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Propensity for Lunar Periodicity in Hamsters and Its Significance For Biological Clock Theories.* (30656)

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Two alternative hypotheses exist for the timing system of the rhythmic daily activity patterns (circadian rhythms) persisting in animals shielded from all ordinary environmental variations. The endogenous hypothesis is that the basic timer is a very stable autonomous oscillation which in constant illumination and temperature runs freely revealing its individual inherited period. This postulated inherent rhythm is assumed to depend upon the daily light and temperature variations (Zeitgeber) for its synchronization to the day. The exogenous hypothesis is that organismic responsiveness to pervasive rhythmic geophysical variations constitutes the basic timer for the persisting circadian rhythms, and that the diverse frequencies observed even among individuals of the same species under the same conditions of unvarying light and temperature are derived through intraorganismic variable frequency transformation. Both rational biological explanations (1,2) and theoretical models(3) for such frequency transformation have been suggested. Thus, either timer hypothesis is now tenable, with personal preference and plausibility determining the choice between them for interpreting any given observed circadian characteristics.

The following two reports of observations of mice with accurate mean 24-hour periodicities illustrate two very different possible interpretations.

It has been noted(4) that in blinded mice held under controlled environmental temperature (24 ± 0.5°C) and on a 24-hour cyclic lighting regimen—12 hours of light alternating with 12 hours of darkness—several circadian rhythms that were desynchronized from the lighting schedule during the first few months after the operation returned to an average 24-hour period about 5 months after blinding. The interpretation favored was that the mice had become responsive to 24-hour variation in such subtle potentially effective factors as unidentified laboratory noises and perhaps odors which, for the same mice, were initially ineffective as synchronizers of postulated inherent rhythms.

In another study of mice(5) under conditions of constant illumination and temperature, and within the same enclosure, in which some of the mice were capable of displaying each its individual odd circadian period there was a tendency for the other mice to maintain an apparent precise mean 24-hour period, sometimes continuing over several months. This tendency was much greater than would have been expected from a random distribution of circadian periods. In mice displaying a circadian period different from 24 hours, there was demonstrated to be present simultaneously a characteristic underlying variation in activity related to hour-angle of the sun. This became evident when one examined units of data just long enough to randomize the circadian component. It was postulated that those mice displaying a 24-hour cycle were locking their circadian activity pattern to the 24-hour, presumably geophysically dependent component.

The two foregoing proposed explanations

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possess a common denominator, one widely agreed upon by students of circadian rhythms, namely that when rhythms display an accurate mean period synchronized with the solar-day under arbitrarily set levels of constant temperature and illumination, this is adequate reason to presume that the organism has not been completely shielded from all periodic information(6,7). Such a possibility can, of course, never be denied with confidence, especially since even such obvious factors as, for example, sound can never be completely excluded. Observed circadian periods are generally highly variable, usually falling within the range of 19 to 29 hours. They are functions of levels of constant illumination and temperature, of genotype, and even of previous history of the individual. The probability of precise mean synchrony of any fully autonomous endogenous oscillation with a natural geophysical period is understandably remote.

Evidence that a circadian periodicity in unvarying illumination and temperature is related in any manner to a subtle environmental variation other than one directly or indirectly effected by activity of man himself is obviously far more convincing when a lunar-day (24.8-hr) rather than a solar-day period is involved. No artificial cues from ordinary environmental factors associated with laboratory or experimental routines can be expected to exist for the lunar-day period. Therefore the following observations of two hamsters during extended sojourns in unvarying illumination become of great interest and significance. It is important to emphasize that these data were not specially selected series from among numerous recordings. These were the only 2 hamsters studied. A small male (A) and a large male hamster (B) were placed on Nov. 21, 1960, in running wheel actographs constructed of 0.25″ hardware cloth. The wheels were oriented N-S and were located in an inside room of a large laboratory building. The temperature of the room during the cooler months of the year was thermostatically held at about 25°C and during the warmer months irregularly drifted above this temperature even to about 29°C during prolonged periods of warm weather. In the absence of forced ventilation, however, no significant 24-hour variations in temperature occurred at these times, and lunar-day temperature variations are highly improbable. The illumination to which the hamsters were subjected was continuously less than 2 foot-candles maintained constant through a voltage-regulated power supply. At several-day intervals the food and water, always present in excess, were replenished. The two hamsters were about 6 feet apart in the same enclosure, screened from the sight of one another, but presumably each was able to hear activity of its partner. Continuous recording of activity was carried out for each hamster. The recording of one of the hamsters was terminated by its death of July 10, 1961; recording of the other was terminated on August 29, 1961.

