error as the deviation of the fringes from the vertical becomes smaller.

The results of 24 diffusion experiments with three gases at nine pressures are given in Table 1. The diffusion coefficients rank the gases in the inverse order of their molecular dimensions (*20*), the same order as in water solutions (*13*), as expected from Stokes' law (*21*). Graham's inverse relation between the root of a solute's molecular weight and its diffusion coefficient (*22*) was not observed.

Neither the absolute nor the relative solubilities, in perfluorotributylamine, of the three gases examined [$CO_2 > O_2 > N_2$ (*3*)] were related to the diffusivities found. This emphasizes the point that the two parameters must be considered as distinct physical entities, a fact which is also evident from their opposite temperature coefficients in certain instances (*23*).

The diffusion coefficients of respiratory gases in a perfluorocarbon liquid will make it possible to estimate the time needed to saturate pure fluorocarbon liquids or their dispersions under a given partial gas pressure, and also the time or gradient, or both, required to release these gases again. As a consequence, the rate of flow necessary for maintaining a desired gas pressure in a flow system and the optimum quantity of liquid in circulation can be calculated.

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Localization of a Circadian Pacemaker in the Eye of a Mollusc, *Bulla*

Abstract. *The eye of the marine mollusc* Bulla *contains a circadian pacemaker which, along with critical entrainment pathways, is located among a small group of neurons at the base of the retina. Long-term intracellular recording from cells of the organized photoreceptor layer, which constitutes most of the retinal volume, indicates that these cells are not involved in generating the rhythm since rhythmic changes in membrane potential were not observed. In addition, surgical removal of the entire photoreceptor layer does not alter the period of the circadian rhythm and does not prevent phase shifts by light pulses.*

The eye of the marine gastropod *Aplysia*, which expresses a circadian rhythm in the frequency of spontaneous optic nerve impulses (*1*), has been important in studying the cellular basis of circadian rhythms. The retina exhibits robust free-running circadian rhythms in vitro which can be phase shifted by the application of light pulses (*2*). The exact location of the pacemaking system is not known (*3*), but it is clear that a small fragment of retinal tissue containing photoreceptors and higher order cells will support a circadian rhythm (*4*).

In *Bulla*, the cloudy bubble snail, the eye expresses a circadian rhythm similar to that of *Aplysia* (*5*), but the retina of *Bulla* has fewer and larger cells than that

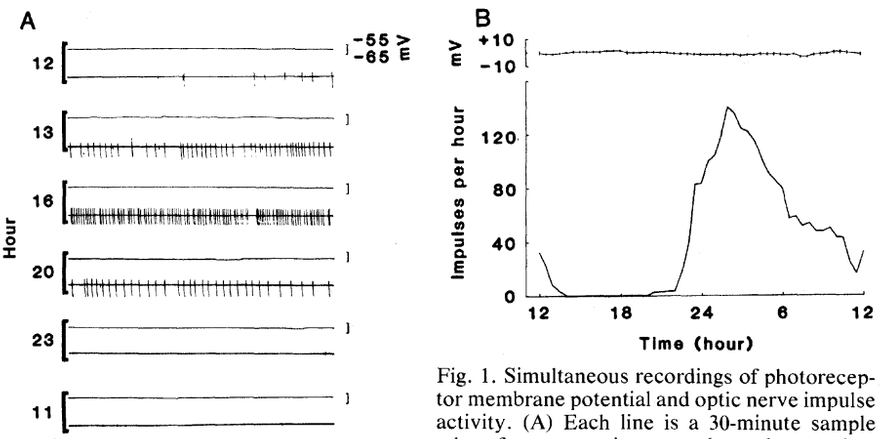

Fig. 1. Simultaneous recordings of photoreceptor membrane potential and optic nerve impulse activity. (A) Each line is a 30-minute sample taken from a continuous polygraph record at the hour specified. The upper trace for each pair is the actual measured resting membrane potential of a photoreceptor; the lower trace is an extracellular recording from the optic nerve. For long-term recording the lens was removed to make the receptors accessible, and the eye was suspended in petroleum jelly to minimize vibrations. Intracellular recordings were obtained with 20- to 40-megohm glass microelectrodes. The bathing medium was artificial seawater (Instant Ocean, 30 m*M* Hepes) maintained at 16°C. All recordings were made in continuous darkness, although eyes received 5 to 10 minutes of high-intensity light during impalement; the previous dawn was at 1400 hours in a light-dark cycle of 12 hours of light and 12 of darkness. (B) Average membrane potential and optic nerve impulse frequency from four preparations. The upper trace is a moving average (bin width = 0.25 hour, window = 0.5 hour) showing relative resting membrane potential, vertical bars represent standard deviations. The lower trace is the mean optic nerve impulse frequency from the four preparations. All records were made in darkness; previous dawn was at 2400 hours.

