The seismicity increased approximately concurrently with a change in the pattern of strain accumulation in southern California. The change is one that would favor strain release on the right-lateral strike-slip or normal faults having a northwesterly to northerly trend which characterize the San Andreas fault system and the Basin and Range faults. The geodetic observations are not extensive or frequent enough to establish the correlation in every locale in which the increase in seismicity has been observed. It appears, nevertheless, that the strain buildup is nonlinear and that there may be periods in which an increased susceptibility to damaging earthquakes may be identified.

We conclude that California is likely to experience one or more $M > 7$ earthquake in the next decade. However, without extensive real-time observations of strain and seismicity in the areas of principal concern, more precise and reliable predictions of such events are unlikely in that time. There is not yet an adequate observational base against which to test physical models of the failure process that leads to great earthquakes. Reliance on empirically established precursory phenomena will still be necessary until a better formulation of a theoretical model is possible. Both as a means of developing the observational basis for better models and collecting data which will have value as precursory signals, an extensive network for closely monitoring and for analyzing strain and seismicity data in real time is imperative.

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Regulation of Circadian Rhythmicity

Joseph S. Takahashi and Martin Zatz

It's been a hard day's night.
I should be sleeping like a log.
—The Beatles, 1964

Daily rhythms in behavior, such as rest and activity, are so familiar and so clearly coupled to the cycle of night and day that they did not elicit systematic scientific investigation until the present century. It was, naturally, believed that they merely reflected responses to the alternation of light and darkness in the environment. This interpretation was first questioned in 1729 when the French astronomer de Mairan observed that the daily leaf movements of a plant persisted in constant darkness (*1*). Two hundred years later, the persistence of periodicity in the activity of wild mice housed in constant light led Johnson to postulate the presence of a "self-winding and self-regulating physiological clock" (*2*). The existence of endogenous clocks did not become widely accepted, however, until the 1950's (*3*). The fact that the periods of free-running rhythms (that is, those observed under constant conditions) differ from those of all known environmental cycles and differ among individuals excludes the possibility that cryptic environmental cues drive or time these rhythms. By now, daily rhythms in a variety of organisms and in many functions within an individual have been shown to free run in nonperiodic environments (*4–6*). Such self-sustained oscillations with periods close to 24 hours are called circadian rhythms.

Although circadian rhythms are generated endogenously they are regulated by exogenous cycles, especially those of light and darkness. The effects of environmental cycles on two circadian rhythms in humans are illustrated in Fig. 1. During the first 6 days, while the subject was living under natural conditions, his sleep-wake cycle and body-temperature rhythms expressed period lengths of 24 hours and maintained stable phase relationships to the day-night cycle. On the seventh day, the subject was isolated underground without access to any time cues (*7*). During the subsequent 17 days of isolation, his sleep-wake cycle and body-temperature rhythms drifted toward later times each day and expressed a free-running period of 25.4 hours. After returning to natural conditions on the 24th day, his rhythms again became synchronized to the 24-hour day. If the subject subsequently made a transcontinental flight from Europe to America, his rhythms would shift (while he experienced "jet lag") to match the phase of the local environmental cycle. The imposition of period and phase con-

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trol by environmental cues is called entrainment. Daily rhythms observed in the field result from the entraining action of the periodic environment on the organism's internal oscillators (*8*).

In people, sleep and wakefulness, cognitive and motor performance, body temperature, serum hormone levels, and urinary excretion vary rhythmically (*9*). Alterations in human circadian rhythms are important in some clinical conditions (*10*). The susceptibility of animals to toxic agents varies dramatically with time of day (*11*). Similarly, the sensitivity of many sensory and regulatory processes such as photoreception (*12*), synaptic excitability (*13*), and receptor-mediated events (*14*) vary with a circadian rhythm. Clearly, the physiological state of an organism varies throughout the day. Oscillatory systems permit organisms to anticipate periodic events in the environment and to initiate slow processes before they are required (*15, 16*). Circadian systems thus provide a framework for the temporal organization of the animal; as Pittendrigh has emphasized, "they organize 'a day within,' that is, an evolved match to the periodicity of the external world" (*17, 18*).

Questions about the physiology of circadian rhythms fall into three major categories: (i) those exploring the functional organization of circadian systems; (ii) those exploring the anatomical location and physiological basis of circadian pacemaking systems; and (iii) those exploring the cellular and biochemical mechanisms that generate the oscillation itself. We first describe some of the general properties of circadian rhythms and some of the concepts and constraints that they provide for experiments. We then summarize recent work on the anatomical basis of circadian rhythmicity in mammals. Finally, we describe two model systems, the pineal gland and the *Aplysia* eye, which permit both physiological and biochemical analysis of circadian oscillators. Recent progress in locating circadian pacemakers (*19–23*) and in investigating circadian rhythms in explanted organs (*23*) promises to deepen our understanding of circadian systems.

