ADAPTATION TO ABRUPT TIME SHIFTS OF THE OSCILLATOR[S] CONTROLLING HUMAN CIRCADIAN RHYTHMS

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SUMMARY

1. Thirty-six subjects in an isolation unit were subjected to time shifts of 12 hr, or of 8 hr in either direction.

2. The rhythms of body temperature and excretion of eight urinary constituents were studied before and after the shift, both on a usual nychthemeral routine and during 24 hr when they remained under constant conditions, awake, engaged in light, mainly sedentary activity, and consuming identical food and fluid every hour.

3. The rhythms on nychthemeral routine were defined by fitting cosine curves. On constant routine the rhythm after the shift was cross-correlated with the original rhythm, either with variable delay (or advance) or with an additive mixture between this variably shifted rhythm and the unshifted or a fully shifted rhythm. The process yielding the highest correlation coefficient was accepted as the best descriptor of the nature of adaptation.

4. A combination of two rhythms was observed more often for urinary sodium, chloride and phosphate than for other variables.

5. Adaptation appeared to have proceeded further after westward than eastward shifts, and this difference was particularly noticeable for urinary potassium, sodium and chloride.

6. Partial adaptation usually involved a phase delay, even after an eastward shift when a cumulative delay of 16 hr would be needed to achieve full adaptation and re-entrainment.

7. Observations under nychthemeral conditions often gave a false idea of the degree of adaptation. In particular, after an eastward shift the phase of the rhythms appeared to shift in the appropriate direction when studied under nychthemeral conditions whereas the endogenous oscillator either showed no consistent behaviour or, in the control of urate excretion, a shift in the wrong direction.

8. The implications for people undergoing time shifts, in the course of shift work or transmeridional flights, are indicated.

INTRODUCTION

It is now generally accepted that most physiological variables which oscillate with a 24 hr period are influenced from two sources, an endogenous oscillator and a variety of direct influences from the usual nychthemeral habits. Sleep (Mill, Minors & Waterhouse, 1978), meals, and posture can all influence body temperature and renal

and cardiovascular variables. When these influences are removed, an endogenous rhythm persists. Major exogenous rhythmic influences can be removed by keeping subjects awake, either recumbent or engaged continuously in sedentary activities, and by requiring them to ingest identical food and fluid every hour (Mills & Stanbury, 1954, 1955).

In most studies of adaptation to real time shift after long transmeridional flights, or to simulated time shift, the subjects have followed their usual nychthemeral habits before and after the time shift. It is thus uncertain whether the circadian clock has adjusted its phase or whether the measured responses result from exogenous influences which mask that of the clock. To throw light on this problem we have exposed thirty-six subjects to artificial time shifts. One to four days later we have observed their physiological rhythms under conditions of constancy similar to those used by Mills and Stanbury. The form and phasing of their rhythms was then compared with those observed in a similar 24 hr constant routine before the time shift.

Preliminary accounts of the method (Mills, 1977) and results (Minors & Waterhouse, 1977; Minors, Mills & Waterhouse, 1977) have already appeared

METHODS

Thirty-six subjects, in groups of two to five, have been studied in the isolation unit described by Elliott, Mills, Minors & Waterhouse (1972), and one subject was studied alone on a second occasion. Initially subjects were studied for between 36 and 108 hr living on a customary nychthemeral routine. This consisted of retiring at 23.30 and rising at 07.30 with lights on during the hours of daylight appropriate to the time of year. During this period urine samples were collected every 2 hr while the subjects were awake and as a single sample over the sleep period. Rectal temperature was measured hourly during the hours of wakefulness and monitored continuously during sleep. Following this control period the subjects were awakened at 04.00 (group K at 08.00) and for the next 24 hr remained awake engaged in sedentary activities, and in continuous light. They consumed hourly a biscuit and milk with added sodium chloride. This ration provided a daily intake of 2·88 l. water, 200 m-mole sodium and 80 m-mole potassium, given in twenty-four equal parts. Each hour they recorded their own rectal temperatures and micturated as completely as possible. In group K, blood samples were collected every 2 hr. Nine blood samples were similarly collected from subjects 1, 2, 3 and 4 in Group L. This spell of 24 hr is referred to as a 'constant routine'. When it was completed, at 04.00 (group K at 08.00), subjects were allowed to go to bed and sleep late; no observations were made on the following day, but they began again when the subjects next retired to bed about midnight. After another 16–77 hr of nychthemeral routine, the clock and light–dark cycle were changed by 8 or 12 hr and the subjects continued their customary nychthemeral routine on this new time. An 8 hr advance or retardation of the clock simulates an eastward or westward shift respectively, and for convenience is so described. After a further 24–88 hr they were again woken at 04.00 (group K at 08.00) and repeated the 24 hr constant routine, after which the experiment ended. Details of the different routines and subjects are given in Table 1. Methods of temperature recording and of urine analysis for sodium, potassium, chloride, creatinine, phosphate, calcium and urate, were as in a previous paper (see Mills, Minors & Waterhouse, 1977). Calcium and urate were not measured on groups K and L, and phosphate and urate were not measured on group AD. The plasma 11-hydroxycorticosteroids of the subjects in groups K and L were estimated by the method of Mattingly (1962) as modified by Spencer-Peet, Daly & Smith (1965), and plasma phosphates were estimated by AutoAnalyser as for urine. Cosine curves have been fitted to the data, and cosinors computed, as in previous papers. In addition, since our constant routine provides twenty-four equally spaced points we are able to use the more precise technique of cross-correlation, without any assumption that the rhythm is sinusoidal. If partial adaptation consists of a movement of the rhythm in the expected direction, cross-correlation with a variable delay or advance would yield, as the best estimate of the extent of this movement, the shift which gives the highest correlation. Fig. 1 shows an example in which, after a westward shift,

