Title

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Publication Date

1981

Peer reviewed

EXPERIMENTAL 12-HOUR SHIFT OF THE SLEEP-WAKE CYCLE IN MAN: EFFECTS ON SLEEP AND PHYSIOLOGIC RHYTHMS

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The 24-hour rhythms of normal man govern the alternation of sleep and wakefulness, body temperature, meals, urinary excretion, and most metabolic functions. These rhythms arise from the interactions of multiple endogenous circadian oscillators in association with environmental synchronizers such as light and dark, sleep and social schedules, meal timing, exercise programs, etc. (Wever, 1975).

Experimental shifts of sleep-wake rhythms are of interest for two major reasons. First, they provide a controlled model for medical events which occur in shift workers, air travelers, and others who undergo sleep-wake rhythm schedule changes in society. Second, the dynamic responses of physiologic rhythms to experimental shifts provide data regarding mechanisms of control of these ryhthms, especially their interrelationships within the human.

Previous studies of rhythm phase shifts have demonstrated important effects. When the sleep period is shifted by 8-12 hours, a characteristic sleep disturbance results, characterized by awakening toward the end of the sleep period (i.e., after 5 to 7 hours), a shortened time latency to the first rapid eye movement (REM) sleep period, and increased fragmentation of sleep stage patterns (Bryden & Holdstock, 1973; Chernik & Mendels, 1974; Evans, Christie, Lewis, Daly, & Moore-Robinson, 1972; Globus, Phoebus, & Boyd, 1972; Knauth & Rutenfranz, 1972; Weitzman, Kripke, Goldmacher, MacGregor, & Nogeire, 1970). Sleep-wake cycle shifts of even 2 to 4 hours produce measurable impairment of mood performance (Klein, Bruner, Holtmann, Rehme, Stolze, Steinhoff, & Wegmann, 1970; Klein, Wegmann, Athanassenas, Hohlweck, & Kuklinski, 1976; Klein, Wegmann, & Hunt, 1972; Taub & Berger, 1974; Taub & Berger, 1976). Although a shift of the clocktime in bed produces a rapid shift in the overall sleep-wake rhythm,other body rhythms require several days to weeks to complete the phase change produced by a shift in the timing of sleep. The urine volume and sodium excretion rhythms generally shift their phases within a few days, whereas the body temperature rhythm requires one to 2 weeks and urinary potassium and steroid rhythms require several weeks before achieving a complete shift (Sharp, 1960; Sharp, 1960; Sharp, Slorach, & Vipond, 1961). During the process of phase shifting, certain body rhythms appear to "free run"; the shift is not always achieved by moving in the same direction as the sleep period shift, i.e. by an equivalent advance or delay (Aschoff, Hoffmann, Pohl, & Wever, 1975; Mills, 1976; Mills, Minors, & Waterhouse, 1978; Minors & Waterhouse, 1976).

Most previous studies were done over short time periods, and therefore questions regarding the duration of phase-shift adjustment could not be an-

swered. We have performed two long-term controlled phase shift experiments. The first, a 3-week study, consisted of one week of baseline, followed by two weeks of observation after an acute 12-hour sleep period shift. The results of the sleep recordings of this study have been reported (Weitzman, 1975; Weitzman, Goldmacher, Kripke, MacGregor, Kream, & Hellman, 1968; Weitzman, Kripke, Goldmacher, MacGregor, & Nogeire, 1970). Since phase-shift effects were clearly not complete after the two weeks studied, and because the results suggested that not all functions had achieved a 12-hour phase shift, a subsequent 9-week experiment was performed consisting of three weeks of BASELINE, three weeks after an acute 12-hour sleep period inversion and an additional three weeks after a 12-hour reinversion of sleep to the baseline clock time. We present here the sleep results of this 9-week study as well as the metabolic data from both the 3-week and 9-week studies.

Method

Subjects. The subjects were 10 healthy young men, ages 22 to 28. Each volunteer signed a written informed consent for the experiment and was paid for his participation, Only one subject was studied at a time.

Three-week protocol: Five subjects lived for 3 weeks on a clinical metabolic research unit (Clinical Research Center), and were only rarely allowed to leave on a pass during their waking hours. For the first 7 nights, BASELINE, each subject was confined to bed in a totally darkened and sound-isolated room from 10 pm until 6 am. On the 8th night, the subject was kept awake until 10 am the next day, and for the next 2 weeks, the subject was allowed to sleep in total darkness from 10 am until 6 pm. This was called REVERSAL. Subjects were observed by research nurses and staff, and sleep was not allowed during the 16 hours when the subject were not in bed. During the daytime, subjects were exposed to both natural and artificial light, and at night, when awake, subjects were exposed to bright artificial illumination from both fluorescent and incandescent lamps. During the first week, meals were served at 8 am, Noon, and 6 pm. During the next two weeks, REVERSAL, meals were served exactly 12 hours later. Meals were prepared in a special metabolic kitchen to insure that the subjects were provided a diet which was stable in calories, fluid, sodium, and potassium from day to day, however, subjects were not required to consume the full diet. They were not allowed to eat any other foods. Activities and visitors were ad libitum. The subjects spent most of their time reading, watching television, or talking with staff and other subjects and patients.

Nine-week protocol: Five different subjects lived on a similar but different metabolic research unit (Clinical Research Center) for 9 weeks. For the first 21 nights of BASELINE, the subjects remained in bed in the dark and were allowed to sleep from 11 pm until 7 am. On the 22nd night, each subject was kept awake and was then allowed to sleep in the dark from 11 am until 7 pm the following day and for the subsequent 20 days. This is called the REVERSAL segment. On the 43rd day, each subject was kept awake until 11 pm and was then allowed to sleep until 7 am for that night and for an additional 20 nights. This is called BACKREVERSAL. Meals were carefully regulated as in the 3-week protocol, and activities, television viewing and visitors were allowed ad libitum.

Data Collection

Polygraphic recordings: A half hour before each bedtime, electrodes were applied to the scalp, lateral to each eye, and under the chin to record an electroencephalogram (EEG),horizontal electro-oculogram (EOG),and a chin electromyogram (EMG). The subjects were put in bed and the lights were turned out precisely at the planned bedtimes, and lights were turned on, and subjects were aroused exactly 8 hours later. The EEG, EOG, and EMG were continuously recorded throughout each lights out period and were scored by standard criteria as established by Rechtschaffen and Kales (1968). The duration of any wake or sleep stage "episode" was determined from the number of sequential polygraphic pages (20 seconds) of that stage.

Body temperature: Rectal body temperatures (9 subjects) and oral temperature (1 subject), were obtained just at the time of arising and exactly every 4 hours thereafter until bedtime, i.e., 5 times daily. Subjects were not disturbed during the lights out period for temperature or urine sampling.

Urine: Urine was collected immediately at the time of arising and exactly every 4 hours thereafter until bedtime, i.e., 5 times daily. If the subject awoke and urinated during the lights out period, that specimen was also measured. Volumes were measured and aliquots were frozen for chemical determinations of sodium, potassium, creatinine, and 17-hydroxycorticosteroids (17-OHCS). The rate of excretion per hour of water, sodium, potassoim, creatinine, and 17-OHCS were subsequently computed. These were temporally assigned to the midpoint-times between the times of voidings.

Plasma samples: In the 3-week study an indwelling venous catheter was inserted just prior to every other sleep period and blood samples (4 cc) were obtained every 20 min (approximately 25 samples/sleep period) for cortisol and growth hormone. A blood sample was also obtained by direct venipuncture every 4 hours for the next 16 hours following each catheter study to obtain a 24-hour sample. Cortisol was measured with a competitive protein binding method and growth hormone was measured by radio-immunoassay. Plasma steroid results will not by presented in detail because they were more fragmentary than 17-OHCS results although consistent.

Data analysis: The daily and weekly mean values were computed for the descriptors of sleep stage patterns as well as for the 6 metabolic variables (temperature, urine volume, sodium excretion, potassium excretion, creatinine excretion, and 17-OHCS excretion). These values were paired and were contrasted by a t-test for paired samples (N = 5 subjects for each study). Since occasional "significant" differences may occur randomly when many such comparisons are performed, only those differences which were consistently significant will be reported.

