Table 1. Urease activity of whole cells. There was no bacterial growth.

Organism	Units of urease activity*
Porphyridium aerugineum	None
Cyanidium caldarium	None
Plectonema calothricoides	0.396
Phormidium luridum	.244
Phormidium luridum†	.144

* Micromoles of urea hydrolyzed per milligram (dry weight) of cells per hour. Values represent the average of six determinations. The urease assay is sensitive to 0.1 μmole of urea hydrolyzed, with a precision of about 3 percent. † This culture was grown in the presence of 0.001 percent urea.

zyme was added. Usually this procedure was repeated four times. Extraction by repeated freezing and thawing was equally successful, and this method was used with algae not sensitive to lysozyme and as a control with those sensitive to lysozyme. The supernatant solutions were concentrated by vacuum dialysis with Ficoll at 3°C. The concentrated solutions were dialyzed against phosphate (*p*H 7.0, $\mu = 0.1$) at 3°C. The assay for urease was a modification of the procedure of Bernt and Bergmeyer (*6*). Cell suspension or cell extract (1 ml) was added to 1 ml of 0.5 percent recrystallized urea solution. The mixture was incubated at 37°C for 3 hours, at which time an equal volume of 10 percent trichloroacetic acid was added. After the precipitate had settled for 30 minutes, the samples were centrifuged for 10 minutes. The supernatant was decanted and neutralized with NaOH. Ammonia was determined by a modification of the Nessler technique (*7*).

Table 2. Urease activity of extracts.

Source of extract	Units of specific activity*
Cyanidium caldarium	None
Porphyridium aerugineum	None
Porphyridium cruentum	0.30
Plectonema calothricoides	6.93
Synechococcus lividus	4.50
Deuterio *Phormidium luridum*	1.80
Protio *Phormidium luridum*	
Extract 1	1.50
Extract 2	1.10
Extract 3	3.10
Extract 4	0.60
Partially purified *Phormidium luridum* urease	75
Jack bean urease† (partially purified)	280

* Micromoles of urea hydrolyzed per milligram of protein per hour. Values are averages of three or more determinations. The urease assay is sensitive to less than 0.1 μmole of urea hydrolyzed, with a precision of about 3 percent. †Urease powder (jack bean), Mann Research Laboratories, Inc.

The urease activity detected in whole cell cultures is listed in Table 1. In addition, urease activity was detected in cultures of *Synechococcus lividus* and to a very slight extent in *Porphyridium cruentum*. However, the meaning of this is questionable since there was some bacterial contaminant in these cultures. The total urease activity in whole cells of *Phoridium luridum* was recovered after cell lysis and extraction. The total activity in cell extracts was approximately 130 percent of that found in the whole cells, and the extraction procedure was carried out over a period of 4 days. The *Phoridium luridum* grown in the presence of urea did not have greater activity than that grown under normal conditions. This alga did not grow if the concentration of urea was greater than 0.001 percent. The lack of growth in the presence of these urea concentrations raises the question as to what the physiological role of urease may be. Hydrolysis of urea would give a source of CO_2 and NH_3 for use in photosynthesis and nitrogen metabolism; however, our study does not indicate that this is necessarily a prime physiological role of the urease present. Nitrogen starvation and then induction of urease activity were not attempted. Jeffries has demonstrated that bacterial ureases can be induced (*8*).

Since the phycocyanin preparations showed urease activity, it was assumed that the enzyme is a soluble one. Extracts of various algae were tested and activity was detected (Table 2). Other proteins (egg albumin, bovine serum albumin, lysozyme, and carbonic anhydrase) were tested and showed no activity. Fully deuterated algal cells also showed activity, thus making available a fully deuterated enzyme. The rate of NH_3 production was investigated as a function of total protein concentration on dialyzed extracts and was found to be linear with protein concentration. This would be a further indication that the production of NH_3 was catalyzed by a macromolecule. The urease activity of the extracts was in general highest in the first, second, and third extracts and almost negligible in the fourth (Table 2). The washings from whole algal cell preparations did not contain urease activity, a further indication that the urease is an intracellular component.

These data clearly demonstrate the presence of urease in algal cell cultures and extracts and strongly suggest that the activity is of algal origin, the greatest activity being present in the Cyanophyta, with some indications that there is activity in higher algae.