TABLE 1. Details of subjects and of experimental routine. A common key letter or letters indicates a group of subjects studied together. *A*, *B*, *C* indicate the number of hours in the unit before the first constant routine, between this and the time shift, and after the time shift before the second constant routine. *D* indicates the clock change which effected the time shift

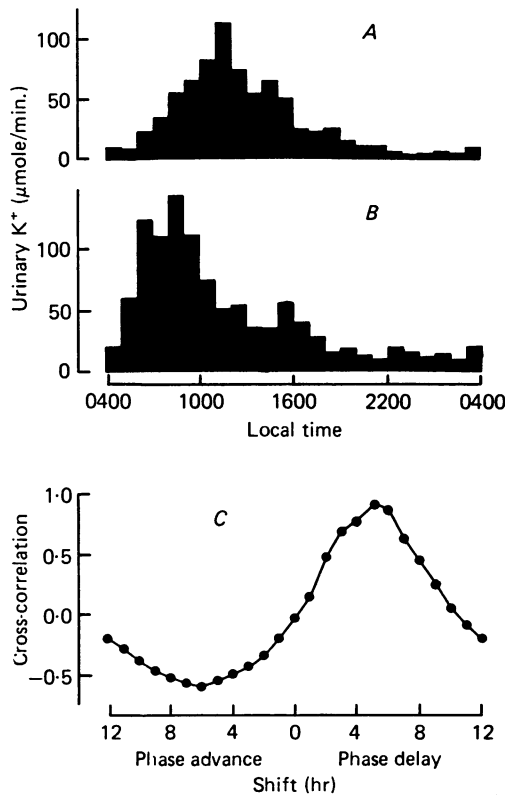| Group	No. of subjects	Sex	Ages	A	B	C	D
K	4	F	18–20	40	56	24	16.00–08.00
L	5	F	20–21	60	36	68	16.00–08.00
AK	4	F	18–21	36	72	80	04.00–20.00
AD	3	M	19–22	85	96	88	04.00–12.00
AE	2	F	19–20	108	96	64	04.00–12.00
AF	3	M	17–19	36	96	64	04.00–12.00
AG	3	F	18–19	36	97	63	05.00–13.00
AJ	4	M	18–22	36	96	64	04.00–12.00
AN*	1	F	21	36	72	64	04.00–12.00
AL	4	M	18–19	36	48	60	04.00–16.00
AM	4	M	18–19	36	96	60	04.00–16.00

* The same subject as AK1.

Fig. 1. Subject L2, urinary potassium during constant routine, *A* before and *B* 68 hr after an 8-hr simulated westward shift. Since both plots are on local time, the patterns would be identical if adaptation were complete. *C*, cross-correlation coefficient between pattern after time shift, and control pattern altered in phase by different numbers of hours.

the excretory pattern for potassium has been delayed by an amount which falls short of the 8 hr needed for complete adaptation. Fig. 1 also shows the cross-correlation coefficients for different shifts: the highest correlation corresponds to a shift of 5 hr.

Such simple shifts often failed to provide a good correlation, so we tested a second hypothesis: that adaptation was achieved by the gradual emergence of a rhythm in the new phase with a simultaneous waning of the original rhythm. Partial adaptation could then be represented as a mixture, in different proportions, of the non-shifted with a fully shifted rhythm. This procedure only rarely achieved any higher correlation.

However, the data from the second constant routine often appeared to consist of two different rhythms combined in some such way, so we tested two further hypotheses: that the data could be described as a combination of a rhythm shifted by a variable number of hours, with either a non-shifted rhythm or with a fully-shifted rhythm; Fig. 2 shows an example. The highest
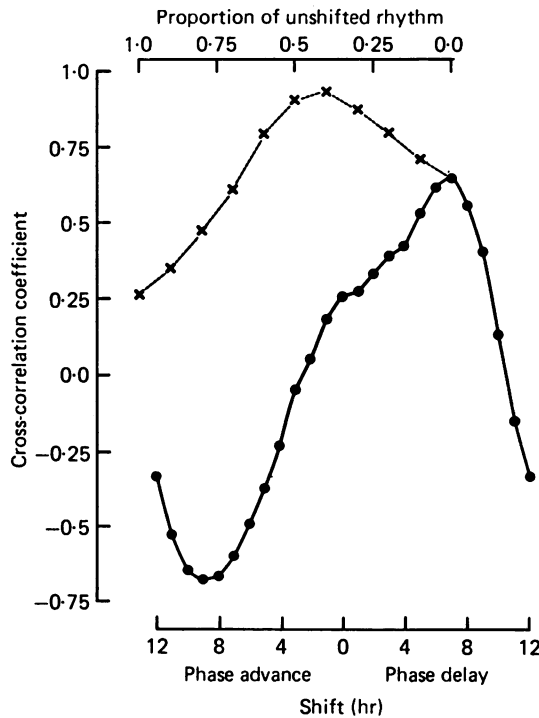

