synthetase cannot charge *E. coli* tRNA with leucine and *E. coli* leucyl-tRNA synthetase cannot charge liver tRNA. The fact that the plasmodial enzyme can charge tRNA from both sources suggests that the major structural change with respect to the enzyme and its recognition site on the tRNA is inborn in the enzyme. It was shown that malarial parasites can take up host cytoplasm by pinocytosis (*7*). Therefore, the lack of charging specificity may permit the parasite to use host tRNA for its protein synthesizing system. However, tRNA is synthesized very rapidly in *P. berghei* in vivo (*8*).

Aminoacyl tRNA synthetase activity was found in the *P. berghei* extract for the following amino acids: tyrosine, histidine, valine, proline, threonine, and lysine (Table 1). High synthetase activity was observed for valine and lysine. Relatively high endogenous activity (without added tRNA) was recorded for almost all amino acids tested. However, when the extract was treated with 0.1 volume of 2 percent streptomycin sulfate, centrifuged for 15 minutes at 30,000*g* to remove RNA, and then passed through a Sephadex G-25 column, no endogenous activity could be observed and there was an absolute dependency on added tRNA.

It should be emphasized that the determinations of aminoacyl-tRNA synthetase activities for the above amino acids were carried out under conditions optimum for leucyl-tRNA synthetase. For example, in *E. coli* the optimum ratio of magnesium ion to ATP for leucyl-tRNA synthetase is 10 while the optimum ratio for the prolyl-tRNA synthetase is 30 (*9*).

Pyrimethamine, hydroxystilbamidine, quinacrine, and acriflavine inhibit the esterification of valine with tRNA significantly at a concentration as low as 0.1 m*M* (Table 2). Chloroquine was without effect. Elucidation of the reactions leading to protein synthesis in malarial parasites may explain the specificity of certain drugs and provide the rationale for the synthesis of new ones.

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Circadian Rhythm of Optic Nerve Impulses Recorded in Darkness from Isolated Eye of Aplysia

Abstract. *The isolated eye of the sea hare* Aplysia californica *shows a circadian rhythm of optic nerve impulses when kept in total darkness. Peak activity on the first day of isolation occurs during the projected "dawn" of the light-dark cycle to which the whole animal had previously been entrained. Eyes from animals previously exposed to constant light show a free-running rhythm. This simple photoreceptor provides a quantized output with an ideal control (the other eye) for studies on rhythms.*

A circadian rhythm in the rate of spontaneous impulses from an isolated eye has not been described for any animal, although a rhythmic migration of pigment occurs in the compound eyes of intact arthropods (*1*). The eyes of certain beetles show a "diurnal rhythm" (circadian) in electrical response to illumination (*2*). The circadian rhythm was thought to be a consequence of pigment migration. Recently, the response of the "sustaining" fibers of the optic nerve of the intact crayfish to light flashes has been demonstrated to show a circadian rhythm as well as the electroretinogram (ERG) (*3*). In the crayfish the analysis of the origin of the rhythm is complicated by the probable influence of several systems within the animal.

The isolated eye and optic nerve of *Aplysia,* described here, has a circadian rhythm of optic nerve activity when kept in constant darkness. Eighteen eyes removed from animals that had been subjected to either constant white fluorescent light (LL) of 195 lux or light-dark cycles (LD, 12 hours light to 12 hours dark) of 165 lux to 0 lux, were used in the experiments. The duration of the exposure to either of these conditions varied from 2 to 10 days. The animals were kept in groups of 4 to 50 at 14° to 15°C in tanks of seawater (380 liters) that were part of a 5680-liter circulating system. They were killed at various times during the day in order to test for the complication of dissection time as a factor in the rhythm. The optic nerves were severed at the cerebral ganglion, and the eyes were removed from the surrounding body wall tissues, leaving 1 cm of optic nerve and the attached eye as the isolated preparation (*4*). Dissection, performed under white light or red light from a 6-volt lamp, was completed in 15 to 20 minutes. The eye was placed in a 100-ml chamber thermostatically regulated at 15°C and containing either seawater filtered through millipore filters (0.22 μ) or sterile culture medium (*5*). This culture medium contained Eagle's minimum essential medium made up in seawater consisting of 20 percent filtered *Aplysia* blood. The medium was buffered at *p*H 7.8; it maintained normal electrical activity of the parieto-visceral ganglion of *Aplysia* for up to 6 weeks (*5*).

The eye was stapled through peripheral connective tissue to a silastic platform, and the severed end of the optic nerve was picked up in a suction electrode consisting of Intermedic polyethylene tubing (PE 20) for recording. The electrical activity was led off by a stainless steel needle and amplified by a Tektronix 122 preamplifier, monitored on an oscilloscope, and recorded with a Grass polygraph. Usually two eyes, either from the same animal or different animals, were tested in the same chamber, but as many as four were sometimes tested together. The surface of the chamber was left open to permit free gas exchange during the 1 to 3 days of continuous recording. Special precautions were taken to insure conditions of total darkness during the recording sessions. After the recording apparatus was properly functioning, the chamber was sealed inside a double light-tight box. This was usually completed within 1 hour after dissection had started.