Properties of Circadian Rhythms

General features. Circadian rhythms are ubiquitous—examples can be found in all eukaryotic classes including unicellular organisms (*24*). Circadian rhythms are genetically determined. Single-gene mutations that alter the period length have been isolated in four organisms: *Drosophila melanogaster* (*25*), *Drosophila pseudoobscura* (*17*), *Chlamydomonas reinhardi* (*26*), and *Neurospora crassa* (*27*). Circadian oscillations are remarkably precise. The variation in cycle length in nocturnal rodents can be less than 3 minutes (standard deviation) (*28*). The steady-state period is temperature compensated, with temperature coefficients (Q_{10}) normally ranging from 0.8 to 1.2 (*3, 29*).

Summary. Daily rhythms in many behavioral, physiological, and biochemical functions are generated by endogenous oscillators that function as internal 24-hour clocks. Under natural conditions, these oscillators are synchronized to the daily environmental cycle of light and darkness. Recent advances in locating circadian pacemakers in the brain and in establishing model systems promise to shed light on the cellular and biochemical mechanisms involved in the generation and regulation of circadian rhythms.

Entrainment. In the experimental investigation of circadian systems, the free-running rhythm is considered to be the "basal" state. Its period reflects the period of the unrestrained endogenous oscillator. Only a few environmental variables can entrain circadian rhythms. Light and temperature are the dominant agents, although in man social cues may be important (*30*). The formal mechanism of entrainment to light cycles has been studied extensively and is well understood (*31–33*). Our understanding derives from the investigation of the effects of brief light pulses on free-running rhythms. Single pulses of light can cause shifts in the steady-state phase of the free-running circadian locomotor rhythm (Fig. 2A). Such phase shifts occur by lengthening or shortening the period of the rhythm for one (or a few) cycles. A standard light pulse can have one of three effects: no shift, delay, or advance. The magnitude and direction of the phase shift depends on the phase of the rhythm at which the stimulus is applied. This relationship is illustrated in the phase response curve (Fig. 2B). For the hamster, the onset of activity is used as a phase reference and is defined as circadian time 12. The hamster is active during "subjective night" and is inactive during "subjective day." Phase response curves have three general features: little or no phase shifts occur after light pulses given during most of the subjective day; phase delays occur after light pulses during the early portion of the subjective night, and phase advances occur during the late subjective night (*34*). Light pulses falling on the delay portion of the

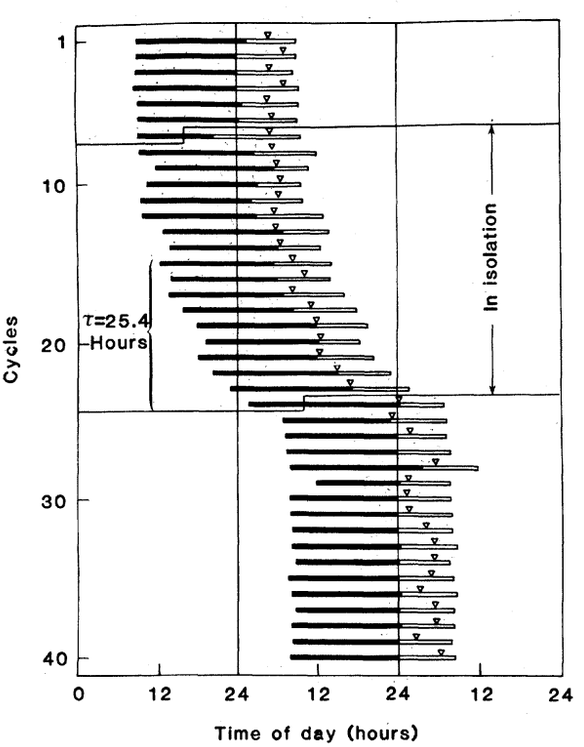

Fig. 1. Circadian rhythms in a male human subject from experiments conducted by Aschoff and Wever in Andechs, West Germany. Each horizontal bar represents one cycle of wakefulness (dark) and sleep (light). Successive cycles are drawn beneath each other. Open triangles indicate the time of minimal body temperature in each cycle; τ represents circadian period. [Redrawn from Aschoff (*7*)]

curve cause the period to lengthen temporarily and those falling on the advance portion cause the period to shorten temporarily. Phase response curves, although obtained by measurements of the overt rhythmic function, reflect the temporal properties of the underlying circadian oscillator (*31, 32*).

Stable entrainment to a light-dark cycle is achieved by repeated daily adjustments of period and phase. Individuals with a free-running period shorter than the period of the light cycle achieve entrainment by way of delays, and individuals with free-running periods longer than that of the light cycle entrain by way of advances. During steady-state entrainment, the daily phase shift is equal to the difference between the period of the free-running rhythm and that of the entraining cycle. For steady-state entrainment to be possible, the amplitude of the phase response curve must be at least as large as the difference between the period of the free-running rhythm and that of the entraining cycle. Thus the locomotor activity rhythm of hamsters will not show stable entrainment to cycles with periods of 21 or 26 hours.