Fig. 2. Subject AK3. Urinary chloride. Cross-correlation coefficient between pattern during constant routine 80 hr after 8 hr simulated westward shift, and pattern before shift, modified: ●——● altered in phase by different amounts; × --- × phase delayed by 7 hr, mixed in different proportions with an unshifted pattern.

correlation on each of these hypotheses was calculated, varying the proportions as to 9:1, 8:2 down to 1:9, and the variable shift at hourly intervals from 0 to 12 hr advance or delay; the highest correlation was taken as the best descriptor of the partial adaptation. These calculations were performed separately for temperature and for each measured urinary constituent of each subject; instances where the highest correlation coefficient was below the 90 % significance level are ignored.

When the observed temporal pattern on the control constant routine showed a single maximum and minimum, as in Fig. 1, these techniques of cross-correlation appear wholly appropriate, and a plot of the correlation coefficient, *r*, against the hours of shift shows a single maximum. In a few instances there were two nearly equal maxima in the control constant routine and two maxima of *r*, similar in magnitude but derived from very different time shifts; the method in

such instances is unreliable, and they have been excluded from further consideration. In a few other instances, particularly for creatinine whose rhythm is usually of very low amplitude, the cross-correlation appeared to rest upon a single very high or low value, such as could have resulted from experimental error; these also were excluded.

<div align="center">RESULTS</div>

'Constant conditions'

To compare adaptation after different time shifts, we have classified the different forms in several ways. We have accepted that an error of up to 2 hr can arise in estimating the magnitude of a shift so that, for example, a mixture of a non-shifted rhythm with one shifted 2 hr would be classified as a non-shifted rhythm; and where a mixture involved 0·9 or 0·8 of one component we have ignored the other component. In this way, undue weight is not given to very small improvements in correlation.

TABLE 2. Incidence of adaptation adequately described as a single shift, or as a mixture of shifts

	Single shift	Mixture of shifts	Total	
Eastward	41	84	125	$\chi^2 = 2.422$
12 hr	27	40	67	$P = 0.298$
Westward	36	48	84	
Total	104	172	276	
Temperature	19	17	36	
Urinary water	15	13	28	
Urinary potassium	17	20	37	
Urinary sodium	8	25	33	$\chi^2 = 24.098$
Urinary chloride	10	25	35	$P = 0.0027$
Urinary creatinine	15	10	25	
Urinary phosphate	6	26	32	
Urinary calcium	6	20	26	
Urinary urate	8	16	24	
Total	104	172	276	

If the correlations are then divided into those adequately described by a single shift and those in which a mixture of shifts provides the best descriptor, we obtain Table 2. Over-all, instances where adaptation could be properly described by a simple shift were significantly ($P < 0.001$) fewer than those where a mixture of two shifts was needed. There is no difference between the proportions observed after eastward, westward or 12 hr shifts, but there are significant differences between different variables. Excretion of chloride, sodium, calcium and phosphate are most often and temperature and excretion of creatinine least often, described by a mixture of two shifts.

The relative ease of adaptation to the different time shifts is most simply assessed by comparing the correlation coefficient between the second constant routine and either the original (completely unadapted) or a fully adapted rhythm. This yields Table 3, in which it is seen that adaptation is better to westward than to eastward shifts, with 12 hr shifts intermediate. There is, however, also a difference between different variables, with temperature least and excretion of phosphate and urate

best adapted. The degree of adaptation after eastward and westward shifts has also been compared for individual variables; those attaining or approaching conventional levels of significance are shown in Table 4. The difference is most clear-cut for excretion of potassium, sodium and chloride. In these comparisons we have not taken account of the differing times elapsing between the time shift and the second constant routine (column C in Table 1). Since the shortest time was for group K, who underwent a westward shift, and the longest for group AD who underwent an eastward shift, these differences would conduce to better adaptation after the eastward shift; the greater ease of adaptation to the westward shift thus occurred despite a bias in the opposite direction. Adaptation was in fact a little worse in group K, who carried

TABLE 3. Incidence of rhythms which are closer to full adaptation or to no adaptation after different time shifts

	Full adaptation	No adaptation	Total	
Eastward	52	86	138	$\chi^2 = 16\cdot414$
12 hr	33	39	72	$P < 0\cdot001$
Westward	63	36	99	
Total	148	161	309	
Temperature	9	28	37	
Urinary water	15	22	37	
Urinary potassium	19	18	37	
Urinary sodium	11	26	37	$\chi^2 = 29\cdot636$
Urinary chloride	16	21	37	$P < 0\cdot001$
Urinary creatinine	23	14	37	
Urinary phosphate	24	10	34	
Urinary calcium	14	14	28	
Urinary urate	17	8	25	
Total	148	161	309	

