(6) also confirm that sea surface temperatures in the eastern equatorial Pacific decreased in a westward direction between March and November 1975.

After measurement of the equatorial wave motions during 1975, it was expected that similar satellite observations could be made each year. During 1976, however, neither the equatorial front nor the long waves were detectable in the satellite imagery. The reason is probably related to the anomalous winds and sea surface temperatures reported in the eastern equatorial Pacific during 1976. From Fig. 2 it is evident that the southeasterly trade winds were considerably weaker in 1976 than in 1975. Ship measurements (6) show that the eastern equatorial Pacific sea surface temperatures between 5°N and 5°S were higher by 4° to 6°C in 1976 than in 1975. Similar anomalously warm water was observed in 1972, 1965, and 1957 in connection with the so-called El Niño. According to Wyrtki (7), excessively strong southeasterly trade winds, during the year preceding the El Niño, increase the east-west slope of sea level by building up water in the western equatorial Pacific. When the winds relax, as during 1976 (see Fig. 2), the accumulated water flows eastward and leads to equatorial warming that extends to the coast of Peru.

Although the limitations of the available satellite images prevented the detection of the motion of the equatorial front during 1976, there is reason to believe that equatorial waves were indeed present. Hansen (8) reports that surface drifter buoys in the North Equatorial Countercurrent (latitude 6°N) followed a meandering eastward path from 150°W to 130°W from December 1975 to March 1976. These observations suggest that the waves observed by satellite during 1975 persisted for at least 3 months in 1976. Although similar time and space scales appear in the data acquired by these different observational methods, simultaneous measurements must be made to properly compare the data. Efforts are presently under way to improve the enhancement of satellite images so that the equatorial front can be detected even when the sea surface temperature gradients are relatively weaker.

One plausible explanation for the westward motion of the waves appears to be that advanced by Philander (9). On the basis of a linear stability analysis of equatorial currents, he predicted that the large latitudinal shear between the South Equatorial Current and the North Equatorial Countercurrent in the Pacific will result in unstable waves with temporal and spatial scales close to the values observed by satellite. Philander (10) has also suggested that the waves observed near the ocean floor by Harvey and Patzert (2) could be neutral waves (equatorially trapped Rossby-gravity waves, for example) that are excited by the instability in the surface layers of the ocean and that propagate vertically downward to the ocean floor. This would explain why the waves observed there and those observed by satellite have the same period and wavelength.

If the long waves in the equatorial Pacific are indeed due to an instability of the currents, then it should be possible to relate their period and wavelength to the shear and hence intensity of the currents. It may be possible to monitor the equatorial currents by measuring the period and wavelength of the waves by satellite. To explore this relation, simultaneous measurements by satellite and by in situ ocean instruments are required.

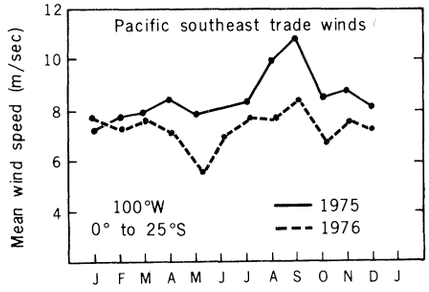

Fig. 2. The southeasterly wind speed during 1975 and 1976 is shown at longitude 100°W as a monthly mean between latitudes 0° and 25°S. These speeds were measured from satellite-observed cloud motions at 900 mbar (approximately 1000 m).

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10. ———, in preparation.