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Neurosecretory Cells: Daily Rhythmicity in Leiobunum longipes

Abstract. *A fluorometric-analysis procedure, used to quantitate indoles, confirmed the presence of 5-hydroxytryptamine (5-HT) in an arachnid; there was unimodal cyclic production of 5-HT in brain and intestinal tissues over a 24-hour period. The same tissues produced 5-HT after 80-day culture; bimodal cyclic output was indicated during continuous 24-hour study. One peak occurred at 0200 hours, at the same time as the peak in vivo, suggesting an endogenously controlled mechanism of secretion. The second peak occurred at midmorning, a time when production in vivo was lowest, suggesting that there is a possible feedback-control mechanism in the organism that inhibits the endogenous output of 5-HT.*

Previous experiments with invertebrates and vertebrates seem to indicate that the quantity of brain secretions undergoes cyclic changes during a 24-hour period. These changes have been shown by measurement of secretion granules after histochemical staining (*1*), by cell size, or by the presence of other cellular inclusions (*2*). Few actual measurements of a specific compound have been reported (*3*). We now report the presence, and measurement,

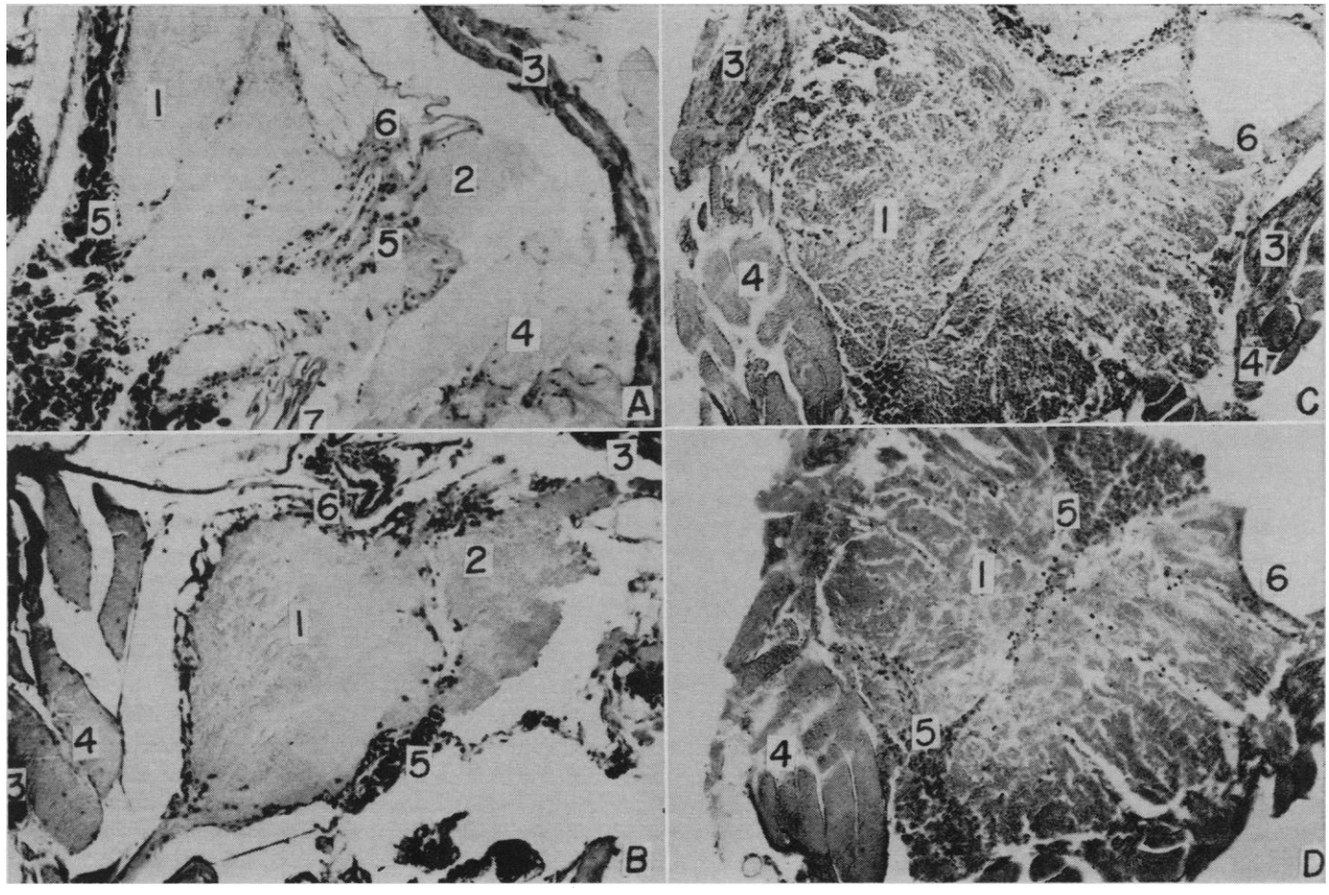

Fig. 1. Sagittal sections of the brain of *Leiobunum longipes*, stained with hematoxylin-eosin. The opilionid brain contains numerous membrane-lined channels containing neurosecretory granules, both in the channels and within the membranes lining them. Several different fixing and staining techniques confirm this peculiar structure. *A*, In vivo brain tissue fixed with alcoholic Bouin's solution (× 80); *B*, in vivo brain tissue fixed with Petrunkevitch's solution (× 80); *C*, in vitro brain tissue fixed in alcoholic Bouin's solution (× 120); *D*, in vitro brain tissue fixed in alcoholic Bouin's solution (× 120). *1*, Subesophageal ganglion; *2*, supraesophageal ganglion; *3*, endosternite; *4*, bundles of muscles; *5*, secretion granules located in the periphery, within the neural membranes, or in channels within the tissue; *6*, esophagus area; *7*, pharynx.

of the neurosecretory compound 5-hydroxytryptamine (5-HT) in an arachnid of the opilionid order, a compound secreted cyclically in mammals (*4*).

Harvestmen, *Leiobunum longipes*, were taken from their natural habitat in woodland and kept in the laboratory under the normal light cycle of midwestern midsummer day (light:darkness, 14:10) at 25°C and a relative humidity of 85 percent. After 0 to 2 days in captivity, arachnids were killed quickly in 70-percent ethanol every 4 hours and the brain and gut dissected from a pair were pooled. Three pooled samples were run for each time period. Extracts of the tissues were separated on paper with a solvent mixture of butanol, acetic acid, and water, areas of the paper containing 5-HT were eluted, and the 5-HT was caused to fluoresce in solution after being heated with ninhydrin (*5*).

