pituitary-adrenal axis, such as stress-evoked responsiveness or responsiveness to exogenous ACTH administration.

The present studies suggest that administration of corticosteroids during a critical period of neonatal development can modify the expression of circadian adrenal periodicity; these results add to the body of evidence indicating that hormones present in excess shortly after birth may have permanent effects in altering the physiological and behavioral state of the individual. Further studies are indicated to determine whether or not such modification of circadian adrenal periodicity (i) persists throughout the adult life of the animals, (ii) is associated with generalized or localized alteration of neurotransmitter content in the central nervous system, or (iii) is associated with altered hypothalamic-pituitary-adrenal responsiveness to various stressor agents. In this regard, Schapiro (*12*) indicated that neonatal administration of cortisol did not alter the response to ether stress (which is manifest at an earlier developmental age than circadian periodicity) in animals 44 days of age. This would be further evidence in support of the thesis (*13*) that different anatomic or chemically mediated pathways are involved in the regulation of adrenal circadian periodicity and stress responsiveness of the hypothalamic-pituitary-adrenal axis.

DOROTHY T. KRIEGER
Neuroendocrinology Laboratory,
Division of Endocrinology,
Department of Medicine,
Mount Sinai School of Medicine,
New York 10029

References and Notes

1. R. Franks, *J. Clin. Endocrinol. Metab.* **27**, 75 (1967); C. Allen and J. W. Kendall, *Endocrinology* **80**, 926 (1967); R. Ader, *Science* **163**, 1225 (1969).
2. Y. Eguichi, in *Physiology and Pathology of Adaptation Mechanisms*, E. Bajusz, Ed. (Pergamon, New York, 1969), pp. 3–29.
3. C. A. Barraclough, *Endocrinology* **68**, 62 (1961); R. A. Gorski, *Amer. J. Physiol.* **205**, 842 (1963).
4. S. Kikuyama, *Annot. Zool. Japan* **34**, 111 (1961); *ibid.* **35**, 6 (1962).
5. B. E. P. Murphy, *J. Clin. Endocrinol. Metab.* **27**, 983 (1967).
6. D. T. Krieger, *Trans. N.Y. Acad. Sci.* **32**, 316 (1970).
7. V. Critchlow, R. A. Liebelt, M. Bar-Sela, W. Mountcastle, H. S. Lipscomb, *Amer. J. Physiol.* **205**, 807 (1963).
8. H. Wells, F. N. Briggs, P. L. Munson, *Endocrinology* **59**, 571 (1956); R. H. Egdahl, B. Richardson, D. M. Hume, *Science* **123**, 418 (1956).
9. M. H. Taylor and E. Howard, *Endocrinology* **88**, 1190 (1971).
10. S. Levine and R. Mullins, *ibid.* **80**, 1177 (1967).
11. A. Vernadakis and D. M. Woodbury, in *Influence of Hormones on the Nervous System*, H. Ford, Ed. (Karger, Basel, 1971), pp. 85–97.
12. S. Schapiro, *Gen. Comp. Endocrinol.* **10**, 214 (1968).
13. G. P. Moberg, U. Scapagnini, J. Groot, W. F. Ganong, *Neuroendocrinology* **7**, 11 (1971); J. Seggie and G. M. Brown, *ibid.* **8**, 367 (1971); M. A. Slusher, *Amer. J. Physiol.* **206**, 1161 (1964); D. T. Krieger, A. I. Silverberg, F. Rizzo, H. P. Krieger, *ibid.* **215**, 959 (1968); D. T. Krieger and F. Rizzo, *ibid.* **217**, 1703 (1969); U. Scapagnini, G. P. Moberg, G. R. Van Loon, J. De Groot, W. F. Ganong, *Neuroendocrinology* **7**, 90 (1971).
14. Supported by PHS grant 2 RO1 NS02853. The assistance of W. Wagman and A. Liotta is gratefully acknowledged.

10 July 1972

Nitrogen Fixation by a Blue-Green Epiphyte on Pelagic Sargassum

Abstract. *Nitrogen fixation by* Dichothrix fucicola, *an epiphyte on pelagic* Sargassum, *was measured in May and June 1972 in the western Sargasso Sea and the Gulf Stream. This is the first report of nitrogen fixation by a heterocyst-bearing blue-green alga in the open ocean, and also the first observation of nitrogen fixation in the genus* Dichothrix. *Cellular carbon/nitrogen ratios suggested that the* Dichothrix *was nitrogen-starved. In dense aggregations of* Sargassum, *such as rafts or windrows, the enrichment of surface seawater with combined nitrogen from nitrogen fixation may be pronounced.*