TABLE 4. Incidence of rhythms of individual variables which are closer to full adaptation or to no adaptation after different time shifts

	Full adaptation	No adaptation	Total	P (exact method of Fisher, 1941)
Urinary K				
Eastward	1	15	16	
Westward	9	4	13	0·00059
Total	10	19	29	
Urinary Na				
Eastward	5	11	16	
Westward	9	4	13	0·0341
Total	14	15	29	
Urinary Cl				
Eastward	4	12	16	
Westward	8	5	13	0·0381
Total	12	17	29	
Temperature				
Eastward	2	14	16	
Westward	6	7	13	0·0547
Total	8	21	29	

out their constant routine only 24 hr after the phase shift, but the difference was not significant.

A time shift of 8 hr is equivalent to a shift of 16 hr in the opposite direction; for example, if adaptation to an 8 hr eastward shift is by a gradual progression, it could be achieved by an 8 hr phase advance or by a 16 hr phase delay. For convenience these will be referred to as shifts in the right direction when 8 hr would be required to complete the shift, or the wrong direction when 16 hr would be needed.
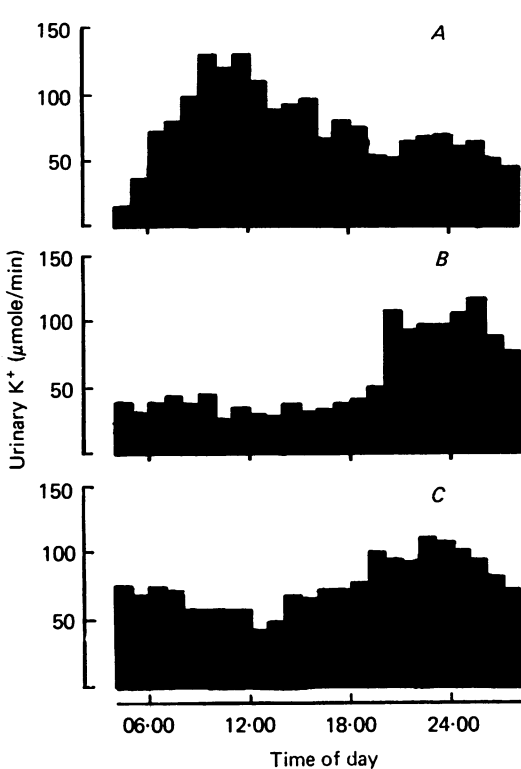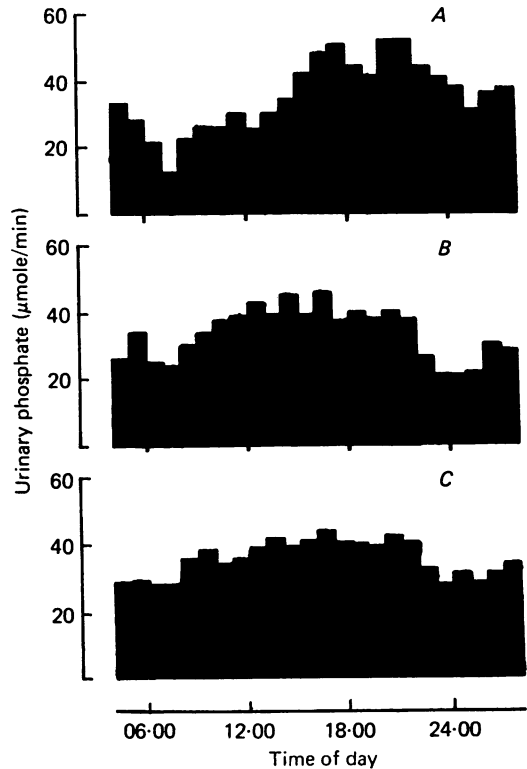

Fig. 3   Fig. 4

Fig. 3. Subject AE2. Urinary potassium during constant routine, *A* before and *B* 64 hr after an 8 hr simulated eastward shift. *C* is the modification of *A* which best cor-relates with *B*: a mixture of 0·3 of an unshifted rhythm mixed with 0·7 of a rhythm delayed by 5 hr. $r = 0.850$. All plots on local time, cf. Fig. 1.

Fig. 4. Subject AF1. Urinary phosphate during constant routine, *A* before and *B* 64 hr after an 8 hr simulated eastward shift. *C* is the modification of *A* which best correlates with *B*: a mixture of 0·4 of a fully adapted rhythm mixed with 0·6 of a rhythm delayed by 8 hr. $r = 0.890$. All plots on local time, cf. Fig. 1.

Fig. 3 shows an example in which the best descriptor is a mixture of an unshifted rhythm and a rhythm moved 5 hr in the wrong direction (that is, still requiring 11 hr shift); and Fig. 4 shows an instance in which a mixture of a fully adapted rhythm and one shifted by 8 hr in the wrong direction (that is, still requiring 8 hr shift) provides the best correlation.