Fig. 1 is a chart in which each hour that a hamster registered any wheel-running activity is shaded. Arbitrarily, no distinction was made in the chart between hours when the animal turned the wheel almost continuously and those hours when only one or a few turns occurred. In Fig. 1 the hourly activity for each animal is plotted on both solar- and lunar-day charts. The lunar-day relationship was obtained by replotting each hour of the solar-day in its natural hourly relationship to the hour of the upper transit of the moon. Just as one method of presenting the data enabled easy visualization of the day-to-day changes in activity relative to time of solar day, so did the other enable comparable visualization of the alteration with respect to time of lunar day.

Hamster A, upon being placed under the recording conditions, rather quickly converted its normal 24-hour activity rhythm into a typical circadian one which persisted for the more than 9-month period of the study. This is evident even from superficial examination of the times of activity onsets in Column 1 of Fig. 1. It is also apparent from inspection that the period of the persistent activity did not remain constant but altered with time.

In Fig. 1, Column 2, in which the same data are plotted in relation to hour of the lunar day it is seen that during the last six days of November, 1960, the frequency of

onset of activity was close to that of the lunar day. Thereafter, until about December 27, the period of the rhythm lay between values for a solar day and lunar day, but from the latter date the hour of onset of activity occurred, with some irregularities, at the same hour of lunar day until about January 21, 1961, when the period became and remained distinctly shorter than the lunar day until about Jan. 24. After this date a lunar-day period again prevailed until Feb. 3. After about 5 days of clearly shortened cycles, a mean lunar-day period in the time of activity onset reappeared and continued until March 12. Following another 3 days of a shortened cycle, an essentially precise mean