The 24-hour rhythm data of the metabolic variables were statistically evaluated. For each 72 hour of sequential data, a best-fitting 24-hour cosine was estimated utilizing a least-squares technique (Halberg, Johnson, Nelson, Runge, & Sothern, 1972). The confidence of a 24-hour cosine component being present was determined using an F-test. If the confidence was 95% or better, it was inferred that there existed for that variable a 24-hour rhythm which was reasonably approximated by a sinusoidal curve. The results of the F-test

therefore served as an approximation of the reliability of the least-squares estimate. The phase of the fitted cosine, defined by the peak value (acrophase), was taken as an estimate of the phase or timing of the 24-hour rhythm of the variable. Acrophases were expressed in negative degrees to indicate a delay of elapsed time after midnight, e.g., - 15 degrees indicated a fitted cosine peak at 0100, - 90 degrees indicated 0600, - 180 degrees indicated 1200, - 270 degrees indicated 1800, etc. "Cosinor" values (Halberg, Yong, & Johnson, 1967) were computed to determine the significance and consistency of the 24-hour rhythms among the subjects for each defined 72-hour interval. In general, when the cosinor was significant ($p < .05$), it was inferred that there was a significant 24 hour rhythm for the subject group. If the cosinor was not significant, then it was assumed that either a 24 hour rhythm was not present, was not sinusoidal, or was not consistent from subject to subject to achieve significance for the small subject group. This cosinor technique is quite sensitive to changes in the phase and amplitude of a 24-hour rhythm but is relatively insensitive to changes in wave-form or mean values.

A second statistical technique was also used for the 9-week data. The mean 24-hour curve for each variable was computed from the final week of BASELINE, i.e., Week 3. Then, day by day, the squared deviations (variance) of the subsequent daily curves from this mean curve were computed, inverting the curve 180 degrees for the REVERSAL data. At some time during the 3 weeks of REVERSAL, the mean curve was recognized to be inverted, e.g., phase-shifted 12 hours (180 degrees) if the squared deviations returned to BASELINE level. Thus, the variances measured from these mean curves provided a measure of the day-by-day deviations of the 24-hour rhythm from the best BASELINE pattern. This measure was therefore applicable regardless of curve-shape and did not require conformation to a sinusoidal curve. The squared deviations are sensitive to changes in the mean values of the variable as well as to the 24-hour rhythm phase and amplitude. During REVERSAL and BACKREVERSAL, this squared deviation method also indicated the extent to which each variable had fully reversed its pattern.

Results

Sleep stage analysis: During the 9-week BASELINE, the minutes of each of sleep stage from week to week were quite stable (Figure 1). An increase in Stage 2 from Week 1 to Week 2 was the only significant change ($p < .02$). During REVERSAL, there was a significant decrease in Stage REM (e.g., Week 3 vs. Week 4, $p < .02$). Although there was some degree of recovery, Stage REM remained below baseline for Weeks 5 and 6. There was a clear rebound during BACKREVERSAL (Week 7 vs. 1,4,5,6,8, and 9, $p < .05$). Stage 2 was also decreased during REVERSAL, but the differences were only borderline significant. Wakefulness was clearly increased during each of the 3 weeks of REVERSAL ($p < .05$). The lowest amount of wakefulness was observed for Week 7, but was not significantly less than for Weeks 1-3 or 8-9. The number of minutes of Stages 1,3, and 4 were remarkably constant during the entire study.

Sleep-stage changes and durations: In general, the numbers of changes of sleep stages were fewer, and sleep stages lasted longer at the end of BASELINE, i.e., Week 3 (Figure 2 & 3). However, there was a major increase in changes of stage in Weeks 4 and 5, compared to Week 3, as well as a marked increase for Weeks 7 and 8, compared to Week 6. These changes in fragmentation of sleep

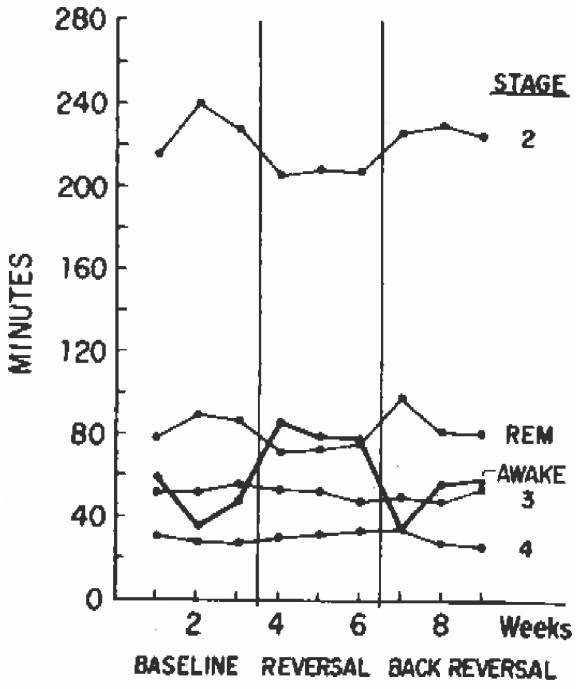

Figure 1. The minutes of each sleep stage (except Stage 1) is shown for each week of the 9-week study.

stages were especially clear for Stage 2 and REM but much less so for Stages 3 and 4 (Figure 2). However, the differences achieved scattered significance for every stage. The decreases in the durations of Stage 2 and REM episodes corresponded to the increased numbers of stage episodes.

Hourly distribution of sleep stages: During BASELINE, the subjects averaged about 30 minutes to fall asleep after lights out but then remained asleep throughout the remainder of the 8 hours in bed (Figure 4). During REVERSAL,the sleep latency was reduced, especially for Week 4, but awakenings were much more prominent during lights-out hours 5 to 8. At that time, early awakenings repeatedly occurred and interrupted the sleep periods. These early awakenings influenced all stages but especially Stage REM (Figure 5). In the first 2 weeks after REVERSAL, Stage REM was clearly markedly increased during the 2nd and 3rd hours and decreased by one-third during the last hour of the lights-out period. During the 3rd week of REVERSAL,this changed pattern had partially recovered. The response to sleep inversion was partially recapitulated during BACKREVERSAL for the first week (Week 7), but the BASELINE pattern unequivocally was re-established during Weeks 8 and 9. Thus there was a clear relationship between early "morning" awakenings and a reduction in REM sleep during the later 3rd of the night in the first 2 weeks of REVERSAL. Therefore, sleep inversion produced a phase shift of REM to an earlier part of the sleep period and a concomitant shift of waking to a later part.

Body temperature: The 24-hour body temperature curves demonstrated great stability and consistency from day to day and from subject to subject throughout the BASELINE periods (Table 1). The individual least-squares cosine fits and the group cosinor analyses were highly significant throughout the 1-week and 3-week BASELINE. A complex pattern of change occurred after acute inversion of the sleep-wake rhythm. The monophasic curve shape of BASELINE was converted into a biphasic curve with two peaks (Figure 6). A fall in temperature occurred 8 hours after awakening and temperature then rose again at 12 and 16 hours after awakening. This was clearly the case for the first 2 weeks of REVERSAL. During the third week of REVERSAL, a monophasic temperature curve was re-established with peak values occurring during the wake time. However, statistical analysis demonstrated that exact 180 degree inversion of the temperature rhythm was not fully established even after 21 days of an inverted sleep-wake cycle. During REVERSAL, the cosinor significant (Table 1) for the temperature rhythms was less consistent. In addition, applying the squared deviation method (see Methods) the reversed rhythm fit imperfectly the baseline curve shape. Considering the cosine fitting results, for the 3-week subjects, the reversal appeared to occur by a cosine phase delay which only achieved about 130 degrees after 2 weeks (Figure 7). For the 9-week subjects, the inversion of the temperature curve better resembled a cosine phase advance, but this advance only achieved 164 degrees after 21 days, (i.e.,a shift from 5 pm to 6 am). It should be emphasized that the temperature rhythm shift did not occur like the progressively moving hands of the clock, forward or backward. Rather the shift occurred by progressive elevation of a new peak and fall of the old temperature peak. This is readily seen in Figure 6. The decrease in amplitude (range) of the circadian body temperature rhythm, the fall of mean waking temprature (.15 degrees F), and the decreased significance of cosinor fits during REVERSAL reflect this pattern of change.

