Preliminary energy calculations and observations of hydraulically generated seismic signals indicate that detectable seismic radiation may be generated from the proposed source. We assume that 10 percent of the kinetic energy of the moving water column is converted to seismic energy as the column is brought to rest. For the Gorner Glacier, 10 percent of the kinetic energy of the water column is 6.4×10^3 J, which produces an equivalent Richter magnitude of -0.4. A signal of this magnitude is detectable at a range on the order of kilometers from its source.

For hydraulic signals to generate the 10^8 J required for an earthquake of Richter magnitude 2.5, the flow quantity must increase substantially. A flow rate of 1000 m^3 sec^{-1} in a subglacial conduit 200 m long and 11.3 m in diameter produces the required energy. Both the flow rates and tunnel dimensions are compatible with values calculated from observed large outruns of water from glaciers (*11*). Seismic signals traceable to hydraulically induced vibrations in the penstock of the Edward Hyatt power plant near Oroville, California, have also been recorded on seismic stations in northern California (*12*).

In the above examples we have used the relatively large value of 10 percent for the energy conversion factor because of the elastic nature of the problem. However, if the conversion efficiency is reduced by an order of magnitude or more, the above arguments still remain valid.

In the case of the volcanic earthquake, we assume that the movement of magma is analogous to the flow of water in glaciers. Quantitatively, the difference between hydraulically generated displacements of lava tubes, as opposed to ice tunnels, is a function of material properties. The properties of the materials required to solve Eqs. 1 and 2 are the elastic constants of the conduit and the bulk modulus, density, and kinematic viscosity of the fluid. A functional description of the frictional interaction of the fluid and the conduit is also required.

Several observations can be made that relate to the seismic waves from both volcanoes and glaciers. The characteristics of the type-II seismic signal indicate that the waves are not associated with ice tectonics. In terms of the wave forms observed, a hydraulically generated signal produces a much more plausible source mechanism. In volcanic earthquakes, it would appear that the A-type volcanic event, which exhibits a wider spectrum of frequencies, is associated with hydraulic fracturing due to magma flow (*6*). However, we interpret the relatively low-frequency signal from B-type earthquakes to be associated with a less catastrophic deformation of the lava tubes.

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10. To solve Eqs. 1 and 2, we consider that the Gornersee represents a reservoir that is at a constant level. The orifice of the ice conduit is considered to be at a constant piezometric head of 50 m ($H = 50$ m at $L = 0$). The steady-state discharge of the tunnel is assumed to be 1 m^3 sec^{-1}, which with a tunnel diameter of 1 m gives a flow rate of 1.273 m sec^{-1}. At a point 100 m from the entrance ($L = 100$ m) of the tunnel, we assume that the flow is terminated. For demonstration purposes, we apply a linear closure to the tunnel which takes place over a period of 0.4 second. Using the elastic properties of ice [K. Neave and J. Savage, *J. Geophys. Res.* **75**, 1351 (1970)], we calculate a wave speed a for the fluid of 1107 m sec^{-1} [(*9*), p. 18]. The damping term in Eq. 1 is considered to be proportional to the square of the velocity. A Darcy-Weisbach friction factor is calculated [H. Rouse and J. W. Howe, *Basic Mechanics of Fluids* (Wiley, London, 1953), p. 141], based on a conduit roughness k of 15. The value of α is taken to be 11.5°.
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12. On 17 June 1976 the Edward Hyatt power plant at the Oroville dam site, Oroville, Calif., was temporarily closed as a result of a faulty valve seal. Vibrations felt in the power plant on the morning of 17 June were the reason for closing the plant. Signals emanating from the penstock were recorded at the seismic station of the University of California at Berkeley in the Lake Oroville area. Seismic activity ceased when the flow to the power plant was terminated.
13. We thank Dr. L. Johnson of the University of California at Berkeley for calling our attention to the seismic signals associated with the Edward Hyatt power plant. We also thank W. Curry of the California Department of Water Resources for providing us with the details regarding the valve malfunction. We thank Dr. S. Colbeck, Dr. G. Ashton, D. Calkins, and Dr. W. Hibler of the Cold Regions Research and Engineering Laboratory, who offered many helpful comments.

