shown to be so in some recent experiments in our laboratory. Rats were injected for 25 days with unlabeled Cr-EDTA in amounts that would have been nephrotoxic had the EDTA been given as the calcium chelate. At the end of the experiment the animals were injected with a single dose of Cr^{51}-EDTA, and, since they excreted the labeled Cr as rapidly as normal animals did, their kidney function was presumably unaffected by the prolonged treatment with large quantities of Cr-EDTA. Also, histological examination of the kidneys failed to reveal any abnormalities.

After intraruminal administration very little Cr-EDTA is absorbed from the gut, and Downes and McDonald (*14*) have described how the complex may be used to advantage as a soluble rumen marker. Our results suggest that Cr^{51}-EDTA may also prove of value in the investigation of renal function.

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6. Initially we prepared Cr^{51}-EDTA from $Cr^{51}Cl_3$ by the technique of Downes and McDonald (*14*). Subsequently, supplies of Cr^{51}-EDTA became readily available at relatively low cost from the Isotope Division of the Australian Atomic Energy Commission, Lucas Heights, N.S.W., Australia. Storage of the labeled material (specific activity 0.5 c/g of Cr) for a period of one half-life of Cr^{51} (28 days) caused no obvious change in the renal excretion of Cr^{51}-EDTA.
7. Inulin for injection was obtained from Thomas Kerfoot & Co., Lancashire, England. The purity of the material was examined by paper chromatography of solutions to which were added tracer amounts of C^{14}-fructose. The only measurable component that could be separated from inulin was free fructose, and this was present in negligibly small amounts (<1 percent). We thank Dr. T. W. Scott for advice on these investigations.
8. After priming injections of inulin and Cr^{51}-EDTA, plasma levels were stabilized by constant intravenous infusion. The priming dose of Cr^{51} was 80 μc, and the sustaining infusion was 0.5 μc/min; plasma levels were 5 to 10 mμc/ml. Urine and plasma samples (3 ml) were counted in a NaI (Tl) crystal with an efficiency of 4 percent (count rates corrected for background were > 20 count/sec).
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10. The procedure with the Auto Analyser (Technicon Instruments Corporation, New York) was developed for the determination of inulin by the direct resorcinol reaction without alkali treatment. Details of the method are being prepared for publication by B. W. Wilson of C.S.I.R.O., Division of Animal Physiology, New South Wales, Australia. Estimates of the renal clearance of inulin were similar when the automated technique was compared with the manual method for measuring alkali-stable inulin. Recovery of inulin added to urine or plasma was 98.5 to 101 percent by autoanalysis.
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Urease Activity in Blue-Green Algae

Abstract. *The apparent enzymatic hydrolysis of urea has been detected in whole blue-green algae and in cell extracts. Urease is present as an intracellular component in cultures in which no bacterial contaminants are found. The activity in the cells was recovered from the extracts.*

During an investigation of the physical properties of normal and fully deuterated phycocyanins (*1*), it was observed that when urea was used as a structure perturbant and dissociating agent there was an increase in *p*H of the buffered protein preparations over a period of several hours. This finding raised the question whether the enzyme urease was present in the preparations. No previous report of urease activity in Cyanophyta has been found. There are conflicting reports about the use of urea as a nitrogen source by blue-green algae (*2*, *3*). In addition, the mechanism of urea metabolism in blue-green algae was obscure, for urease activity could not be detected. An investigation of the origin and nature of the degradation of the urea by the phycocyanin preparations was therefore undertaken.

A phycocyanin preparation from *Plectonema calothricoides* in the presence of urea was examined manometrically. A gas was evolved under both basic and acidic conditions in the approximate ratio of 2 : 1, which is the proper stoichiometry for NH_3 and CO_2 evolution from the urease reaction. This fact and the determination of increasing amounts of ammonia with time by a Nessler technique appeared to indicate definite urease activity.

Fresh cultures of *Plectonema calothricoides*, *Phormidium luridum*, *Porphyridium aerugineum*, and *Porphyridium cruentum* were obtained from the Indiana University culture collection and were maintained bacteria-free in appropriate media (*4*). Several cultures obtained from other sources were also used in these experiments; *Cyanidium caldarium* (M. B. Allen, Kaiser Foundation, University of California, Berkeley), and *Synechococcus lividus* (J. Middlebrook, Ling-Temco-Vought Research Center, Dallas, Texas). All the cultures were grown under fluorescent light on rotary shakers and, with the exception of the thermophilic *S. lividus* and *Cyanidum caldarium*, all were cultured at room temperature. Cells harvested by centrifugation were washed twice with phosphate buffer (*p*H 7.0, $\mu = 0.1$) and then were assayed whole or used to prepare cell extracts. Samples of the harvested cell cultures were inoculated into casein hydrolyzate semi-solid agar with thioglycollate (*5*) and incubated at 37°C for 72 hours and at room temperature for another 72 hours. If no growth appeared at the end of the incubation period, the cultures were considered as probably free of most contaminants. Examination of these cultures under high magnification with a phase-contrast microscope did not reveal detectable bacterial contaminants. If any contaminants were present they were extremely minute. Cultures found to be essentially bacteria-free by the thioglycollate test are listed in Table 1. Bacterial contaminants were evident in cultures of *Synechococcus lividus* and *Porphyridium cruentum*.

Cell extracts of *Plectonema calothricoides*, *Phoridium luridum*, and *Synechococcus lividus* were prepared by adding lysozyme and leaving the suspension at room temperature for 24 hours. The suspension was centrifuged at 18,000*g* for 10 minutes, the supernatant was decanted, and more lyso-

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,