Components of circadian systems. The physiological system responsible for circadian rhythmicity must contain at least three major components: (i) an input pathway for entrainment; (ii) a circadian pacemaker that generates the oscillation; and (iii) an output pathway that results in the expression of the overt rhythm that we measure (Fig. 3). For photic entrainment to occur, a photoreceptor must be coupled to the circadian pacemaker, and to generate a rhythmic output, the pacemaker must be coupled to the system that regulates the overt process. As Eskin (*23*) has emphasized, this simple scheme provides a framework for a number of questions. Where is the circadian pacemaker located? How is environmental information coded and transmitted to the circadian pacemaker? How does the circadian pacemaker regulate its outputs? The model is also useful in interpreting results of experiments. For example, the experimental abolition of circadian rhythmicity is, by itself, ambiguous since it could result from effects on the pacemaker or on the output pathway. In fact most treatments that produce this result probably do so by uncoupling the measured output from the circadian pacemaker. This is equivalent to affecting the "hands" of the clock and not its internal mechanism. Even agents that affect the clock itself, such as light, can influence overt rhythms by mechanisms that bypass the circadian pacemaker. Such effects are called "masking." In principle, it is possible to distinguish effects on the output pathway from those on the oscillator or its input by measuring a parameter that reflects the behavior of the pacemaker. There are only two such parameters: the steady-state phase of the oscillation and its period length (*31, 32*). Treatments that affect either of these two parameters can be interpreted unambiguously as having effects on the circadian pacemaker either directly or secondarily via an input pathway.

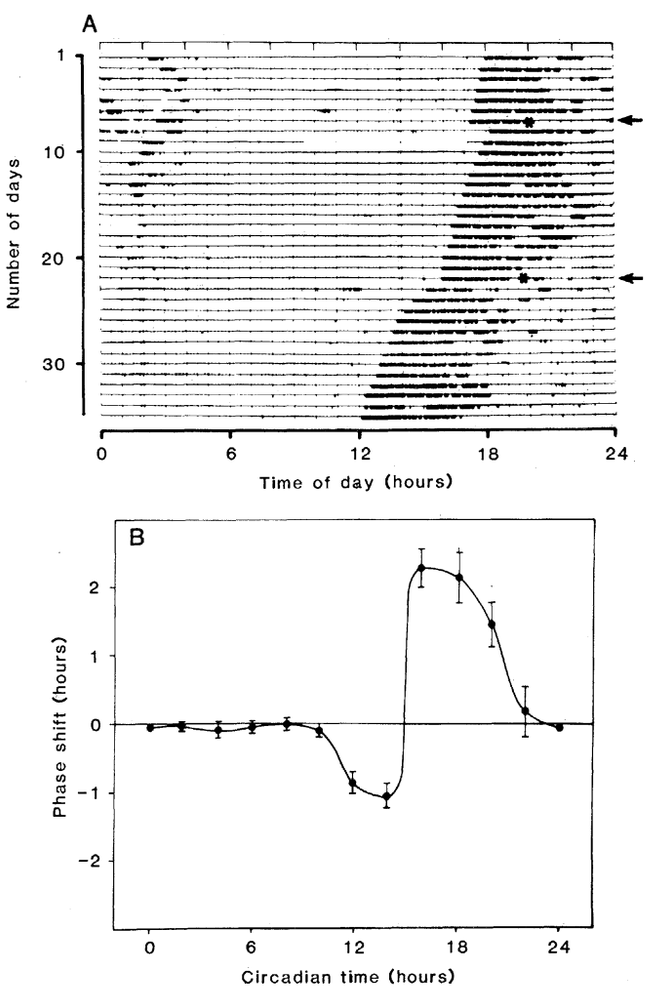

Fig. 2. Effect of short light pulses on the circadian rhythm of hamster locomotor activity. (A) A hamster, free-running in constant darkness, was exposed to light for 15 minutes (on the days indicated by arrows and at the times indicated by asterisks). The first stimulus caused a phase delay and the second caused a phase advance. [From Daan and Pittendrigh (*32*)] (B) A phase response curve illustrating the effect of light pulses (60 minutes) given at various times relative to the hamster's rhythm of locomotor activity. The phase at which the light was turned on was determined relative to the onset of activity which is assigned circadian time 12 by convention. Phase advances are plotted as positive and phase delays are plotted as negative. Each point is the mean ± standard deviation of six animals. Data were obtained by J. S. Takahashi, F. C. Davis, and M. Menaker.

