
	Sodium	<0.084	-214°
Oral Temp.	OC W-R	<0.017	- 23° (-309, - 96)
Rest HR	OC 30M	<0.024	- 85° (-352, -156)
	OC 60M	<0.023	- 95° (-354, -155)
	OC W-R	<0.079	-115°
	HR 60M	<0.09	- 10°
	Ad % Rt.	<0.081	- 71°
Ad # Rt.	Urea N	<0.005	-172° (- 66, -204)
	Creatinine	<0.022	-173° (- 20, -220)
	Volume	<0.066	-178°
Ad % Rt.	Oral Temp.	<0.096	-283°
	OC W-R	<0.011	- 43° (-315, -105)
HR 60M	Chloride	<0.009	-206° (-157, -296)
HR W-R	OC 30M	<0.035	-101° (- 38, -202)
	OC 60M	<0.01	-110° (- 45, -198)
	OP W-R	<0.01	-174° (- 45, -195)
OC 30M	OC 30M	<0.01	- 4° (-348, - 31)
	OC W-R	<0.014	-119° (- 33, -177)
	OP 30M	<0.085	- 2°
	OP 60M	<0.05	- 11° (-224, - 40)
OC 60M	OC 30M	<0.045	-355° (-334, -118)
	OC W-R	<0.012	- 20° (-309, - 90)
	OP 60M	<0.086	- 7°
OC W-R	OC 30M	<0.029	-331° (-271, - 68)
	OC 60M	<0.004	-340° (-272, - 49)
OP 30M	OC 30M	<0.05	- 3° (-265, - 81)
	OC 60M	<0.008	- 6° (-315, - 72)
	OP 60M	<0.063	- 8°

Table 42. - Continued.

Φ Reference	Variable	P	Acrophase* Φ (95% C.I.)
Volume	HR 60M	<0.029	-115° (- 67, -233)
Potassium	HR 60M	<0.061	-129°
Sodium	HR 60M	<0.022	-104° (- 55, -216)
Chloride	HR 30M	<0.057	-127°
	HR 60M	<0.026	-137° (-106, -265)
Urea N	HR 60M	<0.043	-106° (- 47, -220)
Uric Acid	HR 60M	<0.026	-108° (- 53, -218)
Creatinine	HR 60M	<0.033	-106° (- 54, -222)
In. Phos.	HR W-R	<0.069	- 11°
	OC W-R	<0.061	-171°

Ad # Rt.: Number of correct addition problems

Ad % Rt.: Percent of addition problems correct

HR: Heart rate

OC: Oxygen consumption

OP: Oxygen pulse

30M: First 30 min. of work

60M: 60 min. of work

W-R: Work minus rest values

*τ = 24 hr. = 360°, 1 hr. = 15°, Φ reference = indicated parameter

measurements of temperature and resting heart rate were taken solely by the subjects, this may reflect differences in measuring abilities. All of the spring treadmill subjects were familiar with reading a thermometer and feeling for the pulse. Only two of the summer subjects (10 and 13) were so experienced. While the two inexperienced subjects readily learned how to take the measurements, their lack of practice probably made their results less precise. This is supported by the fact that they were the only two subjects (11 and 12) that did not have a statistically significant cosine curve fitted at 24 hours by the method of least squares to their resting heart rate data (Table 5). Subject 11 did not have a significant cosine fitted to his oral temperature data (Table 4).

When a measure of oxygen consumption or oxygen pulse was used as the reference, significant cosinors were found only for other measures of oxygen consumption and oxygen pulse. Since the data were fitted separately to cosine curves and the possible correlations tested independently, the consistency in the results indicates the precision in the computational procedures. This also applies to the cosinors obtained with W-R heart rate as the reference for W-R oxygen pulse.

It is very important to keep in mind that a significant cosinor using an internal parameter as the reference does not mean that a causal relationship exists between the two rhythms. The apparent connection between them may be due simply to coincidence or they may both be coupled independently to a third function. Extensive testing including phase shifting and/or administration of disruptive agents

would be required to prove or disprove such a relationship. Even if the cycles are not coupled, they can still be used as general indicators if their apparent relationship is not changed.

The present research suggests that one or more of the variables of oral temperature, resting heart rate, and the excretion of water, potassium, urea nitrogen, uric acid, and creatinine might serve as indicators of mental performance as measured by arithmetic tests. The elevation of heart rate or oxygen consumption above rest during physical activity might be monitored by the excretion of inorganic phosphate. Physical performance as indicated by work heart rates may be related to the excretion of water, potassium, sodium, chloride, urea nitrogen, uric acid, or creatinine.

This research cannot be used as conclusive proof that correlation exists between the rhythms of these variables. The differences between the acrophases of an individual's rhythms can easily be calculated. If these rhythms are "synchronized" to midnight or midsleep for most individuals, then group synchronized cosinors for one rhythm to another could be found by chance. Such an apparent "correlation" would be as apt to be coincidental as causal or coupled. In this work, however, group synchronized rhythms were found for some variables when they were referenced to the acrophase of physiological variables but not when referenced to midsleep or midnight. The question must be raised then as to why this occurred. Was it simply an artifact or was there an actual correlation? Is it possible that acrophases for some rhythms occur at different times of day for different people?

This would explain why group synchronized rhythms could be found when referenced to another rhythm but not to midnight or midsleep. Since "day" versus "night" people are recognized, this would be a likely way to look for such differences.

Patkai (1969) had found differences in the adrenaline excretion of morning workers compared to evening workers. The evening workers had higher adrenaline levels at night compared to the morning workers. The evening workers' reaction time was significantly less in the evening after a steady decline during the day and they also scored better on arithmetic tests at night. Halberg (1973) found that plasma 17-hydroxycorticosteriod (17-OHCS) levels usually dropped from 0500 to 2100 for two diurnally-active "larks". Measurements made at the same times for a nocturnally-active "owl" generally showed higher levels of 17-OHCS at 2100 compared to 0500. This is certainly an area where much more research is needed. These differences in day and night types may provide important criteria for selection of shift workers.

Comparison of Analysis of Covariance and Rhythmometry

Analysis of covariance essentially removes the effect of prior history in the experimental response of the subject at any particular point in time during the performance testing sessions. As the session progressed, the effect of the prior work could become more pronounced. Because the analysis of covariance used the elapsed time from the start of the session as the covariate, this technique for data analysis helped to emphasize the effects of any externally or internally in-

duced changes between sessions rather than within sessions. Under the same circumstances where there is a limited amount of performance data, analysis of covariance will help to determine the presence or absence of a variation in the data regardless of its form. Under those conditions, rhythmometry compares the data to a particular form, the sinusoidal curve, and the only possible outcomes are an indication of whether the variation in the data is probably sinusoidal or not. However, if the variation is indeed sinusoidal, the rhythmometry analysis provides parameters (ϕ, M, A) for its description.

There is one situation when rhythmometry could have a very definite advantage over analysis of covariance. If the amplitude of the rhythm is small, it may disappear in comparison to the error mean square in the analysis of covariance. Comparison of the data to a cosine curve by the method of least squares may detect the curve if it is reasonably sinusoidal.

In both techniques, of course, it is possible to make both Type I and Type II errors. Perhaps the simplest way to summarize the differences between the two analysis procedures is that analysis of covariance is basically a qualitative measure of variation while rhythmometry is a quantitative measure if the data varies sinusoidally.

As an example of the differences in results from the two methods of data analysis, rhythmometry revealed a statistically significant circadian varaition in resting heart rate of subjects 1-5 (Table 22 while the analysis of covariance detected none (Table 10, time of day effect). This was very likely due to differences in the number of

observations analysed. The degrees of freedom available for analysis of covariance was limited by the experimental design to those values for resting heart rate obtained immediately prior to work sessions. Rhythmometric analyses utilized those values plus all the other data taken throughout the day as self-measurements. Thus the longer observation time utilized by rhythmometry showed a circadian variation which was not detected from the limited subset of values used in analysis of covariance.

Essentially the reverse was true with regard to evaluation of physical performance by the two statistical procedures. Only one value per treadmill run was used for the least-squares analyses. Thus there were fewer observations of work heart rate and they did not cover as large a time span as the resting heart rate values. Because analysis of covariance utilized time elapsed during the run as a covariate, the average values for each five minute interval could be considered as replications. The increased sample size resulted in increased precision. Therefore, for these experiments, analysis of covariance indicated differences in physiological responses (work heart rate, oxygen consumption, and oxygen pulse) to muscular work better than rhythmometry.