It may be noted that both these examples of shifts in the wrong direction followed

eastward time shifts, whereas the seemingly more rational behaviour represented in Figs. 1 and 2 followed westward shifts. An eastward shift in the right direction involves a phase advance or in the wrong direction a phase delay, and vice versa for a westward shift. Since the spontaneous period of the circadian rhythms of human subjects in isolation normally exceeds 24 hr (Mills, Minors & Waterhouse, 1974; Wever, 1975) it seemed possible that a phase delay, or lengthening of the cycle, would be more readily achieved than a phase advance, and indeed that adaptation

TABLE 5. Incidence of shifts of rhythm in the 'right' or 'wrong' direction after eastward or westward time shift. *A*, rhythm adequately described by a single shift. *B*, rhythm best described by a combination of two components, of which one is non-shifted or completely shifted and the direction of the other is indicated in the table. The discrepancies between the totals in this table and in Table 2 result from rhythms well described by zero or complete adaptation or a mixture of these

	Right	Wrong	Total	
A				
Eastward	2	14	16	$P < 0.0001$
Westward	13	2	15	(Fisher's exact method, 1941).
Total	15	16	31	
B				
Eastward	20	43	63	$\chi^2 = 9.00$
Westward	21	12	33	
Total	41	55	96	$P \approx 0.003$

to an 8 hr eastward shift might be achieved by a progressive phase delay through a total of 16 hr; the phase would then appear to move in the wrong direction. We have therefore tabulated, separately for eastward and for westward time shifts, the rhythmic components which appeared to move in the 'right' or in the 'wrong' direction. Table 5 shows that the process of adaptation after westward shifts more often involves a shift in the 'right' and after eastward shifts in the 'wrong' direction, in conformity with this hypothesis. This difference is clear whether one considers rhythms which are adequately described by a single shift, or those described by a mixture of a variable shift with one of zero or complete adaptation. In contrast, there were no significant differences between different variables in the proportion of shifts in the right or the wrong direction.

One subject experienced, on separate occasions, 8 hr time shifts in either direction. The differences between eastward and westward shifts with regard to ease of adaptation, and to number of partial phase shifts in the 'right' or 'wrong' direction, conformed with the group differences already considered, though the numbers are too small to attain conventional levels of significance.

After a 12 hr shift, movement in either direction would have to proceed through 12 hr to achieve full adaptation. After such shifts, thirty-four of the partial adaptations involved a phase delay and only four a phase advance, which again accords with the hypothesis.

Plasma composition. Blood samples were only collected from groups K and L, both of whom underwent westward shifts.

The plasma 11-OHCS on the control constant routine followed the familiar pattern of a high concentration on waking, a steep fall, and a rise during the following night,

much as is seen during nychthemeral existence except that in group L the concentration was already high in the first sample, perhaps owing to the stress of being awakened at 04.00. During the second constant routine there were often two peaks, corresponding roughly to the original and the shifted time. Cross-correlation has been used in the same way as with renal excretion for assessing the nature and extent of adaptation; for group L this necessitated assuming by extrapolation values in the later hours when blood samples were only collected 4 hourly.
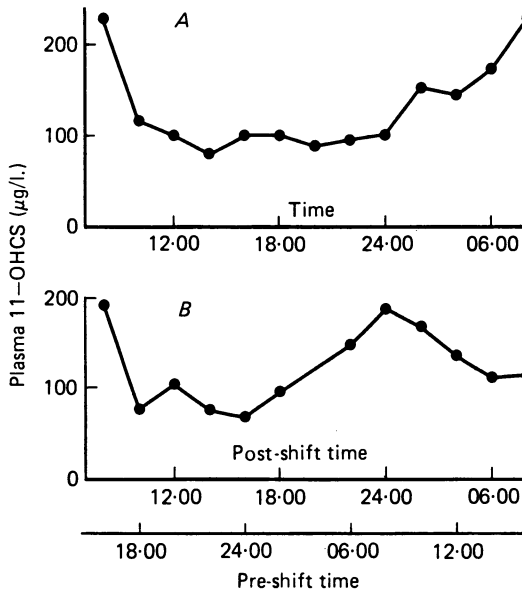

Fig. 5. Subject K3, plasma steroid concentration during constant routine, *A* before and *B* 24 hr after an 8 hr simulated westward shift. Note in second trace two peaks, at 08.00 on pre-shift and on post-shift time.

The correlations were high with a mean, calculated from the z transform, of 0·807 and a range from 0·668 to 0·905. If one accepts the same tolerance limits as those used for urinary constituents, that differences of 2 hr can be ignored, one of the steroid rhythms of group K was wholly unadapted and the other three are best described as a mixture of a completely unadapted and a fully adapted rhythm, whereas in group L only one is fitted by such a mixture and the other three were fully adapted. Fig. 5 shows an example of a two-peaked rhythm with one peak at the time of waking and the other at midnight, which was 08.00 by real time. Cross-correlation indicates a good fit to a mixture of a fully adapted and completely unadapted rhythm. The difference between the degree of adaptation shown by the two groups presumably reflects the different times elapsing between the time shift and the constant routine, one day (24 hr) in group K and three days (68 hr) in group L.

Not all subjects showed clear rhythms in their plasma phosphate on the control day, but after the time shift there were again examples of two peaks in concentration, corresponding to the expected hour on real and on shifted time.