Anatomical Basis of Circadian Systems in Mammals

The suprachiasmatic nucleus. To study the physiological basis of circadian rhythms, one has to locate the components of the circadian system, especially the pacemaker. There are two clear strategies for finding the circadian pacemaker. One can work either "upstream" from the overt rhythm to the pacemaker, or "downstream" from the photoreceptor to the pacemaker (Fig. 3). In mammals, the photoreceptors responsible for entrainment are located in the retina—blinded rodents free run under all lighting conditions (*35*). Seeking the pathway from the retina to the circadian pacemaker, Moore and Eichler, and Stephan and Zucker interrupted both the primary optic tracts and the accessory optic system (*36*). However, these lesions failed to disrupt entrainment. This unexpected result led to the hypothesis that a direct retinohypothalamic pathway, whose existence was unsubstantiated at the time, conveyed photic information to the pacemaker. A reinvestigation of this pathway using autoradiographic tracing methods indeed revealed a direct retinohypothalamic tract terminating in the suprachiasmatic nuclei (SCN) (*37*). In an attempt to interrupt this pathway, both groups lesioned the terminal nuclei of this projection and found that not only was entrainment lost, but in addition, circadian rhythmicity was abolished in three different parameters (*36*).

In the last decade, it has become clear that the SCN play an important role in the maintenance of a diverse number of

circadian rhythms in rodents (*21*). The retinohypothalamic tract clearly plays the dominant role in coupling the mammalian circadian system to the external light-dark cycle to achieve stable entrainment [although some information can reach the circadian system through other pathways (*38*)]. Among the parameters disrupted by lesions of the SCN are wheel-running activity, drinking, feeding, sleep, temperature, adrenal corticosterone, pineal *N*-acetyltransferase, ovulation, estrous cyclicity, and photoperiodic time measurement (*39*). Detailed analysis of long-term locomotor records from hamsters shows that suprachiasmatic lesions severely disrupt entrainment to light cycles and eliminate circadian rhythmicity in constant conditions. Total arrhythmicity, however, is rarely achieved (*40*). Unlike other systems that show plasticity after neonatal injury, circadian rhythms were never reestablished in rats subjected to SCN lesions in the perinatal period (*41*).

Although recent work strongly suggests that the SCN contain self-sustained circadian oscillators, the evidence is as yet indirect. The metabolic rate of the suprachiasmatic nucleus as measured by [2-^{14}C]deoxyglucose uptake oscillates with a circadian rhythm in vivo (*42, 43*). The rhythm of glucose utilization appears to be unique to this portion of the brain (Fig. 4). The spontaneous neural activity of the suprachiasmatic nucleus also oscillates with a circadian rhythm. Using long-term multiple unit recording, Inouye and Kawamura (*44, 45*) have shown that the circadian rhythm of neural activity in the suprachiasmatic nucleus persists for at least 34 cycles after surgical isolation. Outside the hypothalamic island, rhythmic neural activity was abolished. When the retinohypothalamic tract was spared, the neural rhythm of the suprachiasmatic nucleus could be entrained by light cycles and could be phase shifted by single pulses of light. Thus, the rhythm of multiunit activity recorded from the suprachiasmatic nucleus displays all the basic characteristics of circadian rhythms. Although the neural isolation experiments do not exclude hormonal inputs, they strongly suggest that a circadian pacemaker is located within the hypothalamic island.

As a result of the work on the function of the SCN, interest in the anatomy of these nuclei has increased (*46*). The SCN lie adjacent to the midline, ventral and lateral to the third ventricle, and immediately above the optic chiasm. Each nucleus contains about 10,000 small, densely packed neurons (*46*). Numerous neurotransmitters and neuropeptides (or immunoreactivity to antibodies directed against them) have been found in the SCN (*47*). Major afferent projections include the retinohypothalamic tract (*37*), an input from the lateral geniculate body (*48*), and a serotonergic input from the midbrain raphe (*49*). There are two efferent projections within the hypothalamus: a periventricular system to the paraventricular nucleus and dorsomedial hypothalamic nucleus, and a ventral system that descends to the tuberal region (*50*). Extrahypothalamic projections include a prominent one to the periventricular thalamic nucleus and sparse fibers to the medial preoptic area, lateral septal nucleus, and midbrain central gray (*50*). With the exception of the retinohypothalamic tract, the specific functions of the neural connections of the SCN remain to be determined.

Pharmacological approaches to studying the mammalian circadian system appear promising. Gonadal steroids can shorten the free-running period of locomotor activity in rodents (*51*). A cholinergic agonist, carbachol, injected into the lateral ventricle causes phase shifts in both the rhythm of pineal *N*-acetyltransferase in rats and the locomotor rhythm in mice (*52*). Several other substances found in the SCN were ineffective. The phase shifts induced by carbachol are phase dependent and display a phase response curve similar to that obtained with light. Electrical stimulation of the suprachiasmatic nucleus in rodents also produces phase-dependent phase shifts that are similar to those of light (*53*).