In contrast, re-establishment of the BASELINE temperature curve shape occurred very rapidly during BACKREVERSAL. Both the cosinor method and the

TABLE 1
NINE WEEK COSINOR RESULTS

	DAYS	TEMPERATURE Φ	TEMPERATURE C (°F)	TEMPERATURE P	URINE VOLUME Φ	URINE VOLUME C (CC/HR)	URINE VOLUME P	URINE NAT Φ	URINE NAT C (MEQ/HR)	URINE NAT P	URINE KT Φ	URINE KT C (MEQ/HR)	URINE KT P	URINE CREATININE Φ	URINE CREATININE C (MG/HR)	URINE CREATININE P	URINE 17-OHCS Φ	URINE 17-OHCS C (MG/HR)	URINE 17-OHCS P
BASELINE	1-3	-255°	.56	.01	-273°	18	.03	-275°	3.1	.06	-246°	1.3	.03	-296°	9	NS	-218°	.06	NS
	4-6	-257°	.72	.01	-266°	16	NS	-275°	1.9	NS	-229°	1.4	.004	-275°	7	NS	-202°	.07	NS
	7-9	-265°	.70	.005	-244°	13	.04	-255°	2.0	NS	-192°	1.2	.04	-220°	0	NS	-192°	.06	.10
	10-12	-263°	.76	.005	-258°	14	NS	-250°	2.3	NS	-193°	1.1	.08	-224°	6	NS	-193°	.09	NS
	13-15	-249°	.77	.005	-252°	18	.03	-259°	2.7	.08	-216°	1.0	.03	-245°	2	NS	-216°	.06	.07
	16-18	-250°	.85	.003	-263°	17	NS	-248°	2.4	NS	-206°	1.2	.07	-301°	4	NS	-183°	.08	NS
	19-21	-257°	.82	.006	-237°	23	NS	-247°	2.3	.02	-199°	1.8	.02	-277°	7	NS	-207°	.11	.03
REVERSAL	22-24	-242°	.11	NS	-154°	19	.02	-162°	.9	NS	-171°	.52	NS	-349°	2	NS	-176°	.04	NS
	25-27	-105°	.12	NS	-141°	6	NS	-198°	1.2	NS	-163°	.9	NS	-196°	10	NS	-205°	.04	.08
	28-30	- 83°	.29	NS	- 74°	14	NS	- 82°	.8	NS	- 98°	.55	.03	- 26°	4	NS	- 42°	.04	NS
	31-33	-142°	.24	.10	-101°	19	NS	- 96°	1.4	NS	- 99°	.6	.04	-131°	2	NS	- 46°	.03	NS
	34-36	- 95°	.37	.03	- 89°	19	NS	- 91°	2.0	NS	- 77°	.9	NS	-101°	30	NS	- 39°	.06	.05
	37-39	- 94°	.47	NS	- 89°	21	.08	- 89°	2.3	NS	- 93°	.8	.10	-139°	6	.03	- 27°	.07	NS
	40-42	- 91°	.73	.003	- 77°	23	.10	- 82°	2.3	.03	- 80°	.9	.05	-182°	5	NS	- 28°	.07	NS
BACK REVERSAL	43-45	-218°	.42	.003	-322°	9	NS	-320°	.3	NS	- 30°	.2	NS	-311°	10	NS	- 55°	.07	.03
	46-48	-231°	.64	.003	-252°	12	NS	-234°	1.9	.02	-210°	.5	NS	-307°	17	NS	-169°	.02	NS
	49-51	-249°	.78	.002	-267°	15	NS	-261°	2.7	.07	-221°	.8	NS	-288°	18	NS	-158°	.06	NS
	52-54	-258°	1.06	.001	-243°	18	NS	-240°	2.2	.006	-193°	1.0	.06	-239°	9	NS	-166°	.11	NS
	55-57	-261°	.72	.04	-256°	19	NS	-246°	2.3	.07	-220°	.9	.05	-312°	13	NS	-203°	.06	NS
	58-60	-254°	.78	.003	-248°	18	NS	-246°	2.1	.09	-204°	.8	.03	-313°	12	NS	-188°	.08	.03
	61-63	-251°	.96	.03	-232°	6	NS	-211°	1.6	NS	-174°	.8	.08	-211°	5	NS	-174°	.13	NS

Φ = ACROPHASE IN NEGATIVE DEGREES FROM MIDNIGHT

C = CIRCADIAN AMPLITUDE

P = PROBABILITY OF NULL HYPOTHESIS, IE,, NO 24 HR, RHYTHM, NS = NOT SIGNIFICANT, P > .10.

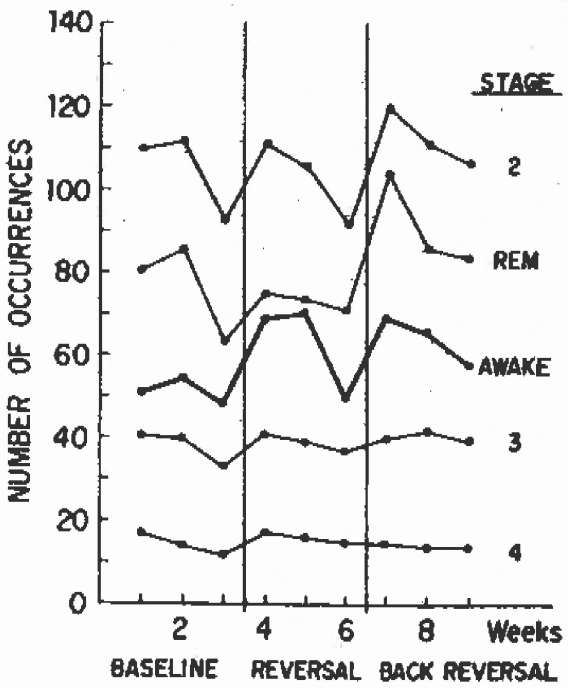

Figure 2. The number of episodes of each sleep stage is shown for each week of the 9-week study.

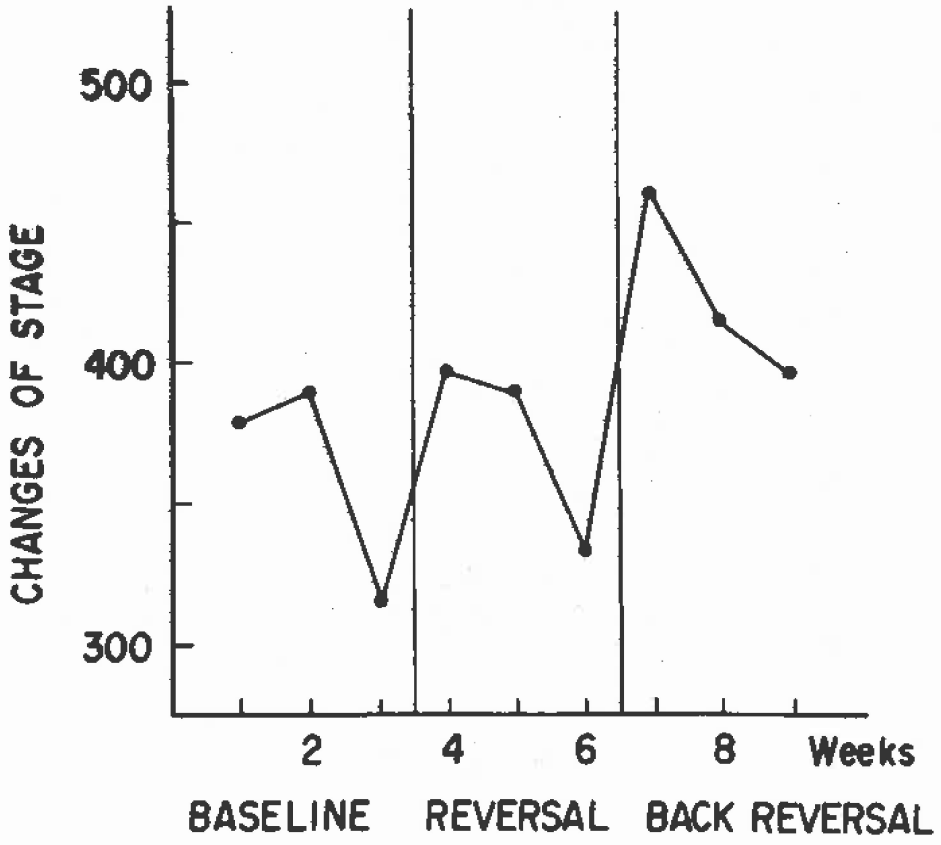

Figure 3. The number of changes of sleep stage per week is shown for the 9-week study.