Chapter V

SUMMARY AND CONCLUSIONS

Thirteen subjects took self-measurements of oral temperature and resting heart rate. They also took a multiplication or addition test throughout the day. Urine volume was measured and specimens were analyzed for potassium, sodium, chloride, urea nitrogen, uric acid, creatinine, and inorganic phosphate. Group synchronized rhythms referenced to midnight or midsleep were found for oral temperature, resting heart rate, urine volume, and the excretion of potassium, sodium, chloride, and uric acid. The duration of the multiplication test taken by subjects 1-9 and the percent of correct addition problems of subjects 10-13 also showed group synchronization. Rhythms in the excretion of urea nitrogen, uric acid, and creatinine during physical and psychomotor performance testing were found only in subjects 10-13. During that period these subjects were on a standardized diet and their activities were regulated for large portions of the day. In contrast, other than the times they had to be in the laboratory for measurement, subjects 1-9 were not regulated in any way.

Psychomotor performance was measured with a tracking task (subjects 6-9) or a target task (subjects 10-13). Equipment malfunction made analysis of the tracking data impossible. A machine resetting problem caused difficulties with analysis of the data from

the target task. Results from the analysis of covariance when the number of resets was not significant as a factor indicate that circadian variability in psychomotor performance was present. Measurement of a perceptual error (selection of the wrong "trigger") which was thought unlikely to have been affected by the resetting problem did show circadian variation with analysis of covariance. Rhythmometric analysis did not show any group synchronized rhythms at 24 hours.

A treadmill was used to simulate physical loading. Subjects worked on the treadmill at alternating rates that were either preselected speeds (subjects 1-5) or work loads based on estimated caloric expenditure (subjects 10-13). Testing of subjects 1-5 was limited to one session per day with measurements made at three different times of day. Subjects 10-13 were measured two or four times per day. Rhythmometric analyses did not find any group synchronized rhythms in work heart rate, oxygen consumption, or oxygen pulse. Analysis of covariance showed circadian variability in work heart rate, oxygen consumption, and oxygen pulse. Between-day variation was statistically significant which suggests that circaseptan rhythms were present as well as circadian rhythms.

Using a screening program written as part of this research, the acrophases for physiological variables and for physical and mental performance were examined for possible internal references. Mean cosinors were prepared for those variables with closely grouped acrophases when referred to the acrophase of another variable. Some

statistically significant mean cosinors were then found. These results mean only that for this research, the acrophases of those variables were consistently found with a particular spacing. Additional research will be needed to determine if these relationships are general characteristics and whether or not they could be applied to screening procedures. Further research is thus indicated to determine if correlations exist between the following variables:

a) addition or multiplication tests as measures of mental performance with oral temperature, resting heart rate, or urinary excretion of water, potassium, urea nitrogen, uric acid, or creatinine;

b) the elevation of heart rate and oxygen consumption above rest levels during physical loading with the urinary excretion of inorganic phosphate; and

c) the level of work heart rate with urinary excretion of water, potassium, sodium, chloride, urea nitrogen, uric acid, or creatinine.

Based on the observations made in this research the following conclusions were reached:

1. Circadian rhythms in oral temperature, resting heart rate, mental performance as measured by a multiplication test, and the urinary excretion of water, potassium, sodium, chloride, and uric acid can be found in subjects following their usual daily routine with no restrictions on meals (either content or timing) or activities including the times of getting up or going to bed. Some restriction of activity and/or of meal quality and spacing facilitates the finding of group synchronized rhythms in urea nitrogen and creatinine.

2. Circadian variation can be demonstrated in physical performance as indicated by the values for work heart rate, oxygen consumption, and oxygen pulse. Evidence of circaseptan variability in physical performance was found.

3. Performance rhythms were correlated to those of physiological variables. These relationships may be suitable for application to screening procedures for the selection of shift workers and the monitoring of performance abilities in critical situations.

4. As a result of this and other studies several implications can by made regarding the application of the knowledge of circadian rhythms to shift work:

a) Relationships between physiological and performance cycles as shown in this research may be used directly to predict performance level by monitoring the circadian state. This knowledge could be applied in two ways. First, in the selection of workers who would probably have a desired level of performance during work on a particular shift. This would basically involve taking advantage of the "owl-lark" differences in the general population. Second, continued monitoring of physiological rhythms of workers in jobs where a very high level of performance is required could be used to detect a decrement in performance due to disruption of circadian rhythms.

b) Circadian rhythms should be utilized in the selection of workers for rotating or night shifts who would be subjected to frequent disruption of their physiological cycles. Performance decrement and impairment of health seem to be related to the extent

of the desynchronization between physiological rhythms and the time required for resynchronization. Therefore potential employees should be tested to select those who would be best suited to shift work.

c) Knowledge of the cycles in performance can also be directly utilized. Production rates, for instance, could be altered to correspond to the worker's performance level. Present rates may not be high enough at times of optimum performance. Slowing the rate when performance level is lower should result in fewer mistakes. For tasks where high precision is required, the work may have to be scheduled just at the time when the performance cycle for the required skills is highest.

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APPENDIX A

Instruction Sheet Given to Subjects

TIMES TO TAKE URINE SPECIMEN, BODY TEMPERATURE, MATH TEST, AND HEART RATE

1. As soon as you wake up.

   Try to take body temperature and heart rate before getting out of bed if possible. Collect urine specimen and then take math test.

2. 9 AM — These times are approximate, but try to come
3. 11-12 AM — within 1 hour--be sure to take measurements
4. 3 PM — before eating.
5. 5-6 PM
6. 9 PM
7. right before going to sleep

If you need to urinate at other times, go ahead and collect the specimen then according to the instructions. Try to drink enough liquids so you can always fill the bottle 1/2 full but not use more than 2 bottles at a time (of course, if you need to use more to empty the bladder, go ahead and use them.)

URINE SPECIMENS

All urine passed should be collected and turned in. Use a separate specimen bottle each time urine is passed. Fill the bottle to within an inch of the top and then use additional bottles as necessary until the bladder is completely empty. Label (in pencil) each bottle with your last name, date and day, and exact time including AM or PM. It is most important that this labeling be exact. If more than one bottle was used; number them, putting the number in a circle so we'll know to look for more than one bottle. Be sure, though, that name, date and time is on EVERY bottle.

Put bottles in refrigerator or on ice immediately. Indicate on your activity sheet the time and that you took a urine specimen.

If you are away from home and cannot get the specimen cold, put 3-4 drops of the preservative in each container and label with a "P" so we'll know preservative was added. Keep the specimens cool and get them refrigerated as soon as possible.

Bring all specimens in every day--twice a day if possible.

HEART RATE

Find your pulse on your wrist or neck using your fingers NOT your thumb. Count the beats for a one minute interval and record the number in the space provided. Count the beats for 15 seconds and record this number in the proper space--DO NOT try to convert this one to beats per minute. Always measure your heart rate before you take the math test.

BODY TEMPERATURE MEASUREMENTS

You should not have had anything hot or cold to eat or drink for 15 minutes before taking temperature.

Hold thermometer in mouth with mouth closed at least 5 minutes.

Carefully read the thermometer and record the temperature and the time it was removed from your mouth on the record sheet.

Shake the thermometer until the mercury is below 96°.

MATH TESTS

Work each problem as rapidly and as accurately as possible without checking answers or correcting mistakes. Show all steps in the space provided. Please try to write neatly and clearly.

Make sure the stop watch is on zero and start it when you start taking the test. Avoid looking at the numbers on the test until you have started the watch. Do not look at the watch while working the test. As soon as you finish the test, stop the watch. Record the time in the space provided on the math test. Make sure your name, date and day, and exact time including AM or PM are on the sheet. Record on your activity sheet the time and number of the math test. Please try to take the tests in order but this is not absolutely necessary. Please do not try to remember times from previous tests and "do better". Just do each one as best you can and don't refer to it again.

Please bring the urine speciment to room 506 in the biology building and put them in the refrigerator. You will find your name on one of the shelves--please try to put all of your specimens in this area. If you need more space use one of the unmarked shelves. Thank you.

APPENDIX B

Forms Used by Subjects for

Self Measurements

_____

Name

_____

Date

Arising Time_____ Retiring Time_____

Body Temperature Measurements

Time	Temp	Previous Activity
1		
2		
3		
4		
5		
6		
7		
8		
9		
10		

Heart Rate Measurement

Time	B/15 sec	B/min	Previous Activity
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			

Arising Time_____ _____

Name

Retiring Time_____ _____

Date

Activities

Time	Activity	Comments

Name

Date

Food and Liquid Intake

Time	Item	Amount

Problem Set #1

Time _____ _____
Name

Test Duration _____ _____

Score _____ Date

Multiply the following; show work on paper.