Multiple oscillators. Under ordinary conditions, the circadian pacemaking system acts as a single oscillator. However, there is compelling evidence for the presence of multiple circadian oscillators (*17, 54*). In man, the sleep-wake cycle and the body-temperature rhythm occasionally dissociate from each other

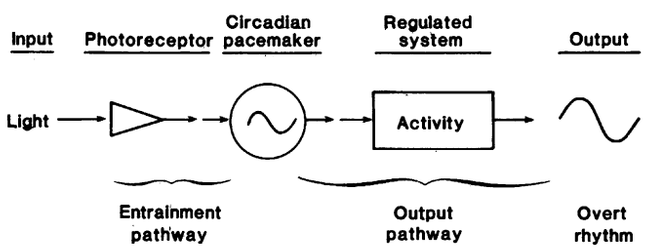

Fig. 3. A schematic model of the components of a circadian system. [After Eskin (*23*)]

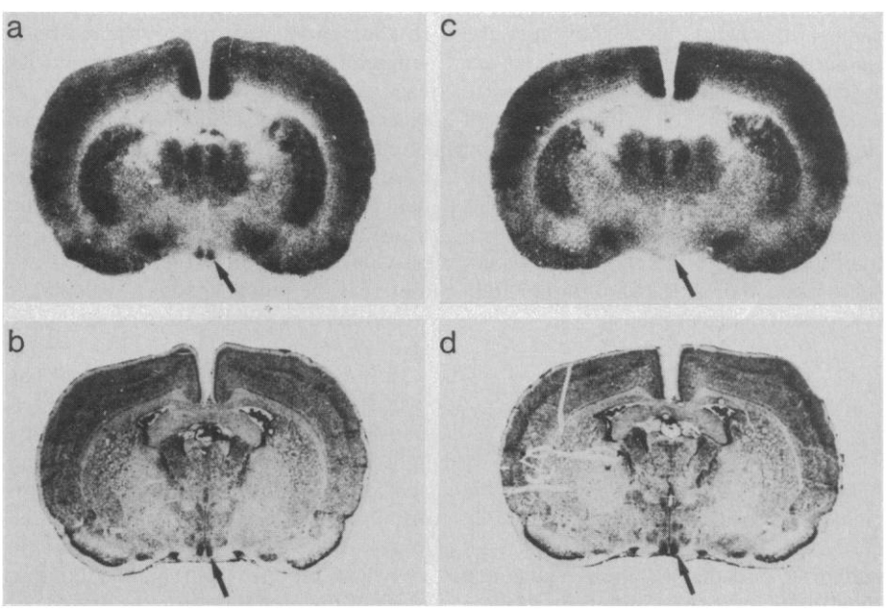

Fig. 4. Daily rhythms in glucose utilization by the SCN. Coronal brain sections from rats injected with radioactive 2-deoxyglucose are shown. The upper sections (a and c) are autoradiographs and the lower (b and d) are adjacent Nissl-stained sections. In the autoradiographs dark areas reflect greater glucose utilization than light areas. The sections on the left (a and b) are from an entrained animal injected during the day, and those on the right (c and d) from an animal injected during the night. The SCN (arrows) are visible in both Nissl-stained sections (b and d). In the autoradiographs, however, they are visible in the day-injected (a) but not in the night-injected (c) animal. Such data indicate increased glucose utilization by the SCN during the day. [From Schwartz and Gainer (*42*)]

and free run with different periods. This phenomenon is called "internal dissociation" (*9*). In other species, the circadian rhythm of locomotor activity splits into two components under certain conditions (*55, 56*). In two recent studies on primates, suprachiasmatic lesions abolished the drinking rhythm (*57*) and the CSF melatonin rhythm (*58*); however, they did not abolish the body temperature (*57*) or cortisol rhythm (*58*). These experiments clearly establish that circadian oscillators located outside the suprachiasmatic region can regulate the body temperature and cortisol rhythms in primates. The location of these oscillators is unknown.

The organization of multioscillator systems is complex both conceptually and experimentally. Two general types of organization could underlie such systems: (i) a hierarchy in which a dominant pacemaker drives the system and imposes its period and phase upon subordinate oscillators (*17*); and (ii) a nonhierarchical, mutually coupled system in which no single oscillator is dominant but redundant oscillators share the pacemaking role (*17, 28*). The organization of cardiac pacemakers illustrates these principles. The system generating the cardiac rhythm is both hierarchical and mutually coupled. The sinoatrial node normally plays a pacemaking role, imposing its period on the cardiac rhythm. In its absence, the atrioventricular node controls the heart rate. When both the sinoatrial and atrioventricular nodes are absent, other cardiac cells continue to pace the heart. In cell culture, groups of cardiac cells beat in unison with a period that depends on the number of cells and their mutual coupling (*59*). The heart analogy illustrates the difficulty in identifying a pacemaker—its removal does not necessarily abolish the rhythm nor fully elucidate its normal role.