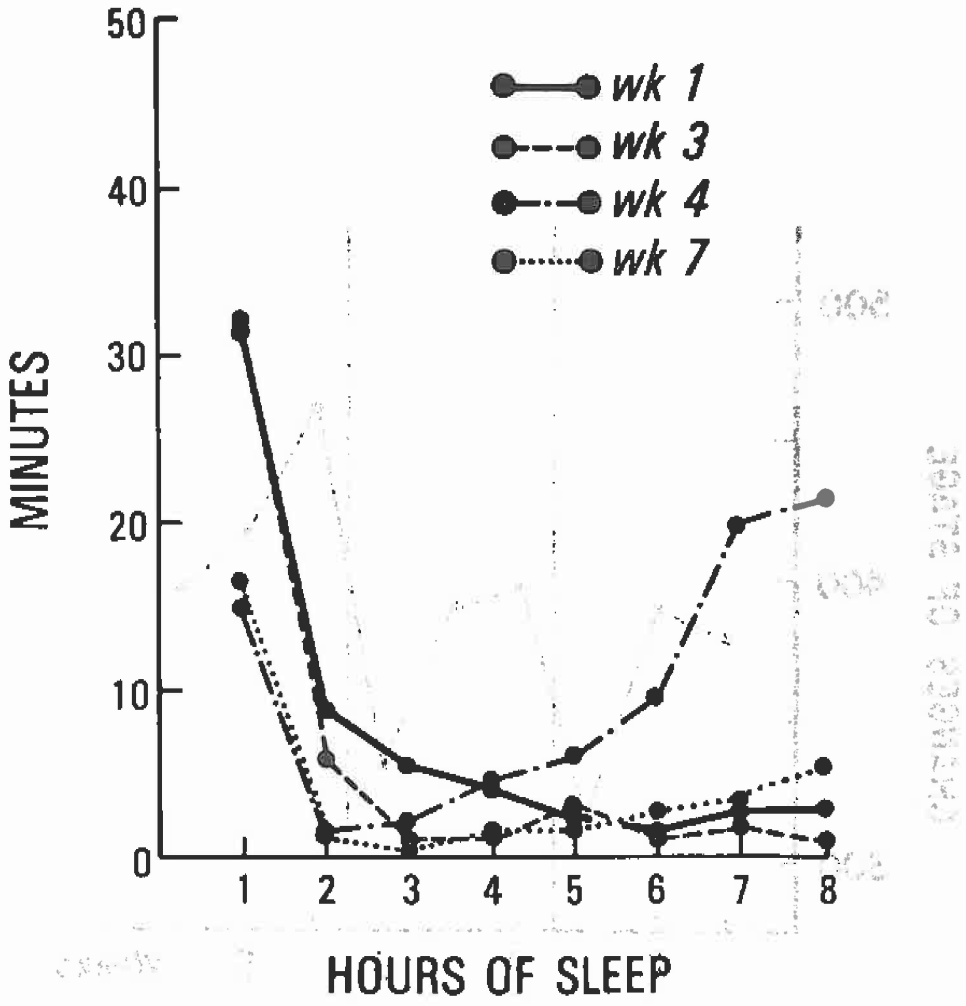

Figure 4. The minutes of Stage Awake per hour of sleep.

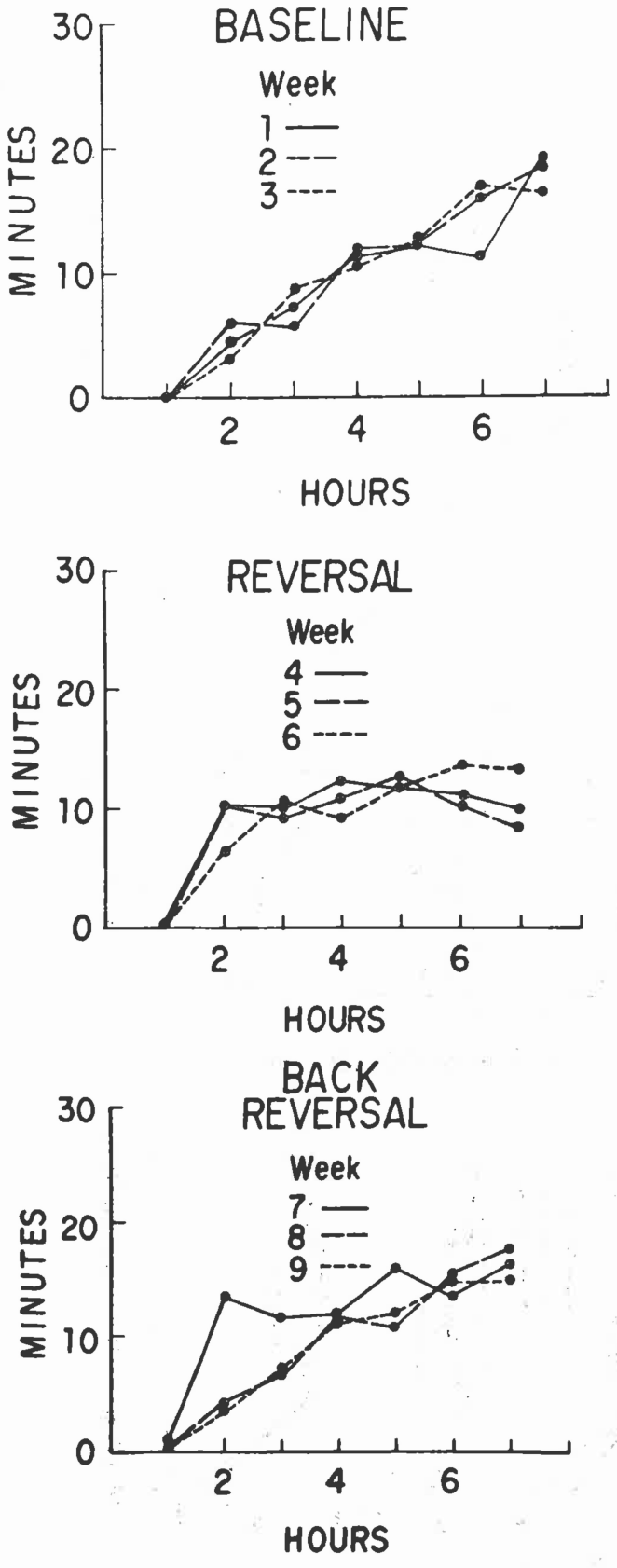

Figure 5. The minutes of Stage REM in each hour of sleep: A) BASELINE, B) REVERSAL, C) BACKREVERSAL.