8875 3566	2633 6082
9504 8374	2799 8268
1838 2171	1844 6602

APPENDIX C

Listing of Acroangle Computer Program

```
C
C                    BETHEA ACROANGLE PROGRAM
C
C
C  REVISED AUGUST 1974
C
C        THIS PROGRAM WAS WRITTEN BY AND IS THE SOLE
C  PROPERTY OF NANCY JO JENSEN BETHEA.  ANY UNAUTHOR-
C  IZED USE, MODIFICATION, PUBLICATION, AND/OR DISTRI-
C  BUTION IS PROHIBITED.  ALL RIGHTS ARE RESERVED BY
C  THE AUTHOR.
C        THIS PROGRAM READS THE ACROPHASES FROM COSINOR
C  CARDS AND CALCULATES THE MINIMUM ANGLE (CALLED THE
C  ACROANGLE) THAT WILL INCLUDE ALL ACROPHASES FOR A
C  PARTICULAR VARIABLE.  THE ACROANGLE IS FIRST CALCU-
C  LATED WHEN MIDNIGHT = 0 DEGREES.  THEN THE ACRO-
C  PHASES ARE ADJUSTED FOR A DIFFERENT VARIABLE = 0
C  DEGREES (E. G., TEMPERATURE) AND NEW ACROANGLES ARE
C  CALCULATED.  THIS PROCEDURE IS REPEATED FOR ALL
C  VARIABLES.  A TABLE OF THE ACROANGLES WHEN EACH
C  VARIABLE = 0 DEGREES IS PRINTED.  ACROANGLES LESS
C  THAN SPECIFIED VALUES AND LESS THAN THE ACROANGLE
C  FOR MIDNIGHT = 0 DEGREES WILL BE INDICATED BY A *
C  OR ** ON THE TABLE.
C        THE PROGRAM WILL ALSO PLOT THE ADJUSTED ACRO-
C  PHASES TWO DIFFERENT WAYS.  IPLOT1 IS A LINE PLOT OF
C  THE ADJUSTED ACROPHASES OF EACH VARIABLE WHEN A
C  SPECIFIED VARIABLE EQUALS 0 DEGREES.  THIS IS RE-
C  PEATED FOR EACH VARIABLE SET EQUAL TO 0 DEGREES.
C  THESE PLOTS CAN BE USED TO SEE WHICH REFERENCE GIVES
C  THE BEST GROUPING OF ACROPHASES FOR VARIABLES OF
C  INTEREST.  IPLOT2 IS A LINE PLOT OF THE ADJUSTED
C  ACROPHASES OF A SPECIFIC VARIABLE WHEN EACH VARIABLE
C  EQUALS 0 DEGREES (I.E., ALL THE POSSIBLE REFERENCE
C  ADJUSTMENTS OF THE ACROPHASES FOR THAT VARIABLE).
C  THIS IS REPEATED FOR EACH VARIABLE.  IT CAN BE USED
C  TO SEE WHICH REFERENCE GIVES THE CLOSEST SPACING OF
C  THE ACROPHASES OF A PARTICULAR VARIABLE.
C        NEW COSINOR CARDS WITH A NEW REFERENCE = 0
C  DEGREES CAN BE LISTED OR PUNCHED.  THESE CARDS ARE
C  PUNCHED IN THE COSINOR FORMAT WITH THE ADDITIONAL
C  INFORMATION OF THE SUBJECT'S ID, VARIABLE, AND
C  REFERENCE ADDED.  THE PROGRAM WILL ALSO SUPPLY SYN-
C  CHRONIZER, TITLE, AND BLANK CARDS IN THE APPROPRIATE
C  PLACES TO PRODUCE A DECK OF CARDS READY FOR USE WITH
C  THE COSINOR PROGRAM AT THE CHRONOBIOLOGY LABORATORY,
C  UNIVERSITY OF MINNESOTA.
C
C              CONTROL CARD ORDER AND OPTIONS
C
```

```
C          MASTER TITLE CARD --- MANDATORY
C
C  COL 1-4:TITL; COL 5-80 ANY ALPHAMERIC TITLE DESIRED
C
C          FIRST CONTROL CARD --- MANDATORY
C
C  COL2-3: NSUBJ = NUMBER OF SUBJECTS.GE.2 AND.LE.20
C  COL5-6: NVAR = NUMBER OF VARIABLES.GE.2 AND.LE.32
C                 INCLUDING MIDNIGHT AS ONE VARIABLE
C  COL 8-10: MAXCUT = MAXIMUM ACROANGLE VALUE TO BE
C              INDICATED BY *
C  COL 12-14: MINCUT = MAXIMUM ACROANGLE VALUE TO BE
C               INDICATED BY **
C
C          SECOND CONTROL CARD --- MANDATORY
C
C  COL 2: NTABLE = NUMBER OF ACROANGLE TABLES TO PRINT;
C                  BLANK = 1 TABLE
C  COL 4: INDAC = 0 FOR PRINT OF ADJUSTED ACROPHASES
C               = 1 FOR PRINT OF ADJUSTED ACROPHASES
C                 WITH IPLOT1
C               = 2 FOR PRINT OF ADJUSTED ACROPHASES
C                 WITH IPLOT2
C               = 3 FOR NO PRINT
C  COL 6: INDANG = 0 FOR PRINT OF ACROANGLES WHEN
C                  CALCULATED
C               = 1 FOR NO PRINT (NOTE-ACROANGLE TABLE
C                 IS NOT AFFECTED)
C  COL 8: IPLOT1 = 0 FOR NO PLOT
C               = 1 FOR LINE PLOT OF ADJUSTED ACRO-
C                 PHASES VS EACH VARIABLE WHEN ONE
C                 VARIABLE = 0 DEGREES
C  COL 10: IPLOT2 = 0 FOR NO PLOT
C               = 1 FOR LINE PLOT OF ADJUSTED ACRO-
C                 PHASES VS EACH VARIABLE = 0 DEGREES
C                 FOR ONE VARIABLE
C  COL 12: LSTCOS = 0 FOR LISTING OF COSINOR CARDS WITH
C                 INTERNAL REFERENCE
C               = 1 FOR NO LISTING AND NO PUNCHING
C  COL 14: MAKCOS = 0 FOR NO CARDS
C               = 1 FOR PUNCHING NEW COSINOR CARDS
C                 (NOTE-LSTCOS MUST = 0 FOR PUNCHING)
C  COL 16-19: MINCOS = MINIMUM ACROANGLE VALUE REQUIRED
C                      FOR NEW COSINOR CARDS TO BE
C                      LISTED AND/OR PUNCHED;
C                      BLANK: MINCOS = MINCUT
C  COL 20-29: BEGINNING OF DARK PERIOD
C             BLANK = -359.9 DEGREES
C  COL 30-39: END OF DARK PERIOD
C             BLANK = -0.01 DEGREES
```

```
C
C          PLOT ID CARD - OMIT IF IPLOT1 AND IPLOT2 = 0
C
C       BEGINNING IN COL 1, GIVE A ONE COLUMN ALPHAMERIC
C   ID FOR EACH SUBJECT TO BE USED ON THE LINE PLOTS
C
C          EXPERIMENT ID CARD - OMIT IF LSTCOS = 1
C
C   COL 1-32: ALPHAMERIC TITLE FOR USE IN LISTING OR
C             PUNCHING NEW COSINOR CARDS
C
C         SUBJECT ID CARD -- OMIT IF INDAC = 3
C                                 AND LSTCOS = 1
C
C      BEGINNING IN COL 1, GIVE A THREE COLUMN ALPHAMER-
C   IC SUBJECT ID FOR USE IN LISTING ADJUSTED ACROPHASES
C   AND ON COSINOR CARDS
C
C          VARIABLE ID CARD --- MANDATORY
C   COL 1-4:VAR=; COL 5: BLANK
C   COL 6-11: SIX COLUMN ALPHAMERIC VARIABLE NAME FOR
C             USE ON LISTINGS, ACROANGLE TABLE, AND NEW
C             COSINOR CARDS
C
C      DATA CARDS FOR PRECEEDING VARIABLE --- MANDATORY
C
C   COL 1-4: THREE BLANKS AND A FINAL PERIOD - USED AS
C            CHECK BY PROGRAM
C   COL 5-6: PR FROM LEAST-SQUARES OUTPUT
C   COL 7: COMMA
C   COL 8-13: MESOR
C   COL 14: COMMA
C   COL 15-20: AMPLITUDE
C   COL 21: COMMA
C   COL 22:- (DASH)
C   COL 23-25: ACROPHASE
C   COL 26-28:.0,  (PERIOD, ZERO, COMMA)
C        THE DATA CARDS USE THE SAME FORMAT AS COSINOR
C   CARDS.  ONLY THE ACROPHASE VALUES ARE USED IN THIS
C   PROGRAM.  OTHER COSINOR DATA IS STORED FOR USE IN
C   MAKING NEW COSINOR CARDS AND COULD BE OMITTED AS
C   LONG AS COL 1-4 ARE PROPERLY CODED (   .).  THERE
C   MUST BE ONE DATA CARD FOR EACH SUBJECT FOR EACH
C   VARIABLE AND CARDS MUST BE IN THE SUBJECT ORDER
C   GIVEN ON THE SUBJECT ID CARD.  IF DATA FOR SUBJECTS
C   ARE MISSING, DUPLICATE COSINOR CARD FOR FOLLOWING
C   SUBJECT AND USE FOR MISSING VALUE.  THIS WILL NOT
C   AFFECT THE ACROANGLE VALUE AND WILL GIVE THE PROPER
C   SUBJECT ID ON PLOTS.
C
```

```
C       REPEAT VARIABLE ID CARD FOLLOWED BY DATA CARDS
C   FOR THAT VARIABLE AS NEEDED
C
C           INDICATOR CARD --- MANDATORY