Model Systems for Cellular and Biochemical Analysis

The pineal gland. Investigation of the pineal gland has been central to the study of the physiology and biochemistry of vertebrate circadian rhythms. The pineal gland appears to play different roles in the circadian systems of birds and mammals. Whereas it acts as a pacemaker in birds, in mammals it merely expresses a driven rhythm. The regulation of pineal biochemistry also differs in these two vertebrate classes. In both birds and mammals, the gland expresses an overt rhythm of indoleamine metabolism that results in the nocturnal synthesis and secretion of the hormone melatonin (*60, 61*). The pivotal step for nocturnal melatonin synthesis is a dramatic (greater than tenfold) increase in the activity of the enzyme serotonin *N*-acetyltransferase (*60, 62*).

In mammals, the rhythm of melatonin synthesis is driven by a circadian pacemaker in the brain (*46, 62*). The gland is coupled to the clock by sympathetic nerve fibers whose cell bodies reside in the superior cervical ganglia (*63*). These fibers transmit the oscillatory information from the hypothalamus to the gland via changes in the release of the neurotransmitter norepinephrine. In a light-dark cycle norepinephrine release is high at night and low during the day (*64*). If the connection between the gland and the spinal cord is cut, the rhythm in indoleamine metabolism is abolished (*46, 62*). Indeed, a lesion anywhere between the suprachiasmatic nucleus and the gland will have the same effect; the gland remains in its quiescent daytime state.

In contrast, the avian pineal is not dependent on neural connections with the brain for rhythmic melatonin production (*65*). Menaker and his colleagues have provided much evidence that the avian pineal gland contains a circadian pacemaker that plays a major role in the overall temporal organization of the bird (*66*). In the house sparrow, removal of the pineal eliminates the free-running rhythms in locomotor activity and body temperature (*67*). Circadian rhythmicity can be restored by transplantation of pineal tissue from a donor into the anterior chamber of the eye of an arrhythmic host. Furthermore, the restored rhythm has the phase of the donor (*68*). The transfer of phase in the restored rhythm provides the strongest evidence available that the pineal acts as a pacemaker within the circadian system of the house sparrow (*69*).

In both birds and mammals, light has two effects on pineal serotonin *N*-acetyltransferase activity and melatonin levels: light-dark cycles entrain the rhythm and acute light exposure at night rapidly reduces enzyme activity and melatonin levels. In the rat, light is effective only in vivo and requires that all the components of the pathway from the eye to the gland be intact (*46, 62*). In contrast, the avian pineal gland is directly photosensitive (*70*); neither the eyes nor sympathetic innervation are required for it to respond to light (*65*).

The regulation of indoleamine metabolism in both the chick and rat pineal has been investigated in organ culture. Both the differences and similarities in these systems are intriguing. In the explanted rat pineal, the nocturnal increase in serotonin *N*-acetyltransferase activity and melatonin synthesis do not occur spontaneously. They can, however, be induced by incubation of the rat pineal with norepinephrine or its analog isoproterenol. The mechanism of action of such stimulation has been extensively investigated (*14, 62*). Very briefly, norepinephrine interacts with the β-adrenergic receptors on the pinealocytes, resulting in the synthesis of adenosine 3′,5′-monophosphate (cyclic AMP). Cyclic AMP, in turn, causes the induction of serotonin *N*-acetyltransferase activity by a process involving RNA and protein synthesis. Thus, cyclic AMP acts as the second messenger for norepinephrine in the β-adrenergic stimulation of the gland. The acute effects of light in vivo (blockade or reversal of the nocturnal increase in enzyme activity) appear to be mediated at the level of the mammalian gland by a reduction in norepinephrine release. These effects can be prevented by the injection of isoproterenol and mimicked by the injection of propranolol (*71*).

The chick pineal gland can also show a "nocturnal" increase in serotonin *N*-acetyltransferase activity and melatonin synthesis in organ culture (*70*). However, unlike the mammalian pineal, such increases are spontaneous and phase-dependent. They are not mediated by β-adrenergic receptors; indeed, incubation with norepinephrine inhibits the increase in enzyme activity (*72*). Furthermore, the gland is sensitive to light in culture (*70*). Exposure to light will inhibit or reverse the increase in enzyme activity or melatonin release. In light-dark cycles, the cultured gland continues to express a rhythm of serotonin *N*-acetyltransferase activity for many days. The rhythm remains robust in light-dark cycles, but damps after the first cycle in constant darkness (*73*). Nevertheless, the rhythm of melatonin release can be detected for at least four cycles in constant darkness (*74*). Thus, the rhythm is circadian. Both the acute and entraining effects of light can be observed in vitro. At present, the regulation of the rhythm of melatonin synthesis in the chick pineal is not well understood. However, it is clear that phototransduction and a major portion of rhythm generation occur within the gland. In contrast, the mammalian pineal appears to have relinquished these functions to the retina and the SCN.