5-Hydroxytryptamine was quantified in a photofluorometer by excitation at wavelength 380 mμ and by allowing maximum transmission at 515 mμ with Corning filters 3-71 and 4-72. The amount of 5-HT in micromicromoles was calculated by conversion of the absorption values of the samples to percentage values of standard 5-HT; standard 5-HT had been used for comparison throughout the procedures.

The same tissues that were used for the analysis in vivo were removed from harvestmen and maintained in vitro (*6*); the gut was placed close to the brain in a Falcon flask that contained tissues from one individual, the culture fluid being the unmodified fluid of Martin and Vidler (*7*). The *p*H, initially 7, became acidic within a few hours or a few days. The culture medium was left unchanged unless obvious conditions, such as unhealthy appearance, seemed to warrant a change.

The culture flask was placed over water in a humidity chamber, with CO_2 replacing air within the chamber; thus the cultures were held in an environment of highly humid CO_2. The chamber was placed in the same light cycle and air-conditioned laboratory as the live captive opilionids.

The tissues used to study secretory activity were all 80 days old; microscopic and macroscopic examination of them revealed very little structural change apart from reduction in number of secretory granules and overall shrinkage of tissue (Fig. 1). For the measurement of 5-HT, the in-vitro tissues were treated the same as those in vivo. In a continuous 24-hour investigation after 80-day culture, six brains and six guts were removed from the flasks and were each pooled in pairs. Three pools were run for each time period; thus in the 24-hour measurement of 5-HT 36 arachnids in all were represented (Table 1 and Fig. 2).

In a test of homogeneity of variances (the *q* test of Foster-Burr) the in vivo and in vitro data were combined, and the hypothesis of homogeneity was rejected at the .05 level of probability. Since there was no homogeneity of

variances overall, the probability of an error in the rejection of the hypothesis when it should have been accepted would be questionable for the tests on the means in an analysis of variance; therefore the data for individuals were not pooled or averaged. The sums for all individuals per time period per day appear graphically in Fig. 2; sums of several individuals should more validly represent a possible rhythm in secretion for *L. longipes* than the value obtained from one individual because of the great individual variation, particularly during periods of peak activity.

The method used to measure 5-HT required the use of entire brains and guts for analysis, so a 24-hour rhythm of secretion for a single individual was unobtainable. Other investigators have found wide variation between individuals as to the time of occurrence of a specific secretion response. Beck *et al.* (*8*), in studying cornborers, found that the probability of an arbitrarily chosen individual showing a secretion response within a 2-hour interval was approximately 67 percent; this figure may be even lower in quantitative studies. Tissues in vitro secreted less total 5-HT than those in vivo. The mass of tissue after 80 hours in vitro was about 25 percent of its original volume.