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Circadian Clock in Culture: *N*-Acetyltransferase Activity of Chick Pineal Glands Oscillates in vitro

Abstract. *N-Acetyltransferase activity was measured in organ-cultured chick pineal glands. A circadian rhythm of enzyme activity persisted in cultured glands for up to 4 days. The phase of the rhythm in vitro closely approximates its phase in vivo. These observations demonstrate that the pineal gland of chicks contains (or is) a self-sustained circadian oscillator.*

The pineal glands of birds and mammals exhibit circadian rhythms in the production of the hormone melatonin. Not only levels of melatonin but also the activity of *N*-acetyltransferase (NAT), the enzyme primarily responsible for its rhythmic production, are significantly higher during the night than during the day (*1, 2*) and the rhythm in NAT activity persists in constant darkness with a period of approximately 24 hours (*1, 3, 4*).

In rats, the circadian rhythm of pineal NAT is generated by rhythmic release of norepinephrine from the sympathetic fibers of the superior cervical ganglia (*5*). The circadian rhythm in sympathetic nerve activity may be generated in turn by an oscillator located in, or transmitting through, the suprachiasmatic nucleus of the hypothalamus (*6*). In contrast, the rhythm of NAT activity in the pineal gland of the chicken does not appear to be generated by the superior cervical ganglia or by any other noradrenergic input. Denervation of the pineal gland does not abolish diurnal rhythms of NAT in the chicken as it does in the rat (*7*). Furthermore, administration of either norepinephrine or isoproterenol to the chick pineal gland in vivo or in vitro does not elicit the increase in NAT activity observed in similar preparations of rat pineal glands (*8*). In the chick, examination of the timing of the spontaneous decline of NAT activity in vitro has revealed a short-term timekeeping ability of the isolated pineal gland (*9*). In the house sparrow (*Passer domesticus*), the pineal gland functions as a biological clock regulating the rhythm of locomotor activity (*10-13*); and melatonin may be involved in this process (*14*). These facts led us to the hypothesis that melatonin synthesis in the avian pineal is controlled by a circadian oscillator located within

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the gland. We report here the results of one test of this hypothesis.

Chicks (*Gallus domesticus*) were raised under a diurnal light cycle of 12 hours of light followed by 12 hours of darkness (LD 12:12) (*15*). At 2 to 3 weeks of age, groups of animals were killed during the light portion of their LD cycle, and pineal glands were removed and placed into culture (*16*). Glands (four per flask) were attached via a chicken plasma clot to the forward base of Falcon culture flasks (Corning). Three milliliters of BJG media (Gibco) supplemented with heat-inactivated 10 percent fetal calf serum (Gibco) and 1 percent antibiotics (PNS, Gibco) were added. The flasks were then gassed with a mixture of 95 percent O_2 and 5 percent CO_2, sealed, and placed in a lighttight incubator set at 37°C which was located in a lighttight room. In the incubator, the flasks were on a rocking platform that tilted them through an arc of approximately 40° every 30 seconds. This allowed the glands alternately to be covered by the media and exposed to the oxygen-rich atmosphere. This technique allowed the rapid removal of the flasks in complete darkness with minimal disturbance to the remaining glands. Flasks, removed from the incubator at desired time points, were immediately frozen on Dry Ice and stored at −70°C until a later time when all glands collected from the experiment were individually assayed for NAT activity by a modification of the method of Deguchi and Axelrod (*17*).

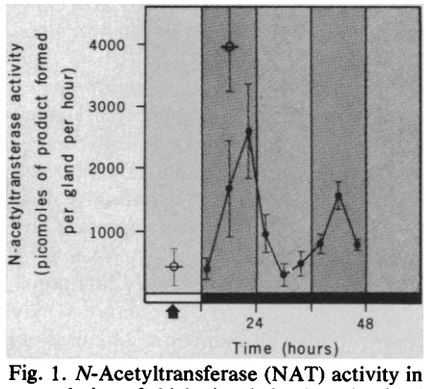

Fig. 1. *N*-Acetyltransferase (NAT) activity in a population of chick pineal glands maintained in vitro in constant darkness (DD). Birds were housed in a light-dark cycle (LD 12:12); pineals were removed and placed into culture 5 to 6 hours prior to projected lights off (solid arrow). The horizontal bar below the graph represents the lighting in the incubator. Lights-off occurred in phase with the birds' prior cycle, and glands were then exposed to continuous darkness. The alternating light and dark shaded portions of the figure designate the "projected" light cycle (that is, the phasing of the cycle if it had been continued). Samples (four glands) were removed at 4-hour intervals, and each gland was assayed independently for NAT activity. Each data point (closed circles) is the mean activity (± S.D.) of four glands. Open circles are means of pineal NAT activity (± S.D.) in intact chicks (six animals per point) killed at mid-light and mid-dark points in the LD 12:12 cycle.