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of *Aplysia*, and there is better spatial separation between morphologically distinct cell populations in *Bulla* (*6*). These attributes permit long-term intracellular recording as well as selective surgical reduction of the *Bulla* retina. Our results indicate that the circadian pacemaker in the *Bulla* eye is not located in the organized photoreceptor layer but resides among a small group of cells at the base of the retina.

The largest and most numerous cells within the *Bulla* retina are the photoreceptors. These elongate cells, approximately 20 by 90 μm, form a layer around the lens and constitute most of the retinal volume. Receptors are impaled with microelectrodes through clearly visible distal segments and, under favorable conditions, continuous recordings of membrane potential can be maintained for 24 to 30 hours. In an effort to determine whether cells in the photoreceptor layer are involved in generating the circadian rhythm in optic nerve impulse frequency, we recorded intracellularly from individual photoreceptors while simultaneously monitoring optic nerve impulse activity. Analysis of long-term recordings did not reveal spontaneous changes in photoreceptor membrane potential which could account for the impulse rhythm recorded in the optic nerve (*7*). In most preparations there are small (±2 mV) fluctuations in receptor potential, but these appear to be random and not to be associated with optic nerve impulse frequency (Fig. 1).

The lack of rhythmic changes in receptor membrane potential suggests that cells in the photoreceptor layer are not involved in driving the optic nerve impulse rhythm. However, it is possible that the circadian rhythm is generated in some electrically distant portion of the receptor or that a special class of cells in this layer was not sampled by our microelectrodes. To investigate these possibilities we surgically reduced eyes by removing the entire region of the retina containing the distal and somatic portions of the photoreceptors; we left only a small fragment of retinal tissue near the origin of the optic nerve (Fig. 2A).

Nineteen of 21 reduced eyes remained spontaneously active, and all of these continued to exhibit a circadian rhythm in optic nerve impulse frequency (Fig. 2B). We found no evidence that the circadian period of the ocular rhythm in reduced eyes differed from that in intact eyes, although peak impulse frequency was typically lower in the retinal fragment (*8*).

Even though the photoreceptor layer had been removed, all reduced eyes remained responsive to light, indicating that light-sensitive neurons are present at the base of the retina. Reduced eyes responded to a light pulse with a transient increase in large amplitude impulse activity which resembled the response of intact eyes. Reduced eyes, however, did not exhibit the low amplitude desynchronized activity that normally accompanies the large impulses in intact eyes (Fig. 2C).

To determine whether the circadian pacemaker in reduced eyes can be phase shifted, light pulses (6 hours in duration) were delivered in the late subjective night (circadian time 2000 hours) during the first in vitro cycle (*9*). We found that the ocular rhythm was phase advanced following the pulse (Fig. 2D), with the mean shift for reduced eyes being +1.1 hours with respect to control eyes. For comparison, light pulses were delivered to intact eyes, and a mean phase advance of 1.5 hours was observed (*10*).

Three conclusions can be drawn from our experimental results: (i) the circadian rhythm in impulse frequency is not due to spontaneous changes in membrane potential of cells in the photoreceptor layer, (ii) a competent circadian pacemaker is present at the base of the retina, and (iii) the organized photoreceptor layer is not necessary for light-induced phase shifts of the ocular pacemaker. Thus, both a circadian pacemaker and its entrainment pathways are located among a small group of cells at the base of the retina, apparently the smallest fragment of neural tissue thus far