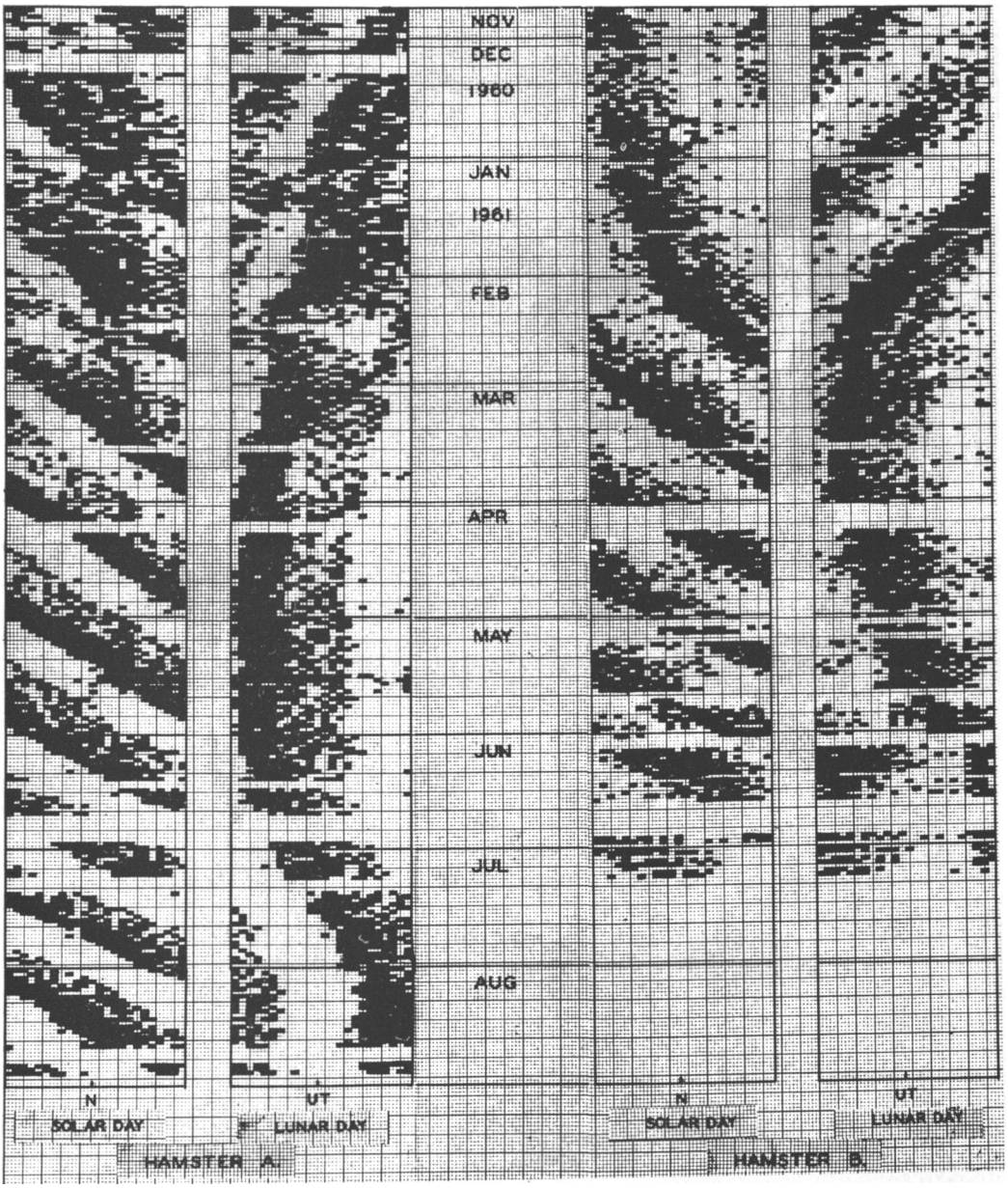

FIG. 1. Solar-day and lunar-day plots of circadian rhythmicity of 2 hamsters. N—noon; UT—upper lunar transit.

lunar-day frequency reappeared about the middle of March and continued steadily until about June 18. At the latter time the period became longer than a lunar-day, and continued with a relatively uniform mean period until about August 2 when the period abruptly shortened to become again a somewhat irregular, but definite average lunar-day period continuing through August 28.

The results obtained with Hamster B are also shown in Fig. 1. From Column 3, it is evident that activity onsets retained for about the first 3 weeks their normal 24-hour frequency. By late December, 1960, and into January and February, 1961, the cycles gradually lengthened. About the middle of February the rhythm altered its period rapidly to become lunar-day whereafter this period was held essentially unchanged until the end of March. By early April the period became longer than lunar-day, except for apparent tendencies to revert to lunar-day for several day intervals during late April and mid-May. About the end of May an abruptly lengthened period appeared and then shortened equally suddenly in less than a week with a readoption of a mean lunar-day period which continued without significant alteration from June 4 until the recording ended on July 8.

It should be noted in comparing the recordings of hamsters A and B that there was virtually no tendency for either the times of the solar day or the lunar day of activity of one of the animals to be correlated with that simultaneously present for the other one, or for the concurrent frequencies of the circadian rhythms of the 2 animals to be the same despite their being within audible range of one another. Hence in evaluating the conspicuous propensities of these 2 hamsters for lunar-day periodicity, it seemed reasonable to consider the 9¼ and 7½ months for the 2 animals as 2 independent series of data.

The remarkable tendency to synchronize with the lunar-day existed despite the fact that the observed hamster circadian periods ranged from 24.0 to at least 25.7 hours. Each of the 2 hamsters, independently, reached the beginning of an extended interval of lunar-day frequency when the time of initiation of its daily cycle of activity came essentially to synchronize at one and the same time with a lower lunar transit and an upper solar transit (full moon). Similarly, both hamsters, independently at different times, later departed from lunar-day to adopt a longer overt period. But later both hamsters regained anew a lunar-day frequency beginning when the time of onset of activity had shifted to occur near a time of simultaneous moonset and sunset (new moon). While both hamsters showed evidence of a capacity to synchronize with the lunar-day in a number of phase-angle relationships it appeared from the remarkable similarity of behavior of the 2 hamsters that synchronization of the activity cycle to lunar-day frequency became most stable when initiated in 2 specific phase-angle relationships, namely with activity onset near lunar 'midnight' and near moonset. The slightly different phase relationships between the 2 hamsters as they adopted a lunar-day period at these two widely different phase angles of the natural cycles are no more different than what is frequently observed between 2 individual mammals responding to the same natural day-night light and temperature cycles.

Suggestive of a solar-day contribution to the foregoing phenomenon is 1) the initial adherence of Hamster B to this period for more than 3 weeks and 2) the observation that the times of initiation of the *major* lunar entrainments, and departures from them, for the 2 hamsters were separated by either close to 30 or to 60 days, and for both hamsters occurred when the initiation of activity was near noon or sunset in the natural solar-day cycle.

The mean period over several monthly intervals during this hamster study is so overtly lunar day as to render unnecessary the use of more elaborate techniques of frequency analysis to establish it.

Substantial lunar variations in activity have been previously reported in two extended studies of male rats(8,9) held in continuous dim illumination. In both studies the rats displayed much greater activity when the moon was below the horizon than when above it, one of the two favored relationships of the hamsters. In one study(8) the variation co-

existed with a conspicuous overt 25¼-hour circadian periodism. In the other study(9) the lunar periodism was itself a large overt one, apparently having synchronized the rat's normal solar-day activity pattern to lunar day.