Nychthemeral rhythms

Urine samples were collected and analysed not only while subjects were adopting the constant routine but also while they were following a customary nychthemeral existence, before and after the phase shift. Before the phase shift a single cosine has been fitted to the whole of each of two control spans, before and after the first constant routine; after the phase shift, overlapping serial sections of 24 hr were fitted, since the rhythm was likely to be changing. The control findings, when compared with the constant routine, might give some indication of how far the periodicities observed under nychthemeral conditions are the direct consequence of external influences; likewise after phase shift some indication is given of how far the adaptation observed by conventional methods (Elliott *et al.* 1972) is a simple reflexion of external influences.

TABLE 6. Phase advance of urinary rhythms observed under constant routine by reference to previous rhythms on nychthemeral routine. Mean ± s.E. (no. of subjects/total possible no. of subjects); values have only been included if, for both routines, $P < 0.01$ that rhythm was absent

Constituent	Mean phase advance (hr)
Phosphate	4.42 ± 0.77 (10/34)
Urate	4.11 ± 0.47 (23/25)
Sodium	2.20 ± 0.60 (20/37)
Chloride	1.41 ± 0.46 (23/37)
Potassium	0.96 ± 0.25 (34/37)

The effect on phase of adopting, for 24 hr, a constant routine has been assessed by calculating the acrophase of the rhythms (maximum of cosine curve fitted to data) during the control period before the first constant routine, and during this constant routine, using only those instances where both yielded a significant rhythm ($P < 0.01$). For temperature and for all urinary constituents this calculation indicated a mean phase advance during the constant routine, but the means for temperature and water were non-significant and for creatinine and calcium were derived from very few subjects, five out of thirty-seven and six out of twenty-eight respectively. The other means are presented in Table 6. The phase advance for phosphate and urate was about 4 hr, which could be a wholly exogenous effect of rising 4 hr earlier. Since subjects also rose 4 hr early in the second constant routine, any such effect does not affect the comparison by cross-correlation between the two constant routines.

That conventional methods may give a false impression of adaptation is well shown in Fig. 6 from subject AE2. On the constant routine the acrophase of urate excretion was in this subject similar to that under nychthemeral conditions. After the phase shift, adaptation appeared to be almost complete immediately, with a phase advance of about 6 hr, but when the subject spent another 24 hr under constant conditions the acrophase was found to have been *delayed* by about 8 hr; and by cross-correlation the rhythm was best described as a simple phase delay of 10 hr.

Rather than presenting further individual examples we have attempted a summary comparison between the observations under nychthemeral and under constant

conditions. For a valid assessment of the extent of adaptation suggested under nychthemeral routine we have required the presence of a significant ($P < 0.05$) fit of a cosinusoidal rhythm to the initial control, and to either the last 24 hr, under nychthemeral conditions after the time shift or at least a continuous 24 hr within not more than 12 hr of the end of this phase. This can only be compared with the extent of adaptation on a constant routine if the latter can be satisfactorily described by a cross-correlation with a simple time shift.
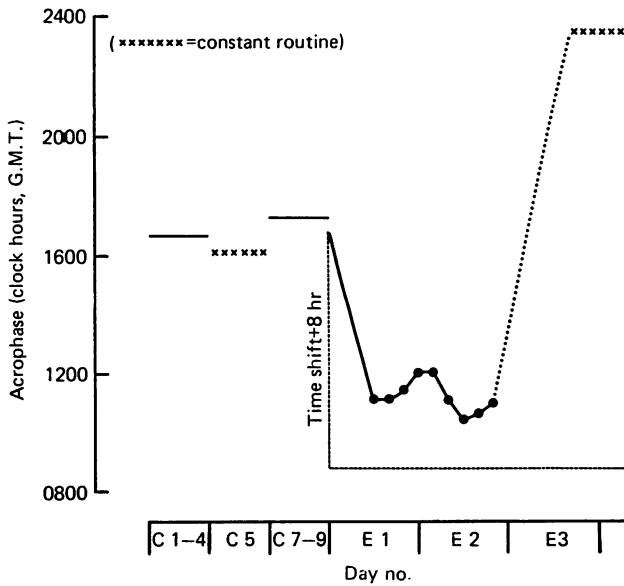

Fig. 6. Subject AE2, acrophases of urinary urate on nychthemeral and on constant routine, before (C1–9) and after (E1–3) simulated 8 hr eastward shift. Control acrophases are derived from a single cosine fit to each stretch of data, covering 3–4 days. After time shift, cosines are fitted pergressively to 24 hr sections with increments of 4 hr. Note irregular compression of time scale on abscissa before time shift.

When for any constituent in any individual these criteria are satisfied, we can calculate two values: the apparent shifts under nychthemeral and under constant routine conditions. When such a comparison can be validly made for any constituent upon a sufficient number of subjects undergoing the same time shift (four is the least number that we have accepted) we can further calculate the mean and standard error of the apparent adaptation under the two routines. However, since the shifts are circular we have not considered that a mean is valid unless all the relevant shifts can be grouped within a restricted arc.