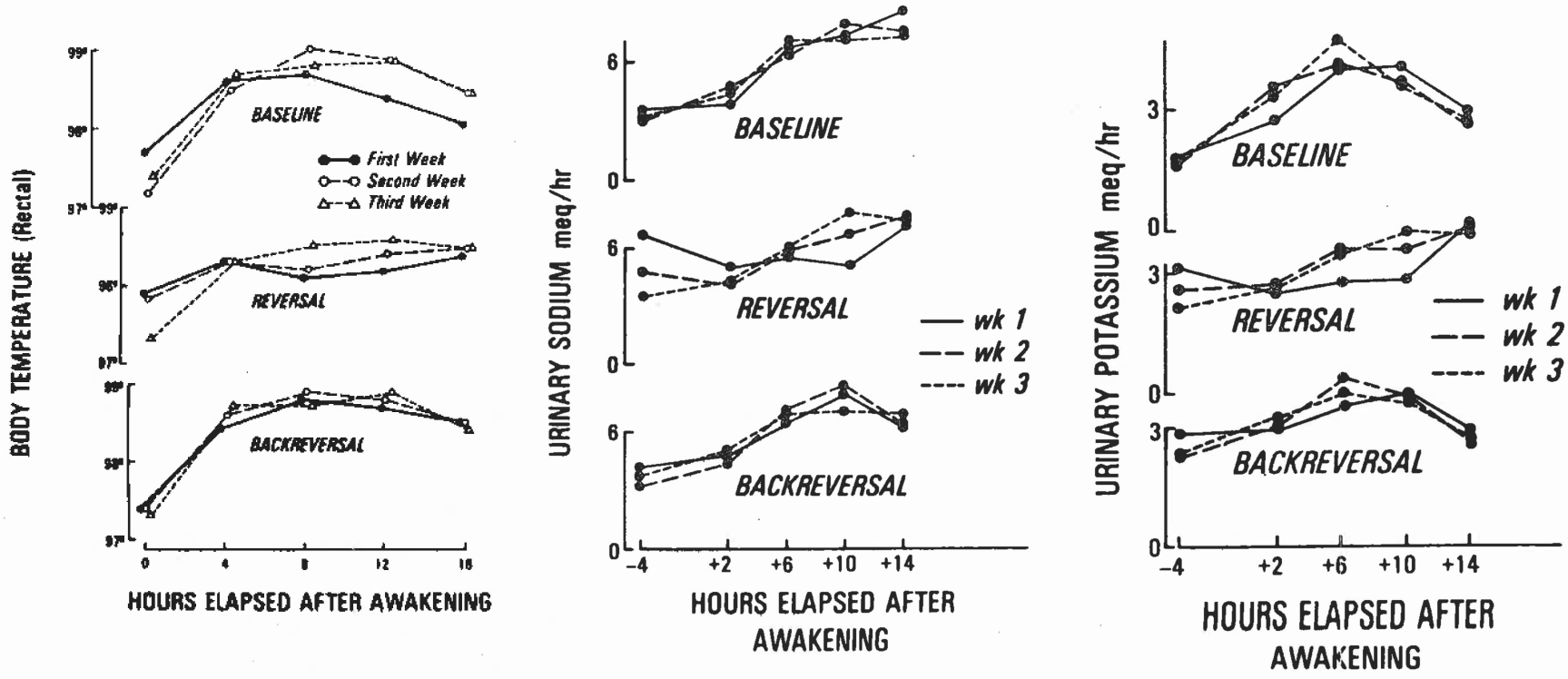

Figure 6. Circadian waveforms are shown for A) rectal temperature, B) urinary sodium, and C) urinary potassium, averaged for 5 subjects week by week.

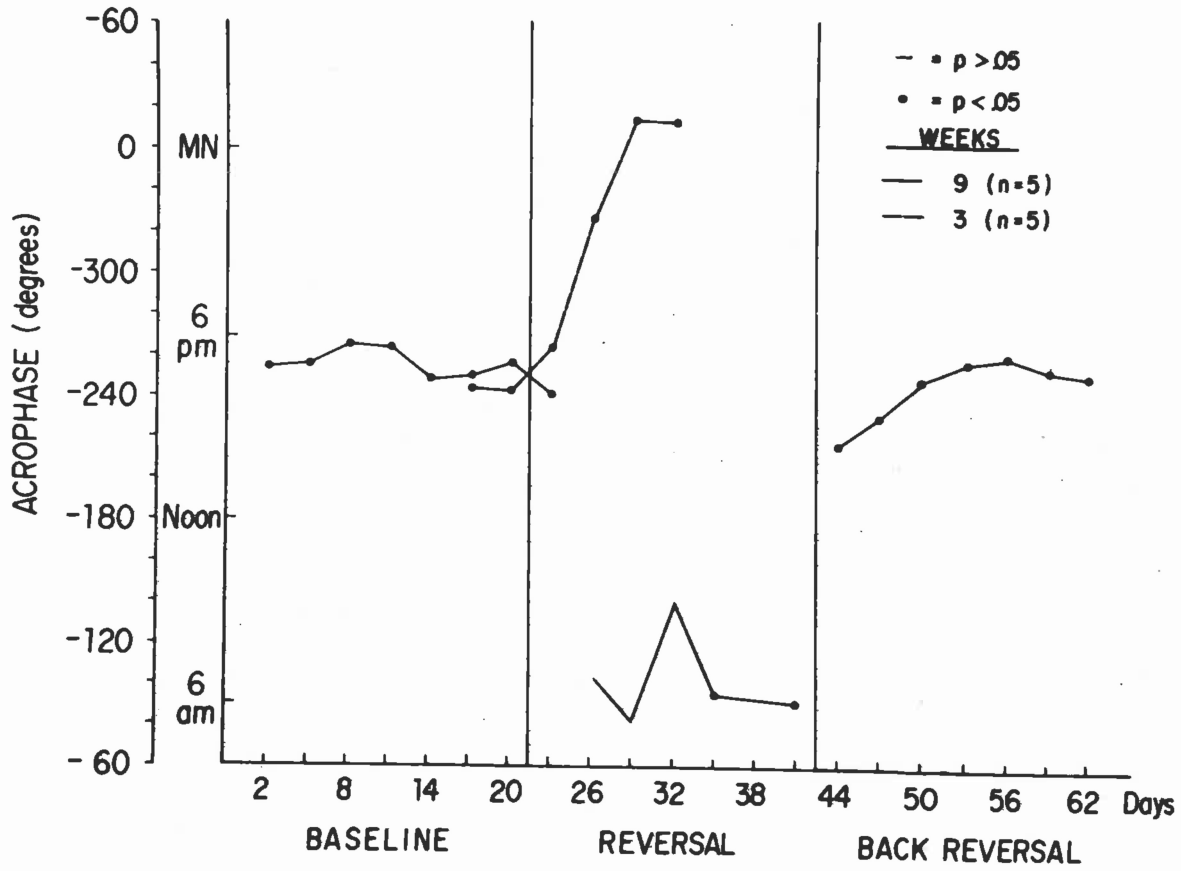

Figure 7. Temperature cosinor phases are shown for both the 3-week and 9-week studies. Each point represents a 72-hour interval, and where the cosinor was significant, a large dot is shown. Consecutive phase estimates were not connected by lines when the discontinuity between consecutive phase estimates was greater than 90 .

curve-fitting technique indicated that the rhythm restoration was complete within 7 to 9 24-hour periods.

Urine volume: During BASELINE the acrophases of the urine volume rhythms were consistent although the cosinor analyses did not always achieve significance in the 9 week study (Table 1, Figure 8). After REVERSAL for both studies, a rapid advance of the acrophase of the rhythm took place. This progressed to about -130 degrees or 0840 am (a 110 degree phase shift) during the 3-week study and to approximately -80 degrees of 0520 am (a 170 degree phase shift) by the end of 3 weeks in the longer study. During BACKREVERSAL, the urine volume rhythm clearly returned to the BASELINE phase angle within 4 to 6 days,but least-squares analysis indicated a lack of consistent rhythms in 2 of the subjects as well as for the group as a whole during BACKREVERSAL. The squared deviation results also indicated that the adjustment was less rapid and complete during REVERSAL than during BACKREVERSAL.

The daily total urine volume was increased for each of the 3-week subjects and for 3 of the five 9-weeks subjects after REVERSAL. Total urine volume was also increased for 3 subjects during Week 7 after BACKREVERSAL. Detailed analyses indicated that these increases in urine volume occurred primarily during sleep when urine output was not markedly decreased as it was during BASELINE.

Urinary sodium excretion: During BASELINE cosinor values were significant for the 3-week subject group and for all 10 subjects measured together. The acrophases were consistent for the 9-week subjects as well but not statistically significant (Figure 9). During REVERSAL, there was a rapid phase advance of approximately 100 degrees during the first 6 days and by the end of 12 days, the 9-week subjects had undergone a 165 degree phase inversion. However, the cosinors did not become significant until the 19th to 21st day. By contrast, during BACKREVERSAL the rhythm was fully shifted within 4 to 6 days. Squared-deviation analyses were consistent with this picture. There were no week-to-week changes in the amount of sodium excreted. The results of analyzing the sodium concentrations and the meq/hour rates were essintially similar.