Persistent lunar-day components have been reported in rhythmic systems of other organisms. Lunar-correlated variations in drift of a circadian peak have been reported for cockroaches(10). Corn beetles(11) have been reported to have a lunar rhythmic component. A large amount of work on fiddler crabs(12, 13), green crabs(14) and sand-crabs(21) has established the conspicuous coexistence of persistent solar-day and lunar-day rhythmic components with indications that their contributions may be in some cases simply additive and in others one may modulate the frequency of the other (monthly or semi-monthly variations). A clear, overt lunar monthly variation occurs in orientational tendency of fresh-water planarians(15,16) and it has been demonstrated(17) that a 180° phase shift in the lunar variation may be effected by a 180° experimental shift in the ambient horizontal magnetic vector.

Published data on *Peromyscus*(18) suggest a semi-monthly variation in the progression of the onsets of activity over the solar-day. A record of drift over the day of the onsets of activity in a flying squirrel(19, Fig. 2) which has, incidentally, often been cited as an outstanding example of the constancy of a circadian period and evidence for basic endogenous timing, exhibits a systematic variation in frequency with about 15 days intervening between maximum and minimum during the 25-day study, suggesting an interaction with an underlying lunar component.

The frequency changes of the hamster rhythms in the constant low illumination between major geophysical frequencies (solar and lunar) and non-geophysical ones may be considered in the perspective of the two clock hypotheses. In terms of either one, a subtle exogenous influence appears most probably to be involved in some manner. If an independent internal oscillator system is present, subtle lunar-day geophysical variations appear under some circumstances able to entrain it, and to do so more readily in some phase-angle relationships of the activity cycles than in others. If no endogenous autonomous oscillation exists there may simply be a continuing subtle balance between two factors in determining period of the persisting daily cycles. One of these factors could be autophasing(1,2) by which the recurring activity patterns are phase-shifted a little each day relative to a reference periodic input or 'carrier' rhythm which may be either the solar or lunar day. The other factor could be an entraining tendency of variations in specific geophysical parameters for the activity cycles.

Of these two postulated explanations of the lunar propensity the one involving exogenous timing is deemed the simpler in view of the current absence, on the one hand, of any secure evidence for the existence of an inherent oscillator system, while on the other hand the same exogenous factor playing the synchronizer role could also be a primary timer. In addition, evidence of several types has established organismic responsiveness to pervasive geophysical factors, especially to magnetic, electric, and electromagnetic components of the natural geophysical environment(2,15,16,20). Even were such direct evidence for organismic sensitivities to subtle atmospheric forces not already available, the exogenous hypothesis would still be as probable as the endogenous one.

In brief, the only plausible explanation for the lunar propensity, so remarkably similar for these two hamsters, is considered to be that a subtle rhythmic geophysical input is contributing to their observed circadian patterns. It seems likely, therefore, that the diverse circadian patterns and frequencies, wherever and whenever they occur, reflect not only differences in the controlled conditions of such obvious factors as levels of constant light and temperature and in the individual organismic reactions to them, but also reflect variations in the uncontrolled subtle geophysical environment.

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Stimulation of Erythrocyte Glycolysis by Nicotinamide Adenylates. (30657)

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The purine nucleosides, particularly adenosine and inosine, have been repeatedly demonstrated to diminish the progressive fall in the glycolytic activity of human erythrocytes which occurs under the conditions of blood storage. These compounds also possess the capacity to replenish the ATP level and thereby restore glucose utilization of cells that have been metabolically depleted(1). The nucleosides have been shown to be freely accessible to the metabolic machinery of the cell whereas the nucleoside phosphates are not generally considered to be as readily permeable and consequently it is not to be expected that they would influence metabolism significantly(2). However, in the course of an investigation of the metabolism of labelled yeast adenylic acid compounds *in vivo*, it was noted that the red cells of the rat and other animals accumulated the label to an unexpected extent. It was of interest to reassess the ability of the adenine nucleoside phosphates to contribute to the support of the metabolism of red cells. This investigation is concerned with effects of these compounds on the glucose metabolism of human erythrocytes. For this purpose the nicotinamide salts of the adenine nucleotides possess distinct advantages in that they do not contribute excess metallic cation and also because nicotinamide has been shown to restore the synthesis of pyridine nucleotides and particularly the pyridine coenzyme levels of aged cells. In addition the nucleotide-organic base combination possesses a potentially useful buffering capacity that could be of significance in maintaining the hydrogen-ion concentration of metabolizing cell suspensions.

That the glycolytic activity of mature erythrocytes is sensitive to the hydrogen-ion concentration is well substantiated(3). As a result of the progressive decrease of pH from the accumulation of lactic acid, the rate of glucose utilization diminishes rapidly. Bishop (4) has shown that if the hydrogen-ion concentration of a suspension of cells is continuously adjusted, the glycolytic activity could be maintained for several days. Although several enzyme reactions of the glycolytic pathway have been implicated, it appears certain that the acid-sensitive reaction is above the glyceraldehyde-3-phosphate step because the addition of nucleosides stimulates glycolysis with an increase of lactate production de-