Indirect evidence suggests that cyclic nucleotides are involved in regulating serotonin *N*-acetyltransferase activity in

the chick pineal. Phosphodiesterase inhibitors, cyclic AMP analogs, and cholera toxin cause increases in enzyme activity (*72*). We have recently obtained direct evidence that cyclic AMP levels oscillate in cultured chick pineals and are correlated with serotonin *N*-acetyltransferase activity under a number of conditions (*75*). Perhaps cyclic AMP levels regulate the rhythm of indoleamine metabolism in the chick pineal as they do in the rat pineal. The difference between the mammalian and avian systems may lie in the mechanisms regulating cyclic AMP. At present, the avian pineal, which acts as a circadian pacemaker in vivo, provides the best model for a vertebrate circadian oscillator in vitro (*76*).

The Aplysia eye. The eye of the marine mollusk *Aplysia californica* displays a robust circadian rhythm in neural output that persists in vitro for many days under constant conditions (*77*). It is clearly a self-sustained circadian oscillator. The rhythm of the isolated eye can also be phase shifted in response to light. Except for the small size of its cells, which do not readily permit intracellular recording, it provides an excellent model for the study of cellular mechanisms involved in the generation and regulation of circadian rhythms.

Pharmacological manipulation of the phase of the rhythm has provided a fruitful approach to the mechanisms of entrainment (*78*). Treatments that cause depolarization have phase shifting effects that are similar to those of light. Both high external potassium concentrations and incubation in the presence of strophanthidin (a blocking agent of sodium-potassium adenosinetriphosphatase that depolarizes the membrane potential) produce phase shifts that are similar to those produced by light. The photoreceptor potential appears to be an important step in the entrainment pathway. Agents that block the electroretinogram also block light-induced phase shifts. Nerve conduction, chemical neurotransmission, and secretion are not required for the effects of light on the oscillatory mechanism. Eskin (*78*) has concluded that the photoreceptor cell either contains the circadian pacemaker or is coupled to it by gap junctions.

Inhibitors of protein synthesis can cause both delaying and advancing phase shifts in the free-running rhythm, suggesting that protein synthesis is involved in the regulation of rhythmicity (*79*). Serotonin, a neurotransmitter present in the *Aplysia* eye, also causes phase shifts (*80*). Its phase response curve differs from that obtained with light, the two being about 180° out of phase. The phase-shifting effects of serotonin appear to be mediated by cyclic AMP. An analog of cyclic AMP (Fig. 5), some phosphodiesterase inhibitors, and an activator of adenylate cyclase (forskolin) each cause phase shifts that are similar to those obtained with serotonin (*81*). These results are consistent with two roles for cyclic AMP: it may be part of an input pathway by which serotonin entrains the pacemaker or it may be part of the circadian pacemaking mechanism itself.

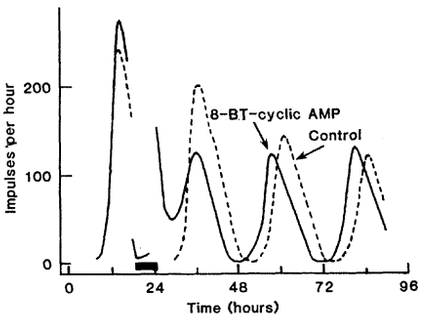

Fig. 5. Phase shift caused by an analog of cyclic AMP. The free-running circadian rhythms in the frequency of compound action potentials generated by each eye of an *Aplysia* were measured in vitro for 4 days. Ordinarily both eyes remain in phase during this period. The experimental eye was exposed to 8-benzylthio–cyclic AMP (8-BT–cyclic AMP) during the interval indicated by the black bar. Although there were effects on the amplitude, the treatment's action on the circadian pacemaker was revealed by the phase advance in the experimental eye. [From Eskin *et al.* (*81*)]

It is intriguing that cyclic AMP is involved in mediating the output of the circadian pacemaker in the avian pineal gland and the input to the pacemaker in the *Aplysia* eye. These systems differ markedly; however, if cyclic AMP were involved in a common mechanism that generates circadian oscillations then it might provide a point of convergence for both inputs and outputs. At present, pharmacological data suggest that the key elements involved in the regulation of circadian rhythms in animals are membrane potential, cyclic nucleotides, and protein synthesis (*82*).