Urinary potassium excretion: The BASELINE potassium cosinor values were significant and stable for both studies (Figure 10). During REVERSAL, a progressive slow advance was noted. This had only progressed approximately -140 degrees after 3 weeks. However, during BACKREVERSAL, the return of the acrophase was largely complete within 4 to 6 days. The squared deviation analysis also supported this result. The total amount of excreted potassium was significantly higher during the 3rd and 7th weeks of the 9 week study than during other weeks. Since urine volume was also elevated during this time, the potassium concentration was not increased.

Urinary creatinine excretion: Creatinine cosinor values and least-squares cosine fits for individual subjects indicated that 24-hour creatinine excretion rhythms were unreliable for both the 3-week and 9-week subjects. The phase changes found after REVERSAL and BACKREVERSAL approximated the urine volume pattern but these findings lacked significance and reliability. There were not changes in the mean creatinine excretion from week to week.

Urinary 17-Hydroxycorticosteroids: During BASELINE, the urinary 17-OHCS rhythms were stable and generally significant (Figure 11). Following acute RE-

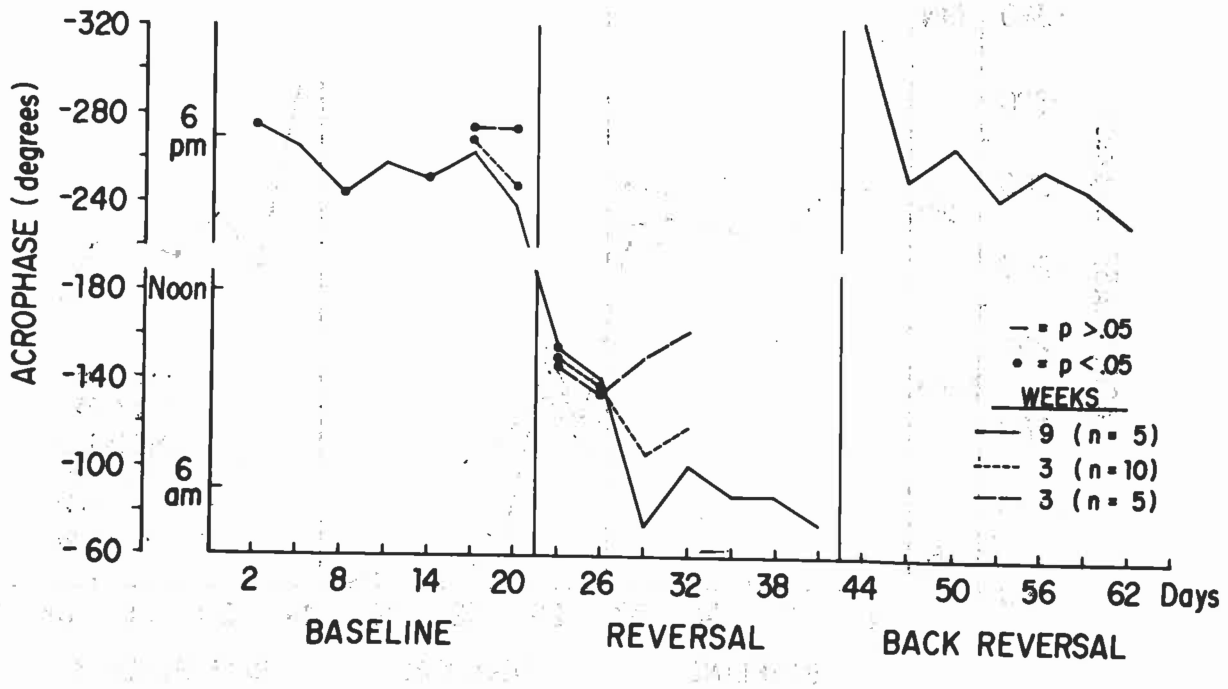

Figure 8. Urine volume cosinor phases are shown as in Figure 7 for the 3-week and 9-week studies, and combined results for both studies are also plotted.

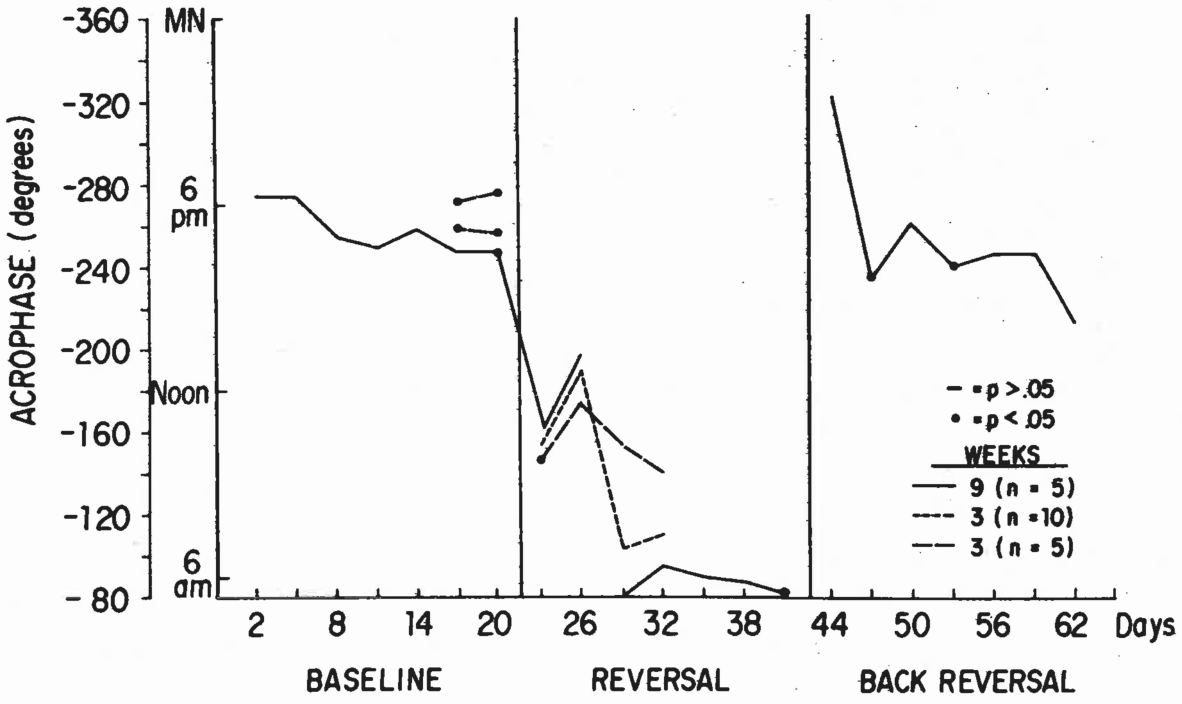

Figure 9. Urinary sodium excretion cosinors are plotted as in Figure 8.