Figure 1 shows the results of an experiment designed to determine whether rhythmicity of NAT activity would persist for 2 days in organ-cultured glands maintained in constant darkness (DD). Chicks were killed, and their pineals were placed into culture 3 to 4 hours prior to the beginning of their dark period. Lighting in the incubator (supplied by a 15-watt utility bulb located 25.4 cm above the flasks containing the glands) was continued until the normal onset of the dark period, and the glands were then held in DD for the remainder of the time in culture. Flasks were removed at 4-hour intervals during 36 hours of DD, and glands were assayed for NAT activity. Two clear peaks of enzyme activity occurred, one in each of the projected nights of the birds' prior LD cycle. The first peak, a five- to sixfold increase in activity relative to both initial values and the trough reached 12 hours later, occurred late in the night. The second peak occurred earlier in the following projected night with a smaller amplitude (three- to fourfold increase).

Practical considerations precluded continuous sampling for more than 48 hours, and, in order to determine whether rhythmicity would persist in culture for more than 2 days, experiments were performed in which glands were held in culture but not sampled until day 3. In order to be able to monitor visually the condition of the glands during the first 2 days in culture, the incubator was placed on LD 12:12 in phase with the previous light regime of the intact animals. The culture medium was changed and the O_2:CO_2 atmosphere in the flasks was renewed, and the flasks were sealed during the light portion of day 3 in culture; at the normal time of lights-off, the glands were placed into DD (Fig. 2, a to c). Sampling at 4-hour intervals revealed persistent circadian rhythms of NAT activity on days 3 and 4 in culture with peaks occurring during the projected night. As in the experiment in which days 1 and 2 in culture were examined, the peak NAT activity on the first day of DD is of higher amplitude than is the peak on the second day. A decrease in amplitude of NAT in DD has been reported in intact animals (*4*, *7*), and similar effects of prior exposure to light versus dark in vitro have been demonstrated on day 1 in culture by Wainwright (*18*). On the other hand, the difference in amplitude between peak 1 and peak 2, which is evident in all our experiments, might be the result of changes in the sealed culture environment.

Our data suggest that some aspect of the culture conditions may have an effect on the phase of the persistent rhythm. When glands are fed and gassed (Fig. 2c) late in the day, the peak of NAT activity seems to occur later in the subjective night than it does when they are fed and gassed in the middle of the day (Fig. 2, a and b). When the glands were fed and gassed, they were removed from the incubator for about 5 minutes, during which time they were exposed to slight changes in temperature and light intensity. Either of these factors or some unspecified change in the culture environment might be responsible for the apparent influence on phase.

A good deal of evidence supports the hypothesis that the avian pineal is a self-sustained circadian oscillator that normally occupies a position at or near the top of the circadian hierarchy. Most of this evidence comes from work with the

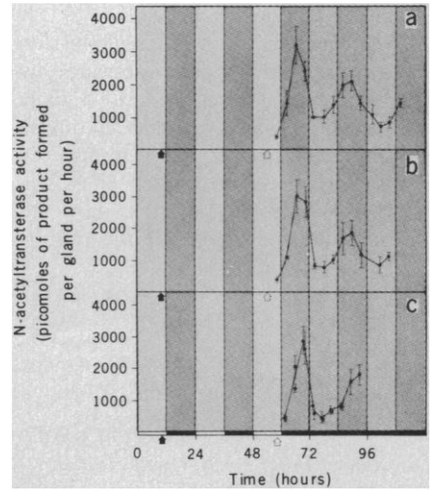

Fig. 2. N-Acetyltransferase (NAT) activity in chick pineal glands on the third and fourth days in organ culture. The experiments were performed and are graphed as described in Fig. 1 with the following exceptions. Pineals were removed from the birds and placed into culture just prior to lights-off (solid arrow), and the light cycle was continued in culture during days 1 to 3 (horizontal bar below graph and shaded and light areas above); the glands were refed with fresh media and regassed on day 3 at the time indicated by the open arrow; at the onset of darkness on day 3 the glands were placed in constant darkness (DD). Sampling at 4-hour intervals (four glands per point) again revealed a circadian rhythm of NAT activity with peaks occurring during the projected night of days 3 and 4 in culture (dark shaded areas). (a and b) Replicate experiments. (c) This experiment was the same as the two above except that refeeding and regassing occurred later in the light portion of day 3, and replicate samples (two vials, four glands per vial) were assayed during the first 24 hours.