11. I thank S. G. H. Philander for his many suggestions and encouragement in the preparation of this report.

22 October 1976; revised 29 March 1977

Timekeeping by the Pineal Gland

Abstract. *N-Acetyltransferase, an enzyme involved in melatonin production in the pineal gland, exhibits a circadian rhythm in chickens with peak values in the dark-time and low values during the light-time, commencing at lights-on. When pineal glands of chickens killed during the dark-time (with high N-acetyltransferase activity) were organ-cultured, there was a decline in enzyme activity to light-time values. Regardless of the time of the dark at which the chickens were killed, the enzyme activity reached light-time levels at precisely the same time.*

Investigators studying house sparrows have demonstrated a physiological role for the pineal gland in circadian rhythms. Pinealectomy, pineal transplants to the anterior chamber of the eye, and melatonin implants provide a convincing case that supports the idea that the pineal gland may be the source of the circadian locomotor rhythm in house sparrows. Furthermore, melatonin, a pineal hormone, may be the means by which rhythmic signals are transmitted to the brain or body of the sparrow (or both) (*1*). Not only is there evidence that the pineal gland may function as a biological clock, but also there is good evidence that the pineal gland has rhythms in melatonin and in the activity of the enzyme involved in melatonin synthesis, *N*-acetyltransferase. *N*-acetyltransferase activity exhibits a daily rhythm in house sparrows and other vertebrates. We have studied in detail the rhythms in *N*-acetyltransferase activity in vivo in chickens, and we have paid special attention to regulation by light and dark: the daily cycle (27-fold in a light-dark cycle) persists in constant dark; the rhythm is damped by constant light; the shape of the oscillation is modified by

day length (photoperiod); peak activity cannot be initiated by dark except during a sensitive period coinciding approximately with the expected dark-time; low activity can be produced by turning on the lights (*2*). These properties are all consistent with pineal gland function as a biological clock or part of a biological clock timing circadian rhythms and involved in photoperiodic time measurement.

Because of the strong case that can be made for the pineal gland as a biological clock, we sought evidence that the pineal gland contains clock function independent of external input. Our initial attempts to demonstrate clock function in the pineal gland were directed toward looking for daily rhythms in pineal gland *N*-acetyltransferase activity in vitro. We began cultures with pineal glands obtained from chickens killed either during the dark-time or the light-time of a light-dark cycle. We noted that the glands from dark-killed chickens maintained relatively high *N*-acetyltransferase activity until about the time of expected lights-on, then dropped to light-time levels and did not rise substantially thereafter (cultures lasted up to 48 hours).

We wondered whether this meant that the glands contained information as to time. This was a testable hypothesis; by killing chickens at different times during the dark-time we could ascertain whether *N*-acetyltransferase activity dropped to the light-time level at one time (Fig. 1a) which would indicate timekeeping ability, or whether *N*-acetyltransferase activity would decline to light-time enzyme activity at different times (Fig. 1b) which would not necessarily suggest timing.

Chicks (*3*) were kept in a light-dark cycle (LD 12 : 12) from day 1 after hatching until they were several weeks of age. At 2-hour intervals during the dark-time one day, groups of chicks were killed and their pineal glands were dissected and placed in organ culture. Glands from each group were harvested from culture at 2-hour intervals and assayed for *N*-acetyltransferase activity (*2*, *4*).

The results of the experiment demonstrate that the pineal glands of the chickens contain timing ability (experimental results correspond to the alternative shown in Fig. 1a). *N*-Acetyltransferase activity decreased from relatively high levels to low, light-time levels at the same time (Fig. 1d, $P < .001$). This concerted decrease means that the glands contain information as to local time and also that the glands are capable of measuring a lapse of time. These two properties are considered by Pittendrigh to be essential prerequisites for a biological clock (*5*). However, the pineal glands in our organ cultures did not oscillate (*6*). It is also noteworthy that the time at which the *N*-acetyltransferase activity reached light-time levels was approximately the hour of lights-on in the light-dark cycle to which the chickens had been exposed prior to killing.

Our results bear on possible interpretations of pineal gland function as a biological clock or part of the circadian organization in vertebrates in the following ways.