C
C   COL 1-4: BLANK IF ANOTHER ACROANGLE PROBLEM FOLLOWS,
C            ANY NON-ZERO CHARACTER IF THERE ARE NO MORE
C
     1 FORMAT (20A4)
     2 FORMAT (2(1X,I2),2(1X,I3))
     3 FORMAT (1X,'NSUBJ NOT SUPPLIED OR TOO SMALL')
     4 FORMAT (1X,'NSUBJ TOO LARGE MUST BE 20 OR LESS')
     5 FORMAT (1X,'NVAR NOT SUPPLIED OR TOO SMALL')
     6 FORMAT (1X,'NVAR TOO LARGE, MUST BE 32 OR LESS')
     7 FORMAT (1X,'MAXCUT NOT SUPPLIED')
     8 FORMAT (1X,'MINCUT NOT SUPPLIED')
     9 FORMAT (7(1X,I1),1X,I3,1X,2F10.2)
    10 FORMAT (1X,20A4,/)
    11 FORMAT (1X,'NSUBJ',1X,'NVAR',1X,'MAXCUT',1X,'MINC
      *UT')
    12 FORMAT(2X,I2,4X,I2,4X,I3,4X,I3)
    13 FORMAT(/,1X,'NTABLE',1X,'INCAC',2X,'INDANG',1X,
      *'IPLOT1',1X,'IPLOT2',1X,'LSTCOS',1X,'MAKCOS',1X,
      *'MINCOS',4X,'BEGDKP',6X,'ENDDKP')
    14 FORMAT (3X,7(I1,6X),I3,1X,2(F10.2,2X))
    15 FORMAT (20A1)
    16 FORMAT (8A4)
    17 FORMAT (20A3)
    18 FORMAT (A4,1X,2A3)
    19 FORMAT (5A4,2X,I3)
    20 FORMAT(A4)
    21 FORMAT(1X,'ACROPHASES FOR ',2A3,' = 0 DEGREES',
      *//,1X,'VARBLE',5X,'SUBJECT')
    22 FORMAT (7X,20(1X,A3))
    23 FORMAT (1X,2A3,30I4)
    24 FORMAT (1X,2A3,I4,1X,30I4)
    25 FORMAT (1X,11X,30(2X,A2))
    26 FORMAT (////,1X,'PLOT OF ADJUSTED ACROPHASES FOR
      *EACH VARIABLE WHEN ',2A3,' = 0 DEGREES',/)
    27 FORMAT(8X,'0',9X,'30',8X,'60',8X,'90',7X,'120',
      *7X,'150',7X,'180',7X,'210',7X,'240',7X,'300',7X,
      *'330',7X,'360')
    28 FORMAT (1X,7X,121A1)
    29 FORMAT (1X,2A3,1X,121A1)
    30 FORMAT(7X,'0000',6X,'0200',6X,'0400',6X,'0600',
      *6X,'0800',6X,'1000',6X,'1200',6X,'1400',6X,'1600'
      *,6X,'1800',6X,'2000',6X,'2200',6X,'2400')
    31 FORMAT (7X,'12AM',7X,'2AM',7X,'4AM',7X,'6AM',7X,
      *'8AM',6X,'10AM',6X,'12PM',7X,'2PM',7X,'4PM',7X,
      *'6PM',7X,'8PM',6X,'10PN',6X,'12AM')
```

```
32 FORMAT (//////)
33 FORMAT(1X,'PLOT OF ADJUSTED ACROPHASES FOR ',
  *2A3,' WHEN ALL VARIABLES = 0 DEGREES',/)
34 FORMAT (1X,'VAR =0',1X,'0',9X,'30',8X,'60',8X,
  *'90',7X,'120',7X,'150',7X,'180',7X,'210',7X,'240'
  *,7X,'270',7X,'300',7X,'330',7X,'360')
35 FORMAT (///)
36 FORMAT (1H1)
37 FORMAT (/)
38 FORMAT (1X,2A3,1X,31I4)
39 FORMAT (8X,31(2X,A2))
40 FORMAT (1X,'LISTING OF NEW COSINOR CARDS BASED ON
  * ADJUSTED ACROPHASES',//)
41 FORMAT(1X,2F10.2,T78,'1')
42 FORMAT (1X,8A4,'/',2A3,' = 0, A= ',2A3)
43 FORMAT(1X,5A4,',-',I3,'.0,',5X,2A3,'= 0, ',2A3,'
  *FOR SUBJECT ',A3)
44 FORMAT (2F10.2,T78,'1')
45 FORMAT (8A4,'/',2A3,' = 0, A= ',2A3)
46 FORMAT (5A4,',-',I3,'.0,',5X,2A3,'= 0, ',2A3,' FO
  *R SUBJECT ',A3)
47 FORMAT (A4)
48 FORMAT (///,1X,'ACROANGLES FOR MIDNIGHT = 0 DEGRE
  *ES AND FOR ',2A3,' = 0 DEGREES',/)
49 FORMAT(1X,'PLOTID = ',20A1)
50 FORMAT(1X,'IDXPER = ',8A4)
51 FORMAT(1X,'IDSUBJ = ',20A3)
52 FORMAT (1X,'ACROPHASES FOR ',2A3,' WHEN ALL VARIA
  *BLES = 0 DEGREES',//,1X,'VAR= 0',5X,'SUBJECT')
53 FORMAT(76X,3(I1,1X),5(I6))
54 FORMAT(1X,'IDVAR IS NOT BEING READ--CHECK CONTROL
  * CARDS OR DATA CARDS AROUND VARIABLE NUMBER ',I1)
55 FORMAT(1X,'MISPUNCHED OR INSUFFICIENT SUBJECT DAT
  *A CARDS FOR VARIABLE ',2A3)
56 FORMAT(1X,'VALUE FOR NVAR TOO SMALL')
57 FORMAT(1X,'TOO MANY DATA CARDS--CHECK NUMBER OF C
  *ARDS AND NSUBJ')
58 FORMAT(8X,31('|',A3),'|')
59 FORMAT(//,1X,'CALCULATIONS ARE COMPLETED FOR PROB
  *LEM: ',19A4)
60 FORMAT (1X,'THE NUMBER OF COSINOR CARDS IS ',I5)
61 FORMAT(1X,'REF.=0 |',4X,'VARIABLE ACROANGLES')
62 FORMAT(//,1X,'********** PROGRAM PROCEEDING TO NE
  *XT PROBLEM **********',/,1X,'MASTER TITLE CARD LA
  *CKED TITL IN COL 1-4 OR CONTROL CARDS WERE OUT OF
  * ORDER. CARD READ AS TITLE CARD WAS: ',/,1X,20A4)
   INTEGER X, Y, D, DB, ANGLE, TMPANG
   INTEGER STAR, DSTAR
   INTEGER TTLCHK, ANGLIN
   DATA TTLCHK/'TITL'/, CHKVAR/'VAR='/
```

```
      DATA CHKDAT/'    .'/, INDCHK/'    '/, BLANK/' '/
      DATA DOT/'.'/, BAR/'¬'/, STAR/' *'/, DSTAR/'**'/
      DIMENSION MASTTL(19), PLOTID(20), COSVAL(32,20,5)
      DIMENSION ANGLIN(32,32), IDXPER(8), IDSUBJ(20)
      DIMENSION PLOT(32,121), UL(121), LINE(32,15)
      DIMENSION IDVAR(32,2), X(32,32), Y(32,32), D(20)
      DIMENSION ANGLE(32,32), TMPANG(32,15)
      NOTITL=0
   90 READ (5,1,END=1200) INDTTL,(MASTTL(I),I=1,19)
      NOTITL=NOTITL+1
      IF(INDTTL.NE.TTLCHK) GO TO 90
      NOTITL=0
      READ (5,2) NSUBJ, NVAR, MAXCUT, MINCUT
      WRITE(6,36)
      WRITE(6,10)(MASTTL(I),I=1,19)
      WRITE(6,37)
      WRITE (6,11)
      WRITE (6,12) NSUBJ, NVAR, MAXCUT, MINCUT
      IF(NSUBJ.LE.1) WRITE (6,3)
      IF(NSUBJ.GT.20) WRITE (6,4)
      IF(NVAR.LE.1) WRITE (6,5)
      IF(NVAR.GT.32) WRITE (6,6)
      IF(MAXCUT.EQ.0) WRITE (6,7)
      IF(MINCUT.EQ.0) WRITE (6,8)
      IF(NSUBJ.EQ.0.OR.NSUBJ.GT.20) GO  TO 90
      IF(NVAR.EQ.0.OR.NVAR.GT.32) GO TO 90
      IF(MINCUT.EQ.0.OR.MAXCUT.EQ.0) GO TO 90
      READ(5,9)NTABLE,INDAC,INDANG,IPLOT1,IPLOT2,LSTCOS
     *,MAKCOS,MINCOS,BEGDKP,ENDDKP
      IF(NTABLE.EQ.0) NTABLE = 1
      IF(MINCOS.EQ.0) MINCOS = MINCUT
      IF(BEGDKP.EQ.0) BEGDKP = -359.9
      IF(ENDDKP.EQ.0) ENDDKP = -0.01
      WRITE (6,13)
      WRITE(6,14)NTABLE,INDAC,INDANG,IPLOT1,IPLOT2,
     *LSTCOS,MAKCOS,MINCOS,BEGDKP,ENDDKP
      WRITE (6,37)
      IF(IPLOT1.EQ.0.AND.IPLOT2.EQ.0) GO TO 100
      READ (5,15)(PLOTID(J),J=1,NSUBJ)
      WRITE(6,49)(PLOTID(J),J=1,NSUBJ)
  100 CONTINUE
      IF(LSTCOS.EQ.1) GO TO 125
      READ (5,16)(IDXPER(I),I=1,8)
      WRITE(6,50)(IDXPER(I),I=1,8)
  125 CONTINUE
      IF(INDAC.EQ.3.AND.LSTCOS.EQ.1) GO TO 150
      READ(5,17)(IDSUBJ(I),I=1,NSUBJ)
      WRITE(6,51)(IDSUBJ(I),I=1,NSUBJ)
  150 CONTINUE
      DATA IDVAR(1,1), IDVAR(1,2)/'MID','NIT'/
```

```
      DO 175 J=1,NSUBJ
  175 X(1,J) = 0
      DO 200 I = 2,NVAR
      READ(5,18) CHK,(IDVAR(I,J),J=1,2)
      IF(CHK.NE.CHKVAR) WRITE(6,54) I
      IF(CHK.NE.CHKVAR) GO TO 90
      DO 200 J = 1, NSUBJ
      READ (5,19) (COSVAL(I,J,M),M=1,5), X(I,J)
      IF(COSVAL(I,J,1).NE.CHKDAT) WRITE(6,55)(IDVAR(I,L
     *),L=1,2)
      IF(COSVAL(I,J,1).NE.CHKDAT) GO TO 90
  200 CONTINUE
      READ (5,20) IND
      IF(IND.EQ.CHKVAR) WRITE (6,56)
      IF(IND.EQ.CHKDAT) WRITE (6,57)
      IF(IND.EQ.CHKVAR.OR.IND.EQ.CHKDAT) GO TO 90
      DO 250 I=1,121
      UL(I) = BAR
  250 CONTINUE
      NSUBM1 = NSUBJ - 1
      IF(NSUBJ.EQ.4) NLOOPS = 2
      IF(NSUBJ.LE.3) NLOOPS = 1
      IF(NSUBJ.GT.4) NLOOPS = ((NSUBJ-3)/2) + 1