The most consistent finding to emerge from this comparison concerns the apparent phase shifts after the clock was advanced by 8 hr (eastward shift). These are shown in Table 7, where it can be seen that for every urinary constituent there was a mean phase advance in the observations under nychthemeral conditions. Only for creatinine and phosphate did this approximate to the full 8 hr of the shift in habits, and for other constituents it appeared to be only a partial adaptation. By contrast, when the observations under constant conditions are examined it can be

seen that the shift was commonly irregular, and the mean never differed significantly from zero except for urate, where there was a phase delay instead of the phase advance which appeared under nychthemeral conditions. After an 8 hr delay in habits simulating a westward flight a phase delay in the urinary rhythms was usual, and there was no consistent difference between the observations under nychthemeral or constant conditions. Only for phosphate, as can be seen in Table 7, was this phase delay approximately equal to the 8 hr phase delay in habits. After a 12 hr shift there were relatively few instances that could be satisfactorily described in terms of a simple phase shift. Of these, the acrophase shift in sodium and potassium excretion under nychthemeral conditions appeared to be not far short of a full 12 hr whereas under constant conditions the shift was irregular and usually took the form of a phase delay. The most consistent behaviour was displayed by urate, which is also included in Table 7. Here under nychthemeral conditions adapation appeared to be virtually complete whereas under constant conditions there was a phase delay much less than 12 hr.

TABLE 7. Mean phase advance ($+$) or delay ($-$) of urinary rhythms after phase shift of habits, assessed under nychthemeral or constant routine, with s.e. and no. of observations. See text for method of calculation

Phase shift of habits	Constituent	Phase change (hr), under:	
		Nychthemeral routine	Constant conditions
8 hr advance	Water	$+4\cdot40 \pm 0\cdot46$ (4)	$-1\cdot75 \pm 1\cdot93$ (4)
8 hr advance	Potassium	$+3\cdot88 \pm 0\cdot43$ (15)	$-0\cdot53 \pm 0\cdot70$ (15)
8 hr advance	Sodium	$+4\cdot44 \pm 1\cdot16$ (11)	$-0\cdot27 \pm 1\cdot42$ (11)
8 hr advance	Chloride	$+3\cdot44 \pm 0\cdot60$ (8)	$-0\cdot88 \pm 1\cdot44$ (8)
8 hr advance	Creatinine	$+8\cdot39 \pm 0\cdot91$ (4)	Irregular
8 hr advance	Phosphate	$+8\cdot66 \pm 0\cdot90$ (4)	Irregular
8 hr advance	Calcium	$+6\cdot70 \pm 0\cdot32$ (6)	Irregular
8 hr advance	Urate	$+5\cdot65 \pm 0\cdot57$ (8)	$-5\cdot75 \pm 1\cdot84$ (8)
8 hr delay	Phosphate	$-8\cdot19 \pm 1\cdot36$ (6)	$-9\cdot33 \pm 1\cdot74$ (6)
12 hr	Urate	$+10\cdot23 \pm 0\cdot70$ (6)	$-6\cdot50 \pm 0\cdot62$ (6)

For the rhythms in deep body temperature, observations under nychthemeral conditions after an 8 hr westward or a 12 hr time shift indicated a greater phase delay than did observations on constant routine. After an 8 hr eastward time shift, the mean change under nychthemeral conditions was a phase advance; on constant routine it was a phase delay. The scatter between individuals was however considerable and consequently none of these differences between nychthemeral and constant conditions was significant. All the mean changes were much less than the imposed time shift.

DISCUSSION

Among the pieces of evidence for the endogeneity of human circadian rhythms (Mills, 1966) are their persistence in the absence of major external periodic influences, and their failure to adapt their phase immediately to a time shift. In view of this apparent independence of external periodicity, few attempts have been made to assess the contribution made to physiological rhythms by such regularly recurring events as meals, activity, rest and sleep. Mills *et al* (1978) have shown that sleep at any time of day or night lowers body temperature and is accompanied by increased

urinary phosphate and decreased potassium. Mills *et al.* (1977), in discussing the simultaneous presence of a circadian and a 21 hr rhythm in subjects living on a 21 hr day, pointed out that the 21 hr influence may be partly or wholly exogenous. Similarly when rhythms are observed after an abrupt time shift (see Elliott *et al.* 1972, and Aschoff, Hoffmann, Pohl & Wever, 1975, for references), their properties and behaviour are compounded with the direct effect of the shifted external influences.

Aschoff *et al.* (1975), reviewing the literature on entrainment in man and other species, mention that, for example, sleep at any hour can be accompanied by a fall of body temperature and thus cause confusion in the interpretation of apparent adaptation; nevertheless they accept the circadian pattern observed after a time shift as evidence of adaptation even when it is observed in subjects following nychthemeral habits. On our constant routine we believe that, by excluding the major external rhythmic influences, we have observed true adaptation of the endogenous rhythms.