The optic-nerve activity consists of impulses which are compound action

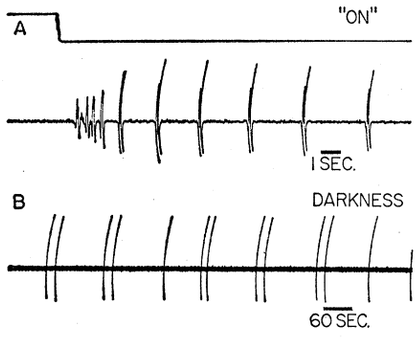

Fig. 1. Compound optic nerve potentials (impulses) from the isolated eye. (A) "On" response to illumination. The top line downward deflection indicates the light is on. The second line shows the shape and frequency of the impulses; there is no "off" response. (B) Spontaneous activity of the same preparation in total darkness. The amplitude and shape of the impulses is unchanged. Negative, upward; impulse amplitude is 50 μv.

potentials (*4*). The impulses occur in response to illumination of the eye (Fig. 1A); they also occur spontaneously at lower frequencies in complete darkness (Fig. 1B). The increase in amplitude of the impulses with time after illumination is probably due to decreasing lateral inhibition between receptors (*4*). These impulses show a double negative peak indicating that they consist of several subpopulations. The impulses may occur singly, in doublets, or up to six impulses per burst. The number of impulses per hour may vary from 0 to 300.

Continuous recording from the optic nerve for 48 hours or longer shows that the occurrence of impulses has a circadian rhythm (Fig. 2). The number of impulses per hour for two eyes from different animals in filtered seawater is shown in Fig. 2A. Both eyes were tested in the same chamber in total darkness and thus were subjected to the same recording conditions. The eye on the top graph had been removed from an animal previously subjected to constant light (LL) for 13 days. The eye on the bottom graph had been subjected to a light-dark cycle (LD 12:12) for 8 days. The black arrows on the time axis indicate the projected dark-light (DL) transition. Both eyes show a definite rhythm. The LL eye has a low-amplitude rhythm with no sharp peaks and a period of about 22.5 hours if the first rise above the base line is taken as a measuring point. The rounded peaks of low amplitude illustrated in Fig. 2A are typical for other LL eyes; additionally, all LL eyes had periods of less than 24 hours. The LD eye has a rhythm of higher amplitude with sharper peaks and a period of about 24 hours if the first upward trend is taken as the point of measurement in the cycle, or 23 hours if the peak is taken. The peak activity precedes the projected DL transition by several hours for both days. Also characteristic of LD eyes tested in filtered seawater is the heightened activity immediately after isolation that rapidly falls to 0 (Fig. 2A, bottom graph).

The eye begins to deteriorate in seawater after 48 hours, so that longer recording periods could not be obtained under these conditions. This is not the case with eyes kept in a culture medium (Fig. 2B). Both LD eyes from the same animal were tested in the same chamber; they are remarkably alike. The upper graph shows the activity of one eye for 2 days, and the bottom graph, the other eye for 3 days (the former eye was damaged on the 3rd day by constriction in the suction electrode). The level of activity is a little lower in eyes kept in culture medium but they clearly last longer. These eyes anticipated the first projected DL transition time but the second peak of activity did not occur until after the second projected transition time. The expression of peak activity was delayed for the second cycle and the third cycle. The period from the first peak to the second peak for both eyes and the period from the second to the third for the lower eye is about 27.5 hours. A period of about 27 hours is typical for eyes kept in culture medium and is probably close to the (*in situ*) period of the free-running rhythm of the eye in darkness.

Eyes kept in seawater typically show a period of 24 hours or less. This finding suggests that metabolic stress (lack of nutrient) in seawater may shorten the period of the rhythm. One would expect the free-running period in a diurnal (day-active) animal subjected to low intensities to be larger than at high intensities [Aschoff's rule (*6*)]. The free-running period of a parieto-visceral neuron of *Aplysia* has been estimated to be 26 to 27 hours (*7*), and this neuron entrains equally well to 24- and 27-hour photoperiods (*8*). This raises the interesting question of the relation of the rhythm in the eye and the rhythm of this central neuron from the same animal. This rhythmic activity of a simple eye could have broad influence over the activity of the whole animal

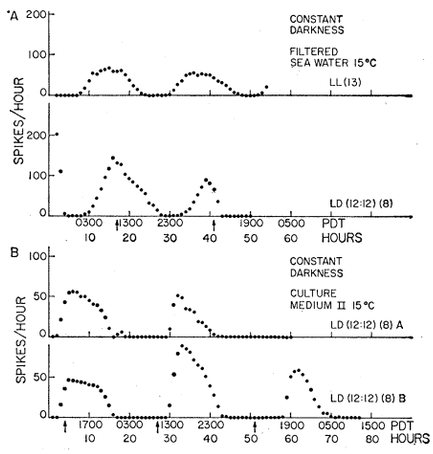

Fig. 2. Plots of the frequency of impulses over several days in total darkness. (A) The eyes were tested in the same chamber in filtered seawater. The upper graph is that of an LL (13 days) eye; the lower is that of an LD 12:12 (8 days) eye. The black arrows on the time axis indicate the projected DL transition time for the LD eyes in (A) and (B). (B) A pair of LD 12:12 (8 days) eyes from the same animal in culture medium.

(that is, locomotion) and the activity of specific central neurons. The phase of the eye rhythm is re-settable at each dawn and thus the eye could set the phase of other coupled rhythms.

The site of the oscillator in the isolated eye is yet undetermined but it is clearly not due to pigment migration (setting-eye sensitivity) since the impulse rate was studied in total darkness. The presence of neurosecretory processes in the retina (*4*) may have significance for the rhythm (*9*) either as conductors of photic stimuli to other systems or as a modulator of retinal excitability.

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