house sparrow. In that species, pineal removal abolishes circadian rhythmicity (*10*) but denervation of the pineal does not (*11*); rhythmicity is restored to pinealectomized birds by the implantation of a donor's pineal into the anterior chamber of the eye (*12*) and the reconstituted rhythm has the phase of the donor bird (*13*); melatonin, administered at constant low levels has dramatic effects on circadian rhythmicity of intact birds (*16*). If, as is implied by these facts, the avian pineal is an oscillator with an hormonal output, then under appropriate conditions it should be possible to measure a continuing oscillation in vitro. The above demonstration of such an in vitro oscillation as well as that of others (*18, 19*) is, in conjunction with the earlier work, the strongest possible evidence that the avian pineal is a circadian clock and opens the way for an investigation of its molecular mechanism. Still to be unraveled are the functional relations between the pineal and other components of the circadian systems of birds. These relationships are known to be complex (*12, 20*) and may vary from one avian species to another (*21*).

Note added in proof: After submission of this manuscript a paper appeared by Binkley *et al.* (*22*) in which "a daily change during day 1 of organ culture in constant dark" was reported to occur in the NAT activity of chicken pineals in vitro. The authors further reported "equivocal persistance" of the rhythm for several days.

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16. The chicks were decapitated, and the heads were dipped into a warm (37°C) solution of disinfectant and then quickly rinsed in warm water. The skullcap was removed under a sterile hood. The pineal was removed, placed into a petri dish containing about 1 ml of Hanks balanced salt solution (*p*H 7.2), and at this time the adhering tissue was removed by dissection. Several small cuts were made in the gland, in order to increase the surface area and promote diffusion of gas and nutrients.
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Microevolution and Clone Structure in *Spartina patens*

Abstract. *Analysis of the clone structures within a population of* Spartina patens *reveals considerable adaptive genetic divergence among adjacent dune, swale, and marsh sites. The dune subpopulation includes a small number of frequently encountered, spatially isolated clones that follow microtopography and have high reproductive output and colonizing potential. The marsh subpopulation consists of a large number of infrequent interdigitating clones with high vegetative biomass and competitive success. The swale subpopulation is generally intermediate for these traits.*

A general pattern has emerged that plant populations are geographically small in extent, with restricted interpopulation migration, and subject to intense selection pressure that varies in direction among populations (*1*). It is therefore no longer surprising to find microevolutionary divergence over distances of meters or even centimeters (*1*). In extending these generalizations to vegetatively reproducing perennial plant populations, a number of ambiguities confound the interpretation of evolutionary patterns. (i) Individual genotypes or clones may reach great age approaching a degree of immortality; (ii) replacement by seed occurs rarely, and selection will operate most strongly among perennating individuals; (iii) migration by vegetative spread may occur over long distances compared to breeding-population areas; and (iv) populations may comprise a small number of clones each with a large number of independent reproductive individuals.

In order to understand the genetic

Table 1. Means analysis on traits of *Spartina patens* plants grown in common environments.

Trait	Subpopulations		
	Marsh	Swale	Dune
Weight (g) per tiller (dry weight)	0.76 ± 0.06*	0.68 ± 0.04	0.58 ± 0.05
Leaf area (cm^2)	30.1 ± 1.9	36.8 ± 2.1	30.8 ± 1.5
Tillers per clone	30.5 ± 0.06	26.7 ± 2.1	35.3 ± 2.6
Seeds per clone	187 ± 33	288 ± 48	401 ± 96
Rhizome (g) per tiller (g) (dry weight)	0.75 ± 0.10	0.97 ± 0.07	1.05 ± 0.11
Index salt tolerance†	0.33 ± 0.05	0.40 ± 0.06	0.57 ± 0.05
Drought tolerance‡	0.41	0.50	0.83

*Mean and standard errors for traits based on a subset of individuals taken from the total sample. Further details of the statistical analyses and methods have been described (*5*). †Ratio of the increment in the length of the longest root over 48 hours in control solution to that in a solution of 1.75 percent synthetic sea salt. ‡Proportion of genotypes surviving 2 months in sand culture and watered every fourth day.

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