1) The pineal gland is a possible source of endogenous circadian oscillations. Our results with pineal organ culture so far do not support this hypothesis as we have not obtained an *N*-acetyltransferase rhythm in organ culture (*6*). However, such negative findings are subject to many criticisms and are not conclusive evidence that the gland does not contain a self-sustaining oscillator.

2) The pineal gland has timekeeping ability. The pineal glands in our cultures clearly exhibited time-measuring capability. If the pineal gland has only this ability (and does not oscillate), is this sufficient to explain the pinealectomy-transplant experimental results? Zimmerman and Menaker (*1*) have shown that pineal gland transplants confer upon a pinealectomized recipient both restored rhythmicity and phase setting.

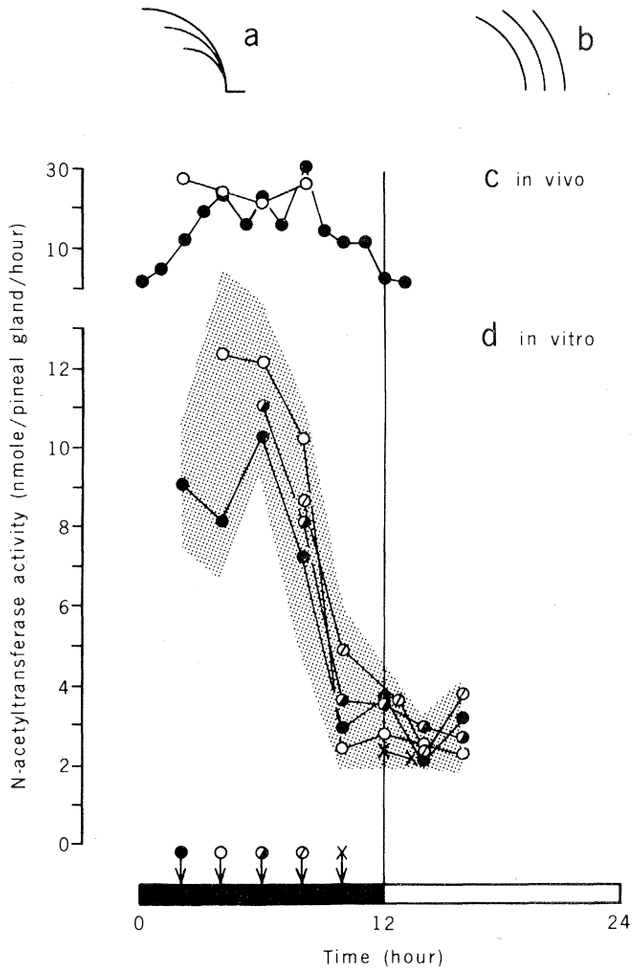

Fig. 1. Two alternative results answer the question of whether the pineal gland can keep time. If the glands keep time, a result such as that indicated in (a) where the responses of the glands converge would be obtained; or, if the glands cannot keep time, some other response, such as that in (b), would be obtained. Chicken pineal glands have rhythms in vivo in a light-dark cycle such as LD 12 : 12; to illustrate this, an LD 12 : 12 light-dark cycle is diagramed in the horizontal bar below the figure and data from an in vivo experiment (*2*) are shown in curve (c) (●). Peak *N*-acetyltransferase activity occurs in the dark-time, and low enzyme activity occurs in the light-time beginning at lights-on. In curve (c) (○) are control in vivo means for the chickens used in the experiment graphed in (d). Graph (d) shows the results of an organ culture experiment demonstrating timekeeping ability of pineal glands. Groups of chicks were killed at indicated intervals throughout the dark (●, ○, ◕, ∅, and ×), and their pineal glands were placed in organ culture. Every 2 hours after the initiation of the cultures, four pineal glands from each group were removed from culture and assayed for *N*-acetyltransferase activity (*4*). The curves connect the means for the five groups. The experimental results follow a pattern (a) indicating timekeeping ability for the pineal gland. The horizontal bar showing a light-dark cycle relates to (d) in that it was the light-dark cycle to which the chicks had been exposed in the days prior to killing. All the groups in (d) reached low light-time *N*-acetyltransferase activity values at the same time, approximately the time of lights-on. In other experiments, not shown, we have found that the low levels persist for at least 48 hours (*6*). A *t*-test of the starting values [(d) groups killed after 2, 4, or 6 hours of dark] compared to the values for the same groups at the time of expected lights-on was significant ($P < .001$). The stippled area was formed by connecting the extremes of the standard errors as an indication of variability.