A technique has been developed for maintaining functionally active tissues of *L. longipes* for long periods. These tissues showed rhythmic secretions. Comparison of the activities of tissues in vitro and in vivo showed similarity in secretion patterns regarding times of greatest activity. However, the in vitro secretion was bimodal; the in vivo unimodal. The cultured tissues exhibited a secondary peak of activity in secretion at a time when production was lowest in vivo. Such results suggest endogenous control of secretion of 5-HT, and a possible central mechanism of feedback control. Is the secretion of 5-HT controlled by photoperiod in this arachnid as it appears to be in vertebrates (*4*)? The phenomenon of cyclic secretion has been noted in the endocrine glands of vetebrates briefly held in vitro (*9*). Confirmation of cyclic activities in tissues long maintained in culture would interest investigators of the nature of the biological clock. Our technique would be useful for examining the question of exogenous versus endogenous control of cyclic phenomena.

Table 1. Sums of 5-HT from 36 brains and 36 guts after about 80 days in vitro. Every 4 hours during a 24-hour period six brains and six guts were removed from Falcon flasks; each sample is a pool of two brains and two guts. Lack of homogeneity of variance prevented statistical handling of the data.

Time (hours)	5-HT ($\mu\mu$mole)	
	In three samples	Total
1000	18.9, 18.9, 18.9	58.7
1400	12.1, 12.1, 12.1	36.3
1800	6.8, 4.6, 2.3	15.9
2200	9.4, 19.0, 28.1	56.5
0200	20.0, 23.6, 27.3	70.9
0600	0 0 0	

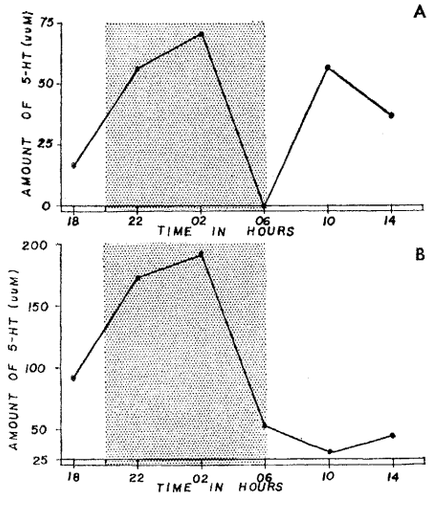

Fig. 2. Daily rhythmicity of the secretion of 5-HT in tissues in vitro. The pattern of secretion is rhythmic, peaking at 0200 hours as in vivo. However, the overall 24-hour pattern is bimodal, with a second peak at 1000 hours (midmorning)—a time when the pattern in vivo is at its lowest point. *A*, In vitro data; *B*, in vivo data for day 1; the curve is representative of all data obtained and is used here for comparison. The stippled areas indicate night during the 24-hour cycles.

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Heme Stimulation of Globin Synthesis in a Cell Free System

Abstract. *In a cell-free system of pigeon erythrocyte nuclei high concentrations of oxygen inhibit globin synthesis because of the inhibition of heme synthesis, which is required for globin synthesis. The effect of heme in overcoming the inhibition of globin synthesis by oxygen is manifested only during the first few minutes of incubation. This period represents the time during which the system is being warmed from 4° to 37°C. The effect of heme seems to be upon some initial assembly or structural process.*

The nuclei of avian erythrocytes carry out the synthesis of globin (*1*, *2*). In cell-free preparations of these nuclei the synthesis of globin is stimulated by heme and by hypoxia (*3*, *4*). This reversible inhibition of hemoglobin synthesis by oxygen is relieved by the addition of heme (*4*). Falk and Porra (*5*) have shown that oxygen limits heme synthesis by inhibiting the formation of uroporphyrinogen from porphobilinogen.

On the basis of these findings, a model was proposed for a direct biochemical regulation of hemoglobin synthesis in which hypoxia stimulates the synthesis of heme which, in turn, increases the rate of globin synthesis (*4*). Further studies on the kinetics of this regulatory process are in agreement with the proposed model and suggest a mechanism of action of heme on protein synthesis.

Nuclei were isolated from pigeon red cells hemolyzed with 0.5 percent saponin in a solution containing 0.25*M* sucrose, 0.003*M* $CaCl_2$, and $10^{-5}M$ ferric ammonium sulfate and centrifuged at 1000*g*. The washed nuclei were incubated in 5-ml serum vials in a medium containing 0.02*M* sodium phosphate buffer, 0.015*M* KCl, 0.03*M* glucose, and 9.6×10^{-4} mmole (0.25 μc) of L-leucine-C^{14} (uniformly labeled) per vial. The vials were closed with rubber-dam stoppers and metal caps and flushed with the various gas mixtures. All of the above procedures were performed while the vials were packed in shaved ice. Incubations were at 37°C in a Dubnoff metabolic shaker. The reactions were stopped by placing the vials in ice and injecting through the rubber dam 1 ml of 10 percent trichloroacetic acid (TCA). The TCA precipitates were purified