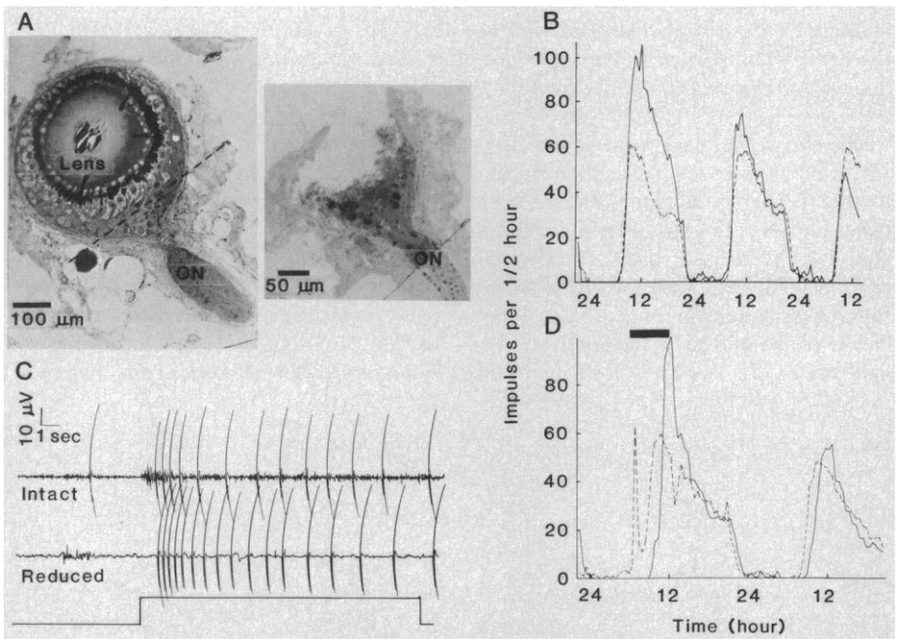

Fig. 2. (A) Thick sections of intact (left) and reduced (right) *Bulla* eye. The stratified nature of the retina is obvious in the intact section. From the lens outward, the retina consists of the distal segments of photoreceptors, pigmented region, nuclei of receptors, neuropil, and neurons at the retinal base near the origin of the optic nerve (*ON*). There are approximately 300 photoreceptors and 50 to 70 cells at the retinal base. These cells, several of which are 20 to 30 μm in diameter, form a cone around the neuropil region in the central portion of the retina. The reduced eye was produced by removing the lens and cutting the eye transversely just above the origin of the optic nerve (shown by the dotted line in the intact eye). Reduced retinas contained 30 to 50 of the cells located at the base of the eye. Retinal tissue was embedded in methacrylate and stained with acid fuchsin and toluidine blue. (B) Circadian rhythm of optic nerve impulse activity in an intact and reduced eye. Electrical activity was recorded by suction electrodes. Both eyes were maintained in darkness at constant temperature (16°C). Previous dawn for the intact animal was 1000 hours. (C) Light evoked optic nerve response in an intact and reduced eye. The responses were obtained from eyes maintained in darkness for approximately 24 hours and then exposed to a brief light pulse (15 seconds, 5000 lux) in the late subjective day. The *Bulla* eye, like the *Aplysia* eye, responds to light with the production of large impulses which appear to be compound since they vary in amplitude as a function of light intensity and duration. Smaller units, visible only in the intact eye record, may be driven by the photoreceptors, which were removed from the reduced eye. The time of the light pulse is indicated by the upward deflection on the lower trace. (D) Phase shift of reduced eye by light. The reduced eye was given a 6-hour light pulse at the time indicated by the black horizontal bar (starting at 0600 hours) on the first cycle in vitro. The onset of light caused a strong sensory response, recorded as a sharp peak in impulse frequency. The net phase shift was measured between midrise points during the second cycle. Previous dawn was 1000 hours.

isolated which exhibits a circadian rhythm and can be phase shifted by light pulses (*11*).

The persistence of an unaltered circadian period in reduced eyes from *Bulla* is significant in that it is not in accord with one proposal that a population of higher frequency noncircadian pacemakers located throughout the retina in *Aplysia* interact to produce a circadian periodicity (*12*). Although our retinal fragments varied in the number of cells remaining, we did not observe any changes in period length. Either the circadian period in *Bulla* is the property of individual retinal cells or is the product of a subpopulation of cells which escaped fractionation in our experiments. Whether or not individual neurons are circadian pacemakers is an important issue. The relatively large size of many of the cells at the base of the *Bulla* retina should be an aid in investigating this question.