Pineal timekeeping ability can account for phase setting. Restoration of a rhythm with a nonoscillating gland would require an interaction between the pineal gland and another structure to produce an oscillation. Another possible explanation is that the pineal gland permits expression of the innate rhythm.

3) The pineal gland measures the length of the dark-time. We have concluded from our previous investigations that the pineal gland may function to measure the length of the dark-time against internal standards established by a combination of prior photoperiod and an internal circadian oscillation (*2*). From the experiment reported here, we suggest that pineal gland timekeeping ability could provide an internal standard. Further experimentation following a variety of photoperiodic treatments is necessary to establish whether the glands contain information as to prior photoperiod.

Our current view of the circadian organization in vertebrates is that the pineal gland plays a crucial role and has at least part of the timekeeping mechanism. At this point we view the system as requiring other structures, perhaps the suprachiasmatic nucleus, which is involved in circadian rhythms in mammals (*7*), and other sites of melatonin synthesis, such as the retina (*8*). We also expect that the exact organization of the system will vary in detail from one species to another in such ways as to account for behavioral differences (nocturnal versus diurnal) and physiological differences (photoperiodism) associated with the environmental adaptations of each species.

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2 May 1977

Ratio of Plasma Alpha Amino-*n*-Butyric Acid to Leucine as an Empirical Marker of Alcoholism: Diagnostic Value

Abstract. *The ratio of plasma alpha amino-*n*-butyric acid to leucine was raised in patients with both alcohol-related and nonalcohol-related liver disease. This ratio appears to act as a relatively nonsensitive index of hepatocellular dysfunction rather than an index of alcoholism.*

It has been shown that the plasma ratio of alpha amino-*n*-butyric acid to leucine (A/L ratio) is raised in chronic, heavy drinkers and the suggestion has been made that this ratio might serve as an objective, empirical marker of alcoholism (*1*).

In order to test this hypothesis we have looked at the A/L ratio in the plasma of 50 control subjects, 43 alcoholics with various degrees of liver damage, and 77 patients with nonalcoholic liver and biliary tract disease. We have also studied the relation between the A/L ratio and several parameters of liver cell damage, including histology, and have observed the changes that occur in the ratio postprandially and diurnally.

Venous samples of blood were taken between 9 and 10 a.m. and placed in heparinized tubes. The plasma was separated and deproteinized with 70 percent sulfosalicylic acid, 50 μl per milliliter of plasma. Norleucine was added to the su-

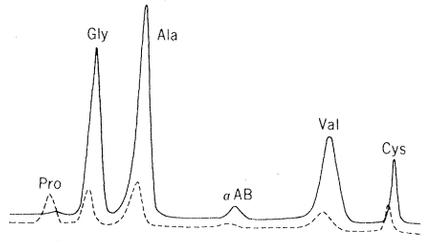

Fig. 1 (left). Part of a plasma amino acid tracing from a control subject showing the small peak of α-amino-*n*-butyric acid. Abbreviations: Pro, proline; Gly, glycine; Ala, alanine; αAB, α-amino-*n*-butyric acid; Val, valine; and Cys, cysteine. The peak area is proportional to the amino acid concentration, the proportionality constant being similar for all peaks shown. Fig. 2 (right). Relation between the plasma A/L ratio and liver histology in 43 patients with alcohol-related liver disease. Histological grading : 1, mild; 2, moderate; and 3, severe liver damage.