      DO 900 M = 1,NVAR
CCCCCCCCC  ACROPHASE VALUES ADJUSTED FOR VARIABLE
CCCCCCCCC  M = 0 DEGREES
      DO 300 J = 1,NSUBJ
      DO 300 I = 1,NVAR
      Y(I,J) = X(I,J) - X(M,J)
      IF(Y(I,J).LT.0) Y(I,J) = Y(I,J) + 360
  300 CONTINUE
CCCCCCCCC  PRINTING OF ADJUSTED ACROPHASES FOR VARIABLE
CCCCCCCCC  M = 0 DEGREES
      IF(INDAC.GT.1) GO TO 401
      WRITE (6,35)
      WRITE (6,21)(IDVAR(M,J),J=1,2)
      WRITE (6,22)(IDSUBJ(I),I=1,NSUBJ)
      WRITE(6,37)
      DO 400 I = 1,NVAR
      WRITE(6,23) (IDVAR(I,J),J=1,2),(Y(I,J),J=1,NSUBJ)
  400 CONTINUE
  401 CONTINUE
CCCCCCCCC  CALCULATION OF ACROANGLE FOR EACH VARIABLE
CCCCCCCCC  WHEN M = 0 DEGREES
      DO 850 I =1,NVAR
      IF(I.EQ.1.AND.INDANG.EQ.0)WRITE(6,48)(IDVAR(M,J),
     *J=1,2)
CCCCCCCCC  SET UP FIELD FOR PLOTS OF ADJUSTED
CCCCCCCCC  ACROPHASES BEFORE REARRANGING
      IF(IPLOT1.EQ.0) GO TO 501
```

```
      DO 450 J = 1,121
  450 PLOT(I,J) = BLANK
      DO 475 J = 1,121,10
  475 PLOT(I,J) = DOT
      DO 500 J = 1,NSUBJ
      KJ = 1.5 + Y(I,J)/3
      PLOT(I,KJ) = PLOTID(J)
  500 CONTINUE
  501 CONTINUE
CCCCCCCCC  ARRANGE ADJUSTED ACROPHASES IN ORDER FROM
CCCCCCCCC  LARGEST TO SMALLEST
      DO 600 J = 1, NSUBM1
      JB = J
      JP = J+1
      DO 550 L = JP,NSUBJ
      IF(Y(I,L).GT.Y(I,JB)) JB= L
  550 CONTINUE
      IF(JB.EQ.J) GO TO 600
      ITEMP = Y(I,JB)
      Y(I,JB) = Y(I,J)
      Y(I,J) = ITEMP
  600 CONTINUE
CCCCCCCCC  FIND THE NUMBER OF DEGREES BETWEEN
CCCCCCCCC  EACH ACROPHASE
      DO 625 L = 2,NSUBJ
  625 D(L) = Y(I,L-1) - Y(I,L)
      D(1) = 360 - Y(I,1) + Y(I,NSUBJ)
CCCCCCCCC  FIND THE LARGEST NUMBER OF DEGREES BETWEEN
CCCCCCCCC  TWO ACROPHASES
      NBIG = 1
      DO 650 L = 2, NSUBJ
      IF(D(L).GT.D(NBIG)) NBIG = L
  650 CONTINUE
      TMPANG (I,1) = 360 - D(NBIG)
      ANGLE(I,M) = TMPANG(I,1)
      IF(NLOOPS.EQ.1) GO TO 701
CCCCCCCCC  FIND ACROANGLE FOR ALL ACROPHASES, THEN
CCCCCCCCC  ANGLES FOR NLOOPS TIMES OF DROPPING THE
CCCCCCCCC  TWO OUTERMOST ACROPHASES AND RECALCULATING
CCCCCCCCC  THE MINIMUM ACROANGLE AGAIN--ONLY FIRST
CCCCCCCCC  ACROANGLE CALCULATED IS GIVEN IN TABLE,
CCCCCCCCC  SMALLER ONES ARE LISTED JUST WHEN CALCULATED
      NP1 = NBIG
      NM1 = NBIG
      NSUB = 0
      DO 700 N = 2, NLOOPS
      NP1 = NP1 + 1
      NM1 = NM1 - 1
      IF(NP1.GT.NSUBJ) NP1 = NP1 - NSUBJ
      IF(NM1.EQ.0) NM1 = NSUBJ
```

```
      NSUB = NSUB + D(NP1) + D(NM1)
      TMPANG(I,N) = 360 - D(NBIG) - NSUB
  700 CONTINUE
  701 CONTINUE
CCCCCCCCC  SET UP LINE FIELD FOR * OR ** INDICATORS
      DO 800 N = 1,NLOOPS
      LINE(I,N) = BLANK
      IF(TMPANG(I,N).EQ.0) GO TO 800
      IF(TMPANG(I,N).LE.ANGLE(I,1).AND.TMPANG(I,N).LT.
     *MAXCUT) LINE(I,N) = STAR
      IF(TMPANG(I,N).LE.ANGLE(I,1).AND.TMPANG(I,N).LT.
     *MINCUT) LINE(I,N) = DSTAR
  800 CONTINUE
      ANGLIN(I,M) = LINE(I,1)
      IF(INDANG.EQ.1) GO TO 850
      IF(I.EQ.1) GO TO 850
      WRITE(6,24)(IDVAR(I,J),J=1,2),ANGLE(I,1),(TMPANG(
     *I,J),J=1,NLOOPS)
      WRITE (6,25) (LINE(I,J),J=1,NLOOPS)
  850 CONTINUE
CCCCCCCCC  PLOTTING OF IPLOT1
      IF(IPLOT1.EQ.0) GOTO 900
      WRITE (6,26) (IDVAR(M,J),J=1,2)
      WRITE(6,27)
      DO 875 I = 1,NVAR
      WRITE(6,28) ( UL(J),J= 1,121)
  875 WRITE(6,29)(IDVAR(I,J),J=1,2),(PLOT(I,J),J=1,121)
      WRITE(6,28) ( UL(J),J= 1,121)
      WRITE(6,30)
      WRITE(6,31)
  900 CONTINUE
CCCCCCCCC  PLOTTING OF IPLOT2
      IF(IPLOT2.EQ.0) GO TO 916
      DO 915 M = 1, NVAR
      DO 907 I = 1, NVAR
      DO 901 J = 1, NSUBJ
      Y(I,J) = X(M,J) - X(I,J)
      IF(Y(I,J).LT.0) Y(I,J) = Y(I,J) + 360
  901 CONTINUE
      DO 905 J = 1,121
  905 PLOT(I,J) = BLANK
      DO 906 J = 1,120,10
  906 PLOT(I,J) = DOT
      DO 907 J = 1,NSUBJ
      KJ = 1.5 + Y(I,J)/3
      PLOT(I,KJ) = PLOTID(J)
  907 CONTINUE
      IF(INDAC.EQ.1.OR.INDAC.EQ.3) GO TO 909
      WRITE (6,35)
      WRITE (6,52)(IDVAR(M,J),J=1,2)
```

```
      WRITE (6,22)(IDSUBJ(I),I=1,NSUBJ)
      WRITE(6,37)
      DO 908 I = 1,NVAR
  908 WRITE(6,23) (IDVAR(I,J),J=1,2),(Y(I,J),J=1,NSUBJ)
  909 CONTINUE
      WRITE(6,32)
      WRITE(6,33) (IDVAR(M,J),J=1,2)
      WRITE(6,34)
      DO 910 I = 1,NVAR
      WRITE (6,28) (UL(J),J=1,121)
  910 WRITE(6,29)(IDVAR(I,J),J=1,2),(PLOT(I,J),J=1,121)
      WRITE(6,28) (UL(J),J=1,121)
      WRITE(6,30)
      WRITE (6,31)
      WRITE (6,35)
  915 CONTINUE
  916 CONTINUE
CCCCCCCCC  WRITE ACROANGLE TABLE
      DO 921 K = 1,NTABLE
      WRITE (6,36)
      WRITE(6,10) (MASTTL(I),I=1,19)
      WRITE (6,37)
      WRITE (6,61)
      WRITE (6,58) (IDVAR(J,1),J=2,NVAR)
      WRITE (6,58) (IDVAR(J,2),J=2,NVAR)
      DO 920 M = 1,NVAR
      WRITE(6,38) (IDVAR(M,J),J=1,2),(ANGLE(I,M),I=2,NV
     *AR)
      WRITE(6,39) (ANGLIN(I,M),I=2,NVAR)
  920 CONTINUE
      WRITE (6,58) (IDVAR(J,1),J=2,NVAR)
      WRITE (6,58) (IDVAR(J,2),J=2,NVAR)
  921 CONTINUE
CCCCCCCCC  PRINT AND PUNCH NEW COSINOR CARDS
      IF(LSTCOS.NE.0) GO TO 1100
      ICARDS = 0
      WRITE(6,35)
      WRITE(6,40)
      DO 1000 M = 2,NVAR
      DO 1000 I = 1, NVAR
CCCCCCCCC  CHECK TO SEE IF ACROANGLE FOR VARIABLE M = 0
CCCCCCCCC  IS LESS THAN FOR MIDNIGHT = 0 AND LESS THAN
CCCCCCCCC  MINCOS; IF SO, CALCULATE ADJUSTED ACROPHASES
CCCCCCCCC  FOR NEW COSINOR CARDS
      IF(ANGLE(I,M).EQ.0) GO TO 1000
      IF(ANGLE(I,M).GT.ANGLE(I,1)) GO TO 1000
      IF(ANGLE(I,M).GT.MINCOS) GO TO 1000
      DO 925 J = 1,NSUBJ
      Y(I,J) = X(I,J) - X(M,J)
      IF(Y(I,J).LT.0) Y(I,J) = Y(I,J) + 360
```

```
  925 CONTINUE
CCCCCCCCC  WRITE SYNCHRONIZER CARD
      WRITE (6,41) BEGDKP, ENDDKP
CCCCCCCCC  WRITE TITLE CARD
      WRITE (6,42) (IDXPER(J),J=1,8),(IDVAR(M,J),J=1,2)
     *,(IDVAR(I,J),J=1,2)
CCCCCCCCC  WRITE DATA CARDS
      DO 950 J = 1,NSUBJ
      WRITE(6,43)(COSVAL(I,J,L),L=1,5),Y(I,J),(IDVAR(M,
     *L),L=1,2),(IDVAR(I,L),L=1,2),IDSUBJ(J)
  950 CONTINUE
      WRITE (6,37)
CCCCCCCCC  PUNCHING OF NEW COSINOR CARDS
      IF(MAKCOS.EQ.0) GO TO 1000
      WRITE(7,44) BEGDKP, ENDDKP
      WRITE(7,45)(IDXPER(J),J=1,8),(IDVAR(M,J),J=1,2),(
     *IDVAR(I,J),J=1,2)
      DO 975 J=1,NSUBJ
      WRITE (7,46)(COSVAL(I,J,L),L=1,5),Y(I,J),(IDVAR(M
     *,L),L=1,2),IDSUBJ(J)
  975 CONTINUE
      WRITE (7,47) BLANK
 1000 CONTINUE
 1100 CONTINUE
      WRITE (6,59) (MASTTL(L),L=1,19)
      IF(IND.EQ.INDCHK) GO TO 90
 1200 CALL EXIT
      END
```