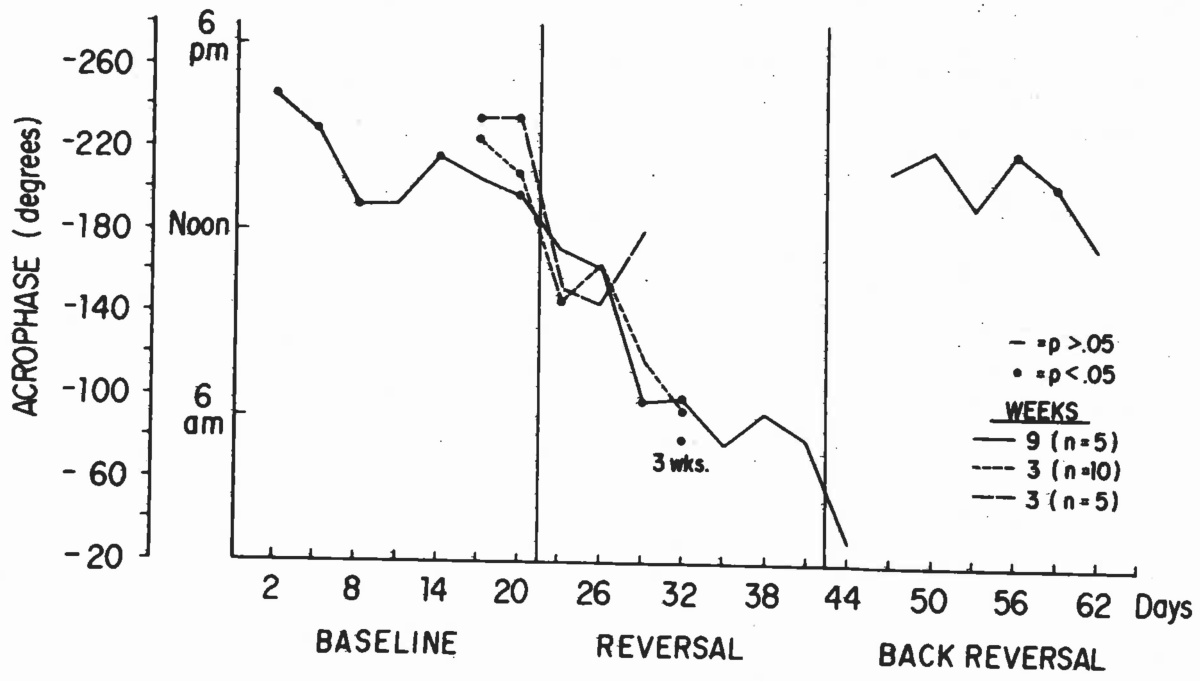

Figure 10. Urinary potassium excretion cosinors are plotted as in Figure 8.

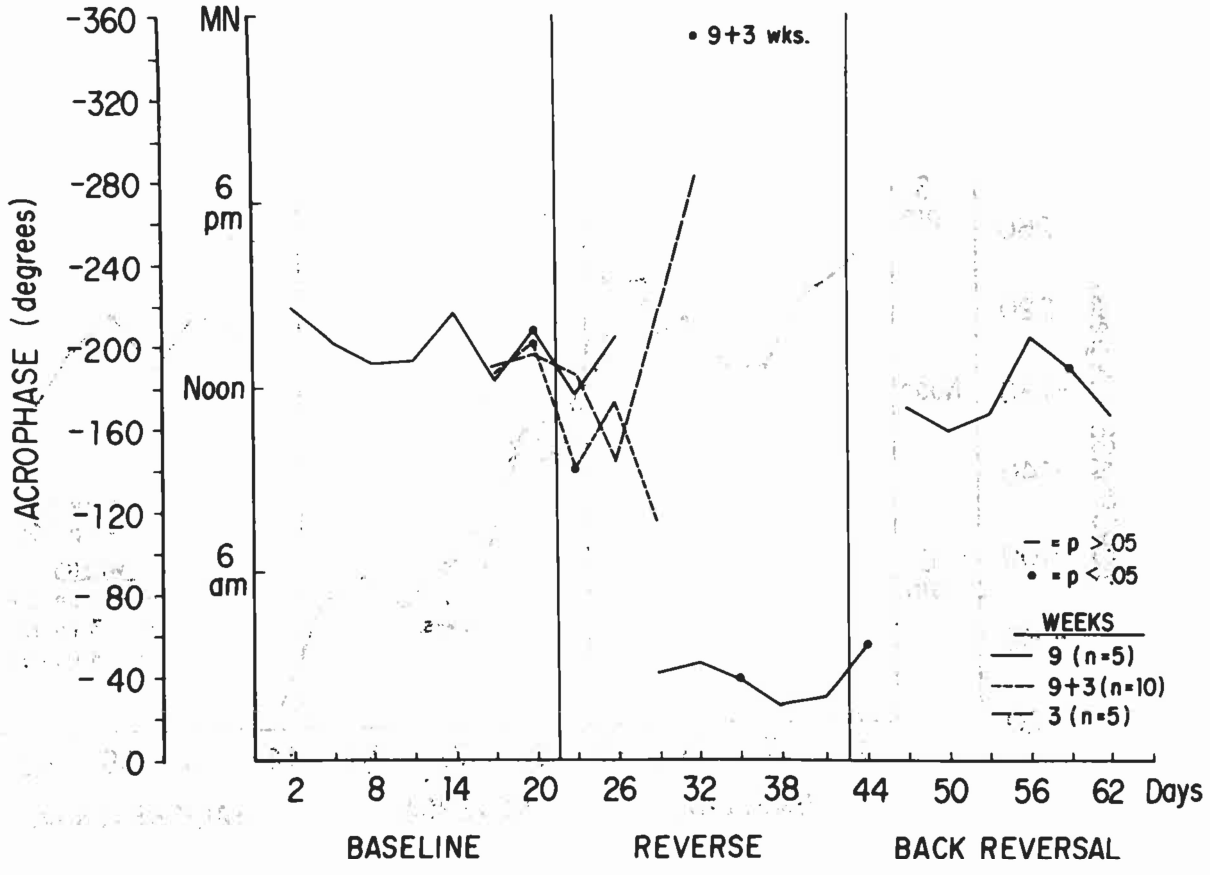

Figure 11. Urinary 17-OHCS excretion cosinors are plotted as in Figure 8.

VERSAL, there was no major change in phase for 4 to 6 days. During the 2nd week of REVERSAL there developed considerable variability among the subjects (some advancing and some delaying). A clear rhythm was not detectable for the group, and rhythm discontinuities occurred in several individual subjects. However, by the 13th to 15th day during REVERSAL among the 9-week subjects, the circadian rhythm had been re-established and a consistent and stable phase shift of about 160 degrees could be identified. In both the 3 week and 9 week studies, a biphasic 24 hour curve was present for urinary 17-OHCS during the reversal process, indicating that the process of shift was not primarily by advance or delay of a single "peak". A rapid re-inversion took place within the 1st week during BACKREVERSAL.

Although no statistically significant changes were identified in weekly steroid excretion amounts for both study groups as a whole, four of the five 3-week subjects had 20 to 50% decreases in 24 hour urinary 17-OHCS at some point between 2-8 days after REVERSAL. This decrease was independently confirmed by plasma cortisol measurement. No clear decrease in 17-OHCS were seen after REVERSAL among the 9-week subjects. However, transient 27 to 70% decreases in 24 hour urinary 17-OHCS excretions did occur in four of the five subjects from 1 to 7 days after BACKREVERSAL. These lasted only 2 to 3 days. In two of these cases, plasma cortisol confirmation was available. The daily steroid excretion was variable and the observed decreases were not consistent for the group as a whole.

Discussion

The 9-week study fully confirmed the previously reported results of the effect of sleep-wake reversal on sleep stages in the 3-week subjects (Weitzman, 1975; Weitzman, Kripke, Goldmacher, MacGregor, & Nogeire, 1970). It is now clear that a twelve-hour inversion of the sleep-wake period under controlled conditions, produces a shortened sleep latency, shortened Stage REM latency, and a increased amount of Stage REM sleep during the first 2 hours of sleep. Early awakenings occur during the 5th to 8th hour of sleep, and all stages of sleep are reduced during the final hours of the sleep periods. In addition an acute sleep reversal produces a transient increase in the number of occurrences of each sleep stage by increasing the number of changes or shifts of stage and by shortening the durations of episodes of each sleep stage. These increased numbers of changes of stage persist approximately for 2 weeks after reversal. However, early awakening and decreases in total Stage REM and Stage 2 sleep persist throughout the 3 weeks of REVERSAL. Although the REVERSAL condition was not a period of sleep deprivation per se, a deficit in sleep and a deficit in Stage REM did develop and immediately after BACKREVERSAL, a rebound increase resulted. The BACKREVERSAL period demonstrated a clear increase in changes of sleep stage which also persisted for 2 weeks. A shortened REM latency and mild but definite morning awakening were also present during the first week, but sleep quantity was not reduced in BACKREVERSAL. Thus, the fragmentation of sleep stage patterns and the altered hourly distribution of sleep stages within the sleep periods is presumably due to the process of sleep-wake phase shift. The "insomnia" present during the REVERSAL condition also is clearly the result of the acute phase inversion, but other factors may be present as well. Since the 8-hour available sleep periods were the same during the BASELINE and REVERSAL conditions and since the sleep environment was equally dark and quiet, no direct environmental disturbances can be impli-

cated in depriving subjects of sleep during the REVERSAL condition. It is probable that certain 24 hours oscillators were not shifted even after 3 weeks of the REVERSAL condition. Concomitant lowered waking body temperatures and increased urine volume during REVERSAL support this supposition. The REVERSAL sleep disturbance is of special interest because early "morning" awakening, fragmented sleep, and reduced REM latency are characteristic of primary depression (Kupfer, 1976; Kupfer, Foster, Coble, McPartland, & Ulrich, 1978) and to some extent narcolepsy (Montplaisir, 1976), two illnesses which may be related (Roth & Nevsimalova, 1975). This phase shift result may serve as an experimental model for these and other sleep disorders.