Biological nitrogen fixation must play a major role in the nitrogen cycle of the ocean (*1*). However, virtually nothing is known of in situ bacterial nitrogen fixation in the open sea, and only one algal genus, *Oscillatoria* (*Trichodesmium*), has been reported to fix atmospheric N_2 (*2*, *3*). Trichomes of this genus do not possess heterocysts—enlarged cells that permit nitro-

Table 1. Nitrogen fixation by *Dichothrix fucicola* epiphytic on pelagic *Sargassum*. The nitrogen fixed per day is given in terms of the amount of cellular N ($\mu g/\mu g$), the amount of *Dichothrix* ($\mu g/mg$), and the area of sea surface ($\mu g/m^2$); all weights are dry weights.

Location	Date (1972)	*Dichothrix*/*Sargassum* (mg/g)	*Dichothrix* sample		N_2 fixed per day		
			N (μg)	C (μg)	$\mu g/\mu g$	$\mu g/mg$	$\mu g/m^2$
			R.V. Knorr, *cruise 25*				
21°05′N 69°35′W	1 May	0.34	33.7	717	0.016	0.100	0.006
			42.5	583	.018	.148	.009
			6.4	80.2	.066	1.50	.092
			3.7	48.1	.162	2.39	.145
			12.5	170	.057	0.802	.049
22°45′N 71°40′W	2 May	0.49	52.1	779	.002	.040	.004
			12.3	225	.004	.100	.009
			39.2	717	.000	.000	.000
23°17′N 71°04′W	3 May	0.41	19.8	252	.015	.403	.030
					.000	.000	.000
			20.2	199	.066	2.45	.181
			3.4	30.8	.191	5.89	.436
			23.5	294	.034	0.707	.052
			25.0	218	.137	5.15	.381
26°18′N 69°51′W	5 May	1.27	4.8	59.9	.102	2.05	.467
			19.1	195	.052		
			9.9	131	.000	0.767	.175
30°22′N 70°37′W	6 May	0.72	61.4	680	.026	.000	.000
			46.8	536	.022	.578	.074
			16.7	160	.037	1.15	1.14
			26.6	433	.007	0.145	0.019
			3.4	30.8	.191	5.89	.436
			23.5	294	.034	0.707	.052
			25.0	218	.137	5.15	.381
34°03′N 70°56′W	7 May	1.53	17.6	201	.053	1.55	.420
			28.4	383	.022	0.573	.158
			37.6	824	.019	.540	.149
			21.5	311	.031	.683	.188
			R.V. Gosnold, *cruise 193*				
37°48′N 70°47′W	7 July	25.1	138	1005	0.008	0.086	0.388
			49.7	500	.023	.098	.443
			392		.003	.057	.257
			42.6	418	.012	.052	.235
			157	1239	.005	.032	.145
			50.3	530	.019	.011	.050
			41.3	321	.014	.067	.303

Table 2. *Dichothrix* standing crop and estimated nitrogen fixation in October 1971. Weights are dry weights.

Stations (*N*)	Location	*Dichothrix*/ *Sargassum* (mg/g)	*Dichothrix*/ sea surface (mg/m^2)	Estimated N_2 fixed ($\mu g\ m^{-2}\ day^{-1}$)
4	Shelf off New Jersey and Delaware	72.3	13.0	10.8
2	Gulf Stream off North Carolina	7.14	1.28	1.06
4	Northwestern Sargasso Sea (north of 30°N)	3.94	0.71	0.591
2	Southwestern Sargasso Sea (south of 30°N)	1.55	.28	.233

gen fixation under aerobic conditions (*4*)—and in this respect it is different from almost all other blue-green algal nitrogen fixers from freshwater and coastal marine areas (*5*). This report describes the first in situ measurements of nitrogen fixation by a heterocyst-bearing blue-green alga from the open ocean.

In May and July 1972, nitrogen fixation was observed with the acetylene reduction technique (*6*) on *Dichothrix fucicola* (Kütz.) Born. & Flah., epiphytic on pelagic *Sargassum* in the western Sargasso Sea. Apparently, this is the first report of nitrogen fixation in the genus *Dichothrix*. About 1 to 5 mg (dry weight) of epiphyte was removed from *Sargassum* and suspended in 1 ml of seawater (filtered through netting of 20-μm mesh size) in a 5-ml wide-mouth serum bottle. The bottles were closed with rubber caps, sealed with silicone grease, and then purged with a mixture of A, O_2, and CO_2 (78 : 22 : 0.04) for 2 minutes. Acetylene (1 cm^3) was