APPENDIX D

Nutritional Schedule

Table 43. Meal schedule and menu items

Time	Meal	Menu Items
7:30 a.m.	Breakfast	Egg, scrambled Toast, white, enriched Orange juice Sweet roll Milk, whole
11:45 a.m.	Lunch	Bread Mayonnaise Ham Cheese Lettuce Tomato Coca cola Grapes
4:00 p.m.	Dinner	Ground round Bun French fries Lettuce Carrots Tomato Ice Milk Canned peach, drained
8:15 p.m.	PM Snack	Formula with whole milk, orange juice, and Carnation Instant Breakfast (vanilla) Peanut cheese crackers
12:00 midnight	Midnight Snack	Peanut bar (Subjects 10, 11 and 12) Whole milk and Peanut cheese crackers (Subject 13)

Table 44. Continued.

12			13		
Protein	Fat	Carbohydrate	Protein	Fat	Carbohydrate
32.0	35.0	93.0	29.2	31.9	86.0
29.0	34.3	98.1	27.7	31.3	84.9
34.5	31.2	98.1	33.8	29.2	79.3
32.5	31.1	100.4	28.8	32.4	84.2
10.2	18.7	27.4	10.6	13.2	25.8
138.2	150.3	417.0	130.1	138.0	360.2
15.5	37.2	47.0	16.0	39.0	45.0
	3574			3203	
	78			69	
	46			46	

APPENDIX E

Tables for Analyses of Covariance of Summer Treadmill Experiment

Table 45. Effects of shift, day of week, and time of day for shifts 1, 2, and 3 on heart rate of subjects 10-13 shown by the significance level from the analyses of covariance of the rest, work (WK), and work minus rest (W-R) values from their treadmill runs.