The different phasing on the constant routine, shown in Table 6, suggests that not all exogenous influences have been removed. Phosphate excretion is known to fall abruptly on getting out of bed at any hour (Conroy & Mills, 1970), and the 4 hr phase advance of phosphate excretion seen in this table could result from the fact that on the constant routine subjects rose 4 hr early. It is possible that a similar explanation applies to urate whose excretory rhythm also has a major external influence, as indicated in the experiments of Mills *et al.* (1977) with subjects living on a 21 or 27 hr day; there is no evidence, however, that the hour of rising is an important influence on urate excretion. Only for these two constituents was there a large difference in the phase between nychthemeral and constant routine.

Other explanations are possible; thus the difference in phasing might result from differences in lighting or sampling regime between nychthemeral and constant routine conditions. Another possibility is that, despite minimizing sleep deprivation by starting the constant routine after the subject had previously been asleep for 4 hr, the small amount of sleep deprivation might be responsible for the shift of phase. Against this possibility is the observation by Aschoff *et al.* (1975) that sleep deprivation usually causes a phase delay rather than the advance observed in our data. Whatever the true explanation might be it does not affect the contrast between the much smaller extent of adaptation shown on the constant routines and that observed under nychthemeral conditions.

Apart from the present paper, we know of no similar attempt to study the effect of time shifts free from the interference of shifted nychthemeral habits, except the observations of Gerritzen (1962) and Gerritzen, Strengers & Esser (1969); these authors used a similar technique on a few subjects flown from Amsterdam to New York and to Anchorage and observed a rather slow adaptation of their urinary rhythms, but did not analyse them in detail.

Our observations suggest that the process of adaptation involves at least two components, shifting at different rates, suggesting that there has been dissociation between different components of controlling oscillators. This is apparent even in the small number of observations upon plasma corticosteroid concentration, for which in four out of eight subjects the pattern after a westward shift appeared to be a mixture of fully adapted and completely unadapted rhythms. Earlier studies with less frequent sampling would only describe this as partial adaptation (Daly, 1970; Elliott *et*

al. 1972). We cannot, however, exclude the possibility that the initial high value in Fig. 5 resulted from the stress of a venepuncture on waking, in which case this rhythm would be wholly unadapted.

The model for adaptation which we describe has many characteristics of the model proposed by Pittendrigh, Bruce & Kaus (1968). However, basic to our model is the assumption that during adaptation the form of rhythm attributed to both oscillators does not change. This is not necessarily the case (see, for example, Wever, 1973). The extent to which our model must be modified to take this into account is not known, but it suggests that a certain degree of caution is necessary.

It is uncertain how far the customary phase-locked rhythms on a usual nychthemeral existence represent a hierarchical system, in which the different components are passively driven by a single master clock (Mills, 1973), or whether there are complex interactions between separate inherently rhythmic organs and tissues (Moore-Ede, Schmelzer, Kass & Herd, 1976). It is clear, however, that many observed rhythms, such as excretion of individual urinary constituents, are influenced by numerous factors, circulatory, chemical and hormonal. If the organization is hierarchical, it involves a complex system of branching causation, with several different influences converging on the kidney; and our observations suggest that at least two of these controlling factors have adapted to time shift at different rates. The different ease of adaptation of different components (Table 3) presumably reflects the properties of these controlling oscillators. The corticosteroid rhythm is probably a major influence on potassium excretion (Mills, 1973; Moore-Ede, Schmelzer, Kass & Herd, 1977), and in the present experiments the extent of adaptation of steroid rhythms paralleled that of urinary potassium. Apart from this single instance, we are as yet in no position to speculate usefully upon the physiological processes represented by these oscillators, but it appears that urinary sodium, chloride and phosphate are most probably controlled by two influences. The situation may indeed be more complex, since several distinct factors can influence renal excretion of most constituents. A full description could involve more than two oscillators, adapting at different rates and even in different directions.

The means whereby an endogenous oscillator can be entrained by rhythmic events in the environment is also unknown, although some such process is continuously operative in maintaining a 24-hr period in rhythms which, in the absence of such environmental periodicities, usually free-run with a period longer than 24 hr (Mills *et al.* 1974; Wever, 1975). A mechanism has been suggested for such 'phase-locking' in small nocturnal mammals and other more lowly organisms, derived from the observed phase shifts which result from single pulses of light, but the suggestion that a similar mechanism operates in man (Palmer, 1976) is devoid of experimental basis. A simple explanation of adaptation to phase shift would be that the oscillators, when out of phase with environmental periodicities, exhibit non-entrained, free-running rhythms until they are again in phase when they become again synchronized or re-entrained. This accords with our observations that re-entrainment is easier after westward than eastward shifts, and that partial adaptation to 12-hr and even to eastward shifts usually proceeds by phase delay, a lengthening of the period. A shift of the temperature rhythm by phase delay after a phase advance of the lighting schedule, has been observed also by Aschoff *et al.* (1975).

The distinction we draw between true adaptation of the endogenous oscillator, and the apparent adaptation observed under nychthemeral conditions, has practical importance. Most situations in which man is exposed to time shifts in his habits are soon reversed; on shift work by return to normal nychthemeral habits at the weekend, after transmeridional travel by travelling home. Any adaptation of an endogenous oscillator would create problems of re-entrainment whereas a simple responsiveness to external periodicities should be rapidly reversible.

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