The 24-hour rhythms of urine volume and urinary potassium were also not fully established even after 3 weeks of REVERSAL compared to BASELINE. In addition, the non-sleep parameters (temperature, urine volume, creatinine, sodium, potassium, and 17-OHCS) were shifted only 140-165 degrees after 3 weeks of REVERSAL; none shifted a full 180 degrees. Although the sleep-wake rhythm was inverted more than the metabolic rhythms, the persistence of mild early-awakening even in the third week of REVERSAL suggested that this rhythm was phase-advanced during the REVERSAL condition in reference to the lights out period. The non-sleep parameters therefore were delayed in reference to the polygraphic stages.

We have considered several alternative explanations for the failure of complete phase inversion of several of the variables studied. One possibility is that one or more endogenous circadian oscillatory pacemakers are so resistant to the phase change ("inertia") that 3 weeks is an insufficient amount of time to produce a 180 degree shift. Since there are data that a more rapid phase shift occurs among air travelers (Klein, Wegmann, Athanassenas, Hohlweck, & Kuklinski, 1976; Klein, Wegmann, & Hunt, 1972) or in other controlled experimental isolation studies (Aschoff, Hoffmann, Pohl, & Wever, 1975), this argues against an "inertial" explanation. Another possibility is that environmental synchronizers over which we had little control exerted substantial effects on the phase of the rhythms. For example, the subjects received visitors and would watch television during the evening hours whether they were sleeping during the day or at night. These waking alerting activities might alter sleep rhythms by delay during day-wake schedules. During REVERSAL,these activities occurred shortly after the subjects awakened and might lead to an advance of components of sleep rhythms. The general difference in levels of stimulation, activity and ambient illumination which the subjects experienced during REVERSAL as compared with BASELINE could also affect the rhythms. Studies in animals have demonstrated a negative correlation between the strength of the synchronizing stimuli and the length of time needed for reentrainment (Aschoff, Hoffmann, Pohl, & Wever, 1975; Erkert, 1976; Hoffmann, 1969). In addition, it has been reported that a complete resynchronization of rhythms took 50% longer for trans-meridian air flight passengers who were kept in relatively isolated hotel rooms, compared with passengers who left the hotel rooms and participated in outdoor activities during the adjustment period (Klein & Wegmann, 1974).

Night shift workers experience similar environmental lighting conditions as well as social synchronizers like those experienced by our subjects. The inability of night shift workers to reverse their social milieu and to experience daylight at night - no matter how carefully and thoroughly sleep patterns

are reversed (bedrooms darkened and sound-proofed and meals reversed) - could lead to disturbances similar to those experienced by our experimental subjects. Not only were our subjects unable to achieve a complete 180 degree reversal of their 24 hour rhythms after 3 weeks of scrupulously maintained sleep period and meal timing reversal, but they experienced a persistent decrease in total sleep and Stage REM durations. Actual night workers experience similar sleep problems (Bryden & Holdstock, 1973), which suggests that the ubiquitous sleep problems of night workers may not be resolved by even the most optimal sleep and meal arrangements and stability of day-sleep patterns even after several weeks. Further studies are needed in man to determine the length of time required to fully re-establish prior relationships of circadian rhythms to the new sleep period after an acute phase shift, under conditions where social synchronizers and other environmental stimuli such as light are not fully shifted. These non-equivalent conditions between the day time and the night time are clearly experienced by the shift worker. It is conceivable that rhythm shifts under these conditions might never be complete.

The rapid physiological shift response to BACKREVERSAL supports the concept that the subjects had not fully adapted even after 3 weeks of the REVERSAL condition. Although the disruption of sleep patterns as indicated by the number of stage changes and the number of Stage 2 and REM episodes was greater for the 1st week after BACKREVERSAL as compared to the 1st week after REVERSAL, the amount of early "morning" awakening during the same periods was less, especially during the last 2 weeks of BACKREVERSAL. Thus, the sleep data suggested that after the end of the REVERSAL period, a combination of a transient phase-shift response plus a rapid rebound-recovery response occurred.

The non-sleep rhythms during BACKREVERSAL returned to the BASELINE pattern with extreme rapidity compared to the adjustments required for the equivalent first REVERSAL. Several explanations should be considered. First, it is possible that environmental factors such as natural light and social synchronizers may have facilitated the BACKREVERSAL process. It is also possible that a more rapid shift during BACKREVERSAL occurred because the REVERSAL shifts had only been incompletely achieved. Indeed, non-sleep variables had only achieved a 140 to 165 degree shift. It is certainly conceivable that an unmeasured endogenous rhythm functioning with strong "inertia" may have been maintained in close conformity to the BASELINE timing during the REVERSAL segment.

Although an attempt was made to reduce many influences of the real world in these studies,the results indicate that very complex and variable responses occur to a phase shift even in experimental settings. Clearly, different body rhythms undergo phase-reversals at very different rates and in different directions. For example, for the 3-week subjects, temperature ostensibly reversed by delay whereas potassium reversed by an advance. Disparities between advance and delay were also noted among each group of subjects for certain variables; moreover, the cosinor analysis revealed substantial disparities between the 3-week and 9-week studies in the transient responses to the REVERSAL phase shift. It should be emphasized however, that the concept of shifting by advancing or delaying the phase of a rhythm is a misleading interpretation arising from an analytical model restricted to sine function model. Indeed it was very clear that temperature and urinary 17-OHCS shifted by a two component mode. A progressive increase in body temperature during the new waking period

occurred along with a progressive decrease of body temperature during the new sleep period. Indeed the rates of these waveform changes appeared to be dissociated, with the fall of the old peak occurring more rapidly than the rise of the new temperature peak. A similar process was apparent for 17-OHCS. A similar process also occurred for urinary sodium, potassium and creatinine since clear decreases in rhythm amplitude and alterations of wave form were observed, although biphasic patterns were not always present for each subject. Aschoff et al. (1975) and Mills et al. (1978) have also concluded that phase-shifts occur through changes in several wave-form components, and dissimilar responses appear in different variables. Thus, depending on the conditions and the individual subject's response, a variety of patterns of internal phase coupling and uncoupling among rhythms may result from a major phase shift.

The factors controlling circadian rhythm phase shifts deserve further study, considering the increasing frequency that man undergoes shift work and rapid jet travel across many time zones. In addition, the occurrence of a persistent insomnia, early "morning" awakening, shortened REM latency, and the failure of certain metabolic rhythms to re-establish a BASELINE phase-angle in relation to the sleep rhythm during a 3-week REVERSAL resembles the sleep disorder associated with depression and narcolepsy. Extremely small alterations of the phase angle between light-dark and activity cycles in hamsters produce remarkable endocrine alterations (Elliott, 1976). Altered internal phase angle relations among body rhythms may also contribute to the seasonal responses of numerous lower species (Pittendrigh, 1974). These results from animals suggest that social synchronizers, lighting, or other factors which might produce subtle perturbations of circadian rhythm organization could have a role in the etiology of affective diseases as well as sleep disorders.

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NASA Contract #NGR33023-032, NIOHS Grant #EC-00341-01, and Clinical Research Center Award #RR-53. In addition, we were supported by a Research Scientist Development award (NIMH K02-MH00117) for Daniel F. Kripke and by the Medical Research Service of the Veterans Administration.