Conclusions

There has been much progress in the past decade in establishing an anatomical and physiological basis for circadian rhythmicity in animals. More or less compelling evidence has implicated specific structures—the mammalian suprachiasmatic nucleus, the avian pineal gland, and the *Aplysia* eye—in the regulation of circadian rhythmicity. Although indirect, a great deal of evidence suggests that the suprachiasmatic nucleus acts as a pacemaker within the mammalian circadian system (*21, 46*). Direct evidence for a pacemaker role exists for the pineal gland of the sparrow (*68*) and for portions of the nervous system of insects (*19*), where it has been possible to surgically transfer the phase or period of the pacemaker from one animal to another. The identification of a pacemaker in a multioscillator system can be a difficult enterprise. As in the case of the heart, the pacemaker is defined by its role in a hierarchical system of oscillators. Establishing that a particular piece of tissue is or contains a circadian oscillator is conceptually easier; a circadian rhythm expressed in vitro (like that of the *Aplysia* eye) provides direct evidence.

Although the conceptual model for the components of a circadian system at the physiological level (Fig. 3) applies to the biochemical level as well, there remain serious difficulties in identifying the biochemical components of a circadian oscillator. In the *Aplysia* eye, cyclic AMP mediates the phase shifting effects of serotonin on the circadian oscillator (*81*). This substance is, therefore, either part of the input pathway for serotonin or part of the oscillatory mechanism itself. The data and the model are inadequate to distinguish between these two roles at present. If cyclic AMP is part of the oscillatory mechanism, then its level must oscillate and perturbations of cyclic AMP must produce phase-dependent phase shifts of its own oscillation. Definitive criteria, however, will have to be derived from specific biochemical models for the oscillatory mechanism.

The physiological investigation of circadian rhythms has made a gradual transition from analyzing the dynamics of the system to identifying its anatomical components. Although attempts to study the biochemical mechanisms of the circadian oscillator have been traditionally restricted to microorganisms (*24*), promising experimental systems have emerged from investigations of multicellular organisms. The cellular mechanisms that underlie circadian oscillators may be the products of convergent evolution and differ among species; alternatively, an ancient mechanism may have been preserved and diversely applied (*83*). Investigations with modern genetic techniques in microoganisms (*27, 84*), and biochemical and cellular techniques in model systems from animals, may reveal these underlying mechanisms.

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Repopulating the Countryside: A 1980 Census Trend

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Summary. Census data confirm that in the 1970's population grew more rapidly outside than inside metropolitan territory, reversing a historic pattern. The new data reveal that the dispersion of population growth beyond metropolitan areas was not so much a movement to small towns as a movement to the open countryside. The trends appear strong enough to suggest a new shift toward rural life-styles.

Censuses are a demographer's microscope, making it possible to examine individual places like towns, villages, cities, and smaller areas within them, including many geographical units that are too small for reliable estimation of intercensal population. Census data show where growth and decline are occurring and how demographic processes are affecting different types of places and altering the various components of the national settlement system. The census microscope can also be focused on specific demographic subgroups which may be of considerable analytical or policy significance but are virtually invisible in sample surveys.

The geographical information provided so far by the 20th decennial census, taken as of 1 April 1980, indicates that major realignments of the spatial structure of the American population are occurring. At one level of analysis, the data confirm various pieces of evidence and hypotheses that the decade 1970 to 1980 was unique in the degree of deconcentration of population beyond the boundaries of metropolitan areas (*1*). The census information also reveals that the dispersion of population beyond the suburban fringes entailed not so much a revival of small towns as a surge of growth outside of incorporated places. In this article we draw on the 1980 census to contrast continuities in basic settlement patterns with new patterns that reflect an unprecedented shift of population toward small urban clusters and rural territory.

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Metropolitan Areas

One aspect of the settlement system that did not change was the continued spatial and demographic expansion of metropolitan areas. A metropolitan area is now defined (by a federal committee) as an urban cluster with a population of at least 50,000 along with the rest of the county and other counties that are linked to the central county through commuting. Everything else is "nonmetropolitan" (*2*).

To some extent the distinction between metropolitan and nonmetropolitan is replacing the traditional urban-rural distinction. Urban and rural are spatial and physical concepts based on residence alone; today only a small proportion of rural residents are farmers. The metropolitan-nonmetropolitan concept embodies both a spatial element (a city and its associated suburbs) and an economic dimension (a more or less unified local labor market). A metropolitan area has both urban and rural parts (see below), as does the nonmetropolitan area.

Between 1970 and 1980 population continued to grow (by 8.8 percent) within the "old" (1970 census) metropolitan boundaries. As in previous decades, the total metropolitan territory increased as nonmetropolitan cities grew and acquired the metropolitan designation and as suburbs of preexisting metropolitan areas expanded into what was formerly nonmetropolitan territory. The newly designated metropolitan areas understandably had a high growth rate—a 21.4 percent increase in population between 1970 and 1980. The counties added to the fringes of the "old" metropolitan areas grew by 33.8 percent between 1970 and 1980.

What was different about the 1970's was that the total population within the updated metropolitan area boundaries grew less rapidly than the residual (nonmetropolitan) territory, reversing a his-

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