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9. Light pulses were delivered to the reduced eyes by means of a plastic light guide placed near the dish with the isolated eye. Pulses were delivered between 2000 and 0200 hours (circadian time). Light intensity varied between 5,000 and 10,000 lux. The phase shift per eye pair was determined during the second in vitro cycle by comparing the midrise time of an eye pulsed with light and one that was not pulsed. The mean phase difference between reduced retinas receiving a light pulse and intact retinas that did not receive a light pulse was compared to the mean phase difference of a control group of reduced and intact eye pairs, neither of which were pulsed with light.
10. The mean phase difference (± standard deviation) between reduced eyes that were pulsed with light and intact eyes that were not pulsed was 1.1 ± 0.8 hours ($N = 7$), whereas the difference between reduced and intact retinas in a control group that did not receive a light pulse was −0.4 ± 0.8 hour ($N = 7$). The mean phase difference between the treatment and control groups was statistically significant [$t(12) = 3.5$, $P < .005$]. In order to compare the phase shift of reduced eyes to phase shifts of intact retinas, one eye of six intact eye pairs was pulsed for 6 hours. The mean phase advance for the pulsed intact eyes was +1.5 ± 0.9 hours, which is similar to the light-induced shift observed in reduced eyes.
11. Cell R-15 in the abdominal ganglion of *Aplysia* expresses one circadian cycle of membrane potential fluctuations after synaptic transmission is blocked with tetrodotoxin and calcium-free seawater (*4*). There is no evidence, however, that the isolated cell rhythm is sustained or can be phase shifted.
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Prevention of Allograft Rejection by Immunization with Donor Blood Depleted of Ia-Bearing Cells

Abstract. *Strain-specific unresponsiveness was induced in adult mice by immunizing them with donor blood treated with antiserum to Ia (I region–associated antigens) prior to the transplantation of islets of Langerhans. This regimen alone produced greater than 100-day survival of islet allografts transplanted across a major histocompatibility barrier.*

Studies in our laboratory have demonstrated that allografts of islets of Langerhans in mice survive indefinitely in nonimmunosuppressed recipients if the islets are treated in vitro with donor-specific antiserum to I region–associated antigens (Ia) and with complement before transplantation (*1*). Because islet cells lack Ia determinants (*2*), the treatment with Ia antiserum and complement apparently removes Ia-bearing donor lymphoid cells (passenger cells) that initiate the rejection reaction. Islet allograft acceptance is promptly terminated by the injection of donor splenocytes into established transplant recipients, thus demonstrating the critical role of donor antigen–presenting cells in eliciting rejection (*1*).

Earlier studies on rats with established islet allografts provided evidence that tolerance had been induced in the recipients (*3*). Recently we reported that strain-specific tolerance, probably mediated by T suppressor cells, was present in mice bearing established allografts of histoincompatible islets (*4*). Since transplants of islet tissue depleted of Ia-bearing cells apparently induced tolerance in the recipients, we decided to determine whether immunization of recipient mice with Ia negative cells would permit the successful transplantation of fresh, untreated islets. We used donor blood treated with antiserum to Ia and with complement for immunization since red blood cells are Ia negative in the mouse.

Male B6 mice (C57B1/6J, H-2^b) were made diabetic by the intravenous injection of streptozotocin (180 mg per kilogram of body weight). Only B6 mice with plasma glucose levels of greater than 400 mg per 100 ml on three successive determinations were used as recipients. Rejection was defined as a plasma glucose concentration greater than 200 mg per 100 ml in three subsequent blood samples.

Whole blood was obtained from the orbital sinus of B10.BR (H-2^k) and B10.S (H-2^s) mice with heparinized capillary tubes. The blood was washed once with phosphate-buffered saline containing 10 percent citric acid and then washed twice in Hanks solution. The washed blood cells were centrifuged and the pellet was incubated for 30 minutes at room temperature with an equal volume of antiserum to Ia. Antiserum to Ia^k (A.TH-anti-A.TL) was used to treat B10.BR blood cells; antiserum to Ia^s (A.TL-anti-A.TH) was used to treat B10.S blood cells. The antiserum-treated red blood cells were washed with Hanks solution and then incubated with a 1 in 3 dilution of guinea pig serum as a source of complement for 30 minutes at 37°C. The preparations were diluted with Hanks solution and 5×10^7 red blood cells (0.5 ml) were injected via the tail vein into B6 recipients according to the schedule shown in Table 1.

Donor B10.BR (H-2^k) islets were isolated by the collagenase technique (*5*), separated on a Ficoll gradient (*6*), and then meticulously handpicked with the use of a Pasteur pipette under a dissecting microscope. A total of 550 to 750 freshly isolated B10.BR islets (H-2^k) were immediately transplanted by the portal vein technique (*7*) into diabetic B6 (H-2^b) animals. Four groups of B6 mice received these freshly prepared allogeneic islets (Table 2). Two groups of recipients were immunized with Ia-depleted blood cells prior to transplantation. Group 1 received donor B10.BR red blood cells (H-2^k); group 2 received irrel-

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