Source of Variation	df	Prework Rest	Combined Light and Moderate Work*		Moderate Work Segment		Light Work Segment	
			WK	W-R	WK	W-R	WK	W-R
Blocks	3	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005
Shift (S)	2	NS	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005
Day (D)	2	NS	<0.025	NS	NS	NS	<0.025	NS
Time (T)	1	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.005	<0.0005
S x D	4	NS	<0.005	<0.0005	<0.1	<0.01	<0.1	<0.05
S x T	2	NS	<0.0005	<0.0005	<0.0005	<0.0005	<0.025	<0.001
T x D	2	NS	<0.05	<0.0005	<0.1	<0.0005	NS	<0.01
S x T x D	4	NS	NS	<0.0005	NS	<0.0005	NS	<0.001
Covariate	1	--	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005
Error df	-	51	824		410		410	

*Work level was a factor and was always significant. Interactions with it were never significant.

Table 46. Effects of shift, day of week, and time of day for shifts 1, 2, and 3 on oxygen consumption of subjects 10-13 shown by the significance level from the analyses of covariance of the rest, work (WK), and work minus rest (W-R) values from their treadmill runs.

Source of Variation	df	Prework Rest	Combined Light and Moderate Work*		Moderate Work Segment		Light Work Segment	
			WK	W-R	WK	W-R	WK	W-R
Blocks	3	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.01
Shift (S)	2	<0.09	<0.0005	NS	<0.1	NS	<0.0005	<0.05
Days (D)	2	<0.003	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005
Time (T)	1	NS	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005
S x D	4	NS	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005
S x T	2	NS	<0.005	<0.1	<0.05	NS	<0.025	NS
T x D	2	NS	<0.05	<0.1	<0.1	<0.1	NS	NS
S x D x T	4	NS	<0.001	<0.1	<0.1	NS	<0.005	<0.025
D x Level	2	--	NS	<0.1	--	--	--	--
Covariate	1	--	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005
Error df	-	51	824		410		410	

*Work level was a factor and was always significant. Only the indicated interaction was significant.

Table 47. Effects of shift, day of week, and time of day for shifts 1, 2, and 3 on oxygen pulse of subjects 10-13 shown by the significance level from the analyses of covariance of the rest, work (WK), and work minus rest (W-R) values from their treadmill runs.

Source of Variation	df	Prework Rest	Combined Light and Moderate Work*		Moderate Work Segment		Light Work Segment	
			WK	W-R	WK	W-R	WK	W-R
Blocks	3	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005
Shift (S)	2	NS	<0.0005	<0.0005	<0.01	<0.0005	<0.0005	<0.0005
Day (D)	2	<0.01	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.025
Time (T)	1	NS	<0.0005	<0.0005	<0.025	<0.0005	<0.0005	<0.0005
S x D	4	NS	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.025
S x T	2	NS	<0.0005	<0.0005	<0.005	<0.0005	<0.001	<0.0005
T x D	2	NS	<0.05	NS	NS	NS	NS	NS
S x D x T	4	NS	<0.0005	<0.0005	<0.01	<0.025	<0.001	<0.01
D x Level	2	--	NS	<0.1	--	--	--	--
Covariate	1	--	<0.0005	<0.0005	<0.0005	<0.0005	NS	<0.025
Error df	-	51	824		410		410	

*Work level was a factor and was always significant. Only the indicated interaction was significant.

Table 48. Effects of week, day of week, and time of day for shifts 1 and 2 on heart rate of subjects 10-13 shown by the significance level from the analyses of covariance of the rest, work (WK), and work minus rest (W-R) values from their treadmill runs.

Source of Variation	df	Prework Rest	Combined Light and Moderate Work*		Moderate Work Segment		Light Work Segment	
			WK	W-R	WK	W-R	WK	W-R
Blocks	3	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005
Week (W)	1	NS	<0.005	<0.0005	<0.01	<0.0005	<0.1	<0.0005
Day (D)	2	NS	<0.005	NS	NS	NS	<0.0005	NS
Time (T)	1	<0.0005	<0.025	<0.0005	<0.05	<0.0005	NS	<0.0005
W x D	2	NS	<0.025	<0.0005	<0.05	<0.005	NS	<0.025
W x T	1	NS	<0.005	<0.0005	<0.01	<0.0005	<0.05	<0.005
D x T	2	NS	<NS	<0.0005	NS	<0.005	NS	<0.025
W x D x T	2	<0.08	NS	<0.0005	NS	<0.0005	NS	<0.0005
Covariate	1	--	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005
Error df	-	33	548		272		272	

*Work level was a factor and was always significant. Interactions with it were never significant.

Table 49. Effects of week, day of week, and time of day for shifts 1 and 2 on oxygen consumption of subjects 10-13 shown by the significance level from the analyses of covariance of the rest, work (WK), and work minus rest (W-R) values from their treadmill runs.

Source of Variation	df	Prework Rest	Combined Light and Moderate Work*		Moderate Work Segment		Light Work Segment	
			WK	W-R	WK	W-R	WK	W-R
Blocks	3	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.01
Week (W)	1	<0.04	<0.0005	NS	<0.1	NS	<0.0005	NS
Day (D)	2	<0.06	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005
Time (T)	1	NS	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005
W x D	2	NS	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.001
W x T	1	NS	NS	NS	NS	NS	NS	NS
D x T	2	NS	NS	NS	NS	NS	NS	NS
W x D x T	2	NS	<0.0005	<0.01	<0.005	NS	<0.0005	<0.0005
Covariate	-1	--	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005
Error df	-	33	548		272		272	

*Work level was a factor and was always significant. Interactions with it were never significant.

Table 50. Effects of week, day of week, and time of day for shifts 1 and 2 on oxygen pulse of subjects 10-13 shown by the significance level from the analyses of covariance of the rest, work (WK), and work minus rest (W-R) values from their treadmill runs.

Source of Variation	df	Prework Rest	Combined Light and Moderate Work*		Moderate Work Segment		Light Work Segment	
			WK	W-R	WK	W-R	WK	W-R
Blocks	3	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005
Week (W)	1	<0.07	<0.0005	NS	<0.005	NS	<0.0005	NS
Day (D)	2	NS	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005
Time (T)	1	NS	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005
W x D	2	NS	<0.001	<0.01	<0.01	<0.005	<0.05	NS
W x T	1	NS	NS	<0.0005	NS	<0.0005	NS	<0.0005
D x T	2	NS	NS	NS	NS	NS	NS	NS
W x D x T	2	NS	<0.0005	<0.005	<0.005	<0.025	<0.0005	<0.01
Covariate	1	--	<0.0005	<0.0005	<0.0005	<0.0005	NS	<0.1
Error df	-	33	548		272		272	

*Work level was always a significant factor. Interactions with it were never significant.

Table 51. Effects of day of week and time of day for shifts 2 and 3 on heart rate of subjects 10-13 shown by the significance level from the analyses of covariance of the rest, work (WK), and work minus rest (W-R) values from their treadmill runs.

Source of Variation	df	Prework Rest	Combined Light and Moderate Work*		Moderate Work Segment		Light Work Segment	
			WK	W-R	WK	W-R	WK	W-R
Blocks	3	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005
Day (D)	2	NS	NS	<0.025	NS	NS	NS	<0.05
Time (T)	3	<0.001	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005
D x T	6	NS	<0.1	<0.0005	NS	<0.01	<0.1	<0.001
Covariate	1	--	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005
Error df	-	33	548		272		272	

*Work level was always a significant factor. Interactions with it were never significant.

Table 52. Effects of day of week and time of day for shifts 2 and 3 on oxygen consumption of subjects 10-13 shown by the significance level from the analyses of covariance of the rest, work (WK), and work minus rest (W-R) values from their treadmill runs.

Source of Variation	df	Prework Rest	Combined Light and Moderate Work*		Moderate Work Segment		Light Work Segment	
			WK	W-R	WK	W-R	WK	W-R
Blocks	3	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.025
Day (D)	2	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005
Time (T)	2	NS	<0.0005	<0.0005	<0.025	<0.005	<0.0005	<0.0005
D x T	6	NS	<0.0005	<0.0005	<0.025	NS	<0.025	NS
Covariate	1	--	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005
Error df	-	33	548		272		272	

*Work level was a factor and was always significant. Interactions with it were never significant.

Table 53. Effects of day of week and time of day for shifts 2 and 3 on oxygen pulse of subjects 10-13 shown by the significance level from the analyses of covariance of the rest, work (WK), and work minus rest (W-R) values from their treadmill runs.

Source of Variation	df	Prework Rest	Combined Light and Moderate Work*		Moderate Work Segment		Light Work Segment	
			WK	W-R	WK	W-R	WK	W-R
Blocks	3	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005
Day (D)	2	<0.001	<0.0005	NS	<0.0005	NS	<0.0005	NS
Time (T)	3	NS	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005
D x T	6	NS	<0.0005	<0.05	<0.005	NS	<0.001	<0.1
Covariate	1	--	<0.0005	<0.0005	<0.005	<0.0005	<0.1	<0.01
Error df	-	33	548		272		272	

*Work level was a factor and was always significant. Interactions with it were never significant.

Table 54. Effects of day of week and time of day for shift 1 on heart rate of subjects 10-13 shown by the significance level from the analyses of covariance of the rest, work (WK), and work minus rest (W-R) values from their treadmill runs.

Source of Variation	df	Prework Rest	Combined Light and Moderate Work*		Moderate Work Segment		Light Work Segment	
			WK	W-R	WK	W-R	WK	W-R
Blocks	3	<0.005	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005
Day (D)	2	NS	<0.0005	<0.01	<0.01	<0.025	<0.005	<0.1
Time (T)	1	<0.006	<0.0005	<0.05	<0.005	NS	<0.05	<0.025
D x T	2	<0.01	NS	<0.0005	<0.1	<0.0005	NS	<0.005
Covariate	1	--	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005
Error df	-	15	272		134		134	

*Work level was a factor and way always significant. Interactions with it were never significant.

Table 55. Effects of day of week and time of day for shift 1 on oxygen consumption of subjects 10-13 shown by the significance level from the analyses of covariance for the rest, work (WK), and work minus rest (W-R) values from their treadmill runs.

Source of Variation	df	Prework Rest	Combined Light and Moderate Work*		Moderate Work Segment		Light Work Segment	
			WK	W-R	WK	W-R	WK	W-R
Blocks	3	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.1
Day (D)	2	<0.1	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005
Time (T)	1	<0.07	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005
D x T	2	NS	<0.005	<0.005	NS	NS	<0.005	<0.0005
D x Level	2	--	<0.1	<0.025	--	--	--	--
Covariate	1	--	<0.0005	<0.0005	<0.0005	<0. 0005	<0.0005	<0.0005
Error df	-	15	272		134		134	

*Work level was a factor and was always significant. Only the indicated interactions were significant.

Table 56. Effects of day of week and time of day for shift 1 on oxygen pulse of subjects 10-13 shown by the significance levels from the analyses of covariance of the rest, work (WK), and work minus rest (W-R) values from their treadmill runs.

Source of Variation	df	Prework Rest	Combined Light and Moderate Work*		Moderate Work Segment		Light Work Segment	
			WK	W-R	WK	W-R	WK	W-R
Blocks	3	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005
Day (D)	2	NS	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005
Time (T)	1	NS	<0.0005	<0.025	<0.01	NS	<0.0005	<0.01
D x T	2	NS	<0.005	<0.005	<0.05	<0.025	<0.025	<0.01
D x Level	2	--	<0.1	<0.05	--	--	--	--
Covariate	1	--	NS	<0.1	<0.1	<0.025	NS	NS
Error df	-	15	272		134		134	

*Work level was a factor and was always significant. Only the indicated interactions were significant.

Table 57. Effects of day of week and time of day for shift 2 on heart rate of subjects 10-13 shown by the significance level from the analyses of covariance of the rest, work (WK), and work minus rest (W-R) values from their treadmill runs.

Source of Variation	df	Prework Rest	Combined Light and Moderate Work*		Moderate Work Segment		Light Work Segment	
			WK	W-R	WK	W-R	WK	W-R
Blocks	3	<0.005	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005
Day (D)	2	NS	NS	<0.0005	NS	<0.025	NS	<0.005
Time (T)	1	<0.01	NS	<0.0005	NS	<0.0005	NS	<0.0005
D x T	2	NS	NS	<0.0005	NS	<0.0005	NS	<0.0005
Covariate	1	--	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005
Error df	-	15	272		134		134	

*Work level was a factor and was always significant. Interactions with it were never significant.

Table 58. Effects of day of week and time of day for shift 2 on oxygen consumption of subjects 10-13 shown by the significance levels from the analyses of covariance of the rest, work (WK), and work minus rest (W-R) values from their treadmill runs.

Source of Variation	df	Prework Rest	Combined Light and Moderate Work*		Moderate Work Segment		Light Work Segment	
			WK	W-R	WK	W-R	WK	W-R
Blocks	3	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.05
Day (D)	2	NS	<0.0005	<0.001	<0.0005	<0.05	<0.0005	<0.001
Time (T)	1	NS	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005
D x T	2	NS	<0.0005	NS	<0.01	NS	<0.005	NS
Covariate	1	--	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005
Error df	-	15	272		134		134	

*Work level was a factor and was always significant. Interactions with it were never significant.

Table 59. Effects of day of week and time of day for shift 2 on oxygen pulse of subjects 10-13 shown by the significance level from the analyses of covariance of the rest, work (WK), and work minus rest (W-R) values from their treadmill runs.

Source of Variation	df	Prework Rest	Combined Light and Moderate Work*		Moderate Work Segment		Light Work Segment	
			WK	W-R	WK	W-R	WK	W-R
Blocks	3	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005
Day (D)	2	NS	<0.0005	NS	<0.001	NS	<0.001	NS
Time (T)	1	NS	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005
D x T	2	NS	<0.0005	NS	<0.01	NS	<0.005	NS
Covariate	1	--	<0.001	<0.0005	<0.005	<0.0005	NS	<0.1
Error df	-	15	272		134		134	

*Work level was a factor and was always significant. Interactions with it were never significant.

Table 60. Effects of day of week and time of day for shift 3 on heart rate of subjects 10-13 shown by the significance level from the analyses of covariance of the rest, work (WK), and work minus rest (W-R) values from their treadmill runs.

Source of Variation	df	Prework Rest	Combined Light and Moderate Work*		Moderate Work Segment		Light Work Segment	
			WK	W-R	WK	W-R	WK	W-R
Blocks	3	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005
Day (D)	2	NS	NS	NS	NS	NS	NS	NS
Time (T)	1	<0.0005	<0.0005	<0.0005	<0.0005	<0.005	<0.0005	<0.0005
D x T	2	NS	<0.005	NS	NS	NS	<0.005	NS
Covariate	1	--	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005
Error df	-	15	272		134		134	

*Work level was a factor and was always significant. Interactions with it were never significant.

Table 61. Effects of day of week and time of day for shift 3 on oxygen consumption of subjects 10-13 shown by the significance level from the analyses of covariance of the rest, work (WK), and work minus rest (W-R) values from their treadmill runs.

Source of Variation	df	Prework Rest	Combined Light and Moderate Work*		Moderate Work Segment		Light Work Segment	
			WK	W-R	WK	W-R	WK	W-R
Blocks	3	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.01
Day (D)	2	<0.03	<0.0005	<0.0005	<0.0005	<0.001	<0.0005	<0.01
Time (T)	1	NS	NS	<0.05	NS	NS	NS	<0.025
D x T	2	NS	NS	NS	NS	NS	NS	NS
Covariate	1	--	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005
Error df	-	15	272		134		134	

*Work level was a factor and was always significant. Interactions with it were never significant.

Table 62. Effects of day of week and time of day for shift 3 on oxygen pulse of subjects 10-13 shown by the significance level from the analyses of covariance of the rest, work (WK), and work minus rest (W-R) values from their treadmill runs.

Source of Variation	df	Prework Rest	Combined Light and Moderate Work*		Moderate Work Segment		Light Work Segment	
			WK	W-R	WK	W-R	WK	W-R
	3	<0.001	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005
Day (D)	2	<0.05	<0.0005	NS	<0.0005	NS	<0.0005	NS
Time (T)	1	NS	<0.1	<0.0005	NS	<0.01	NS	<0.0005
D x T	2	NS	<0.025	NS	NS	NS	<0.0005	<0.1
D x T x L	2	--	NS	<0.1	--	--	--	--
Covariate	1	--	<0.1	<0.005	NS	<0.005	NS	<0.05
Error df	-	15	272		134		134	

*Work level (L) was a factor and was always significant. Only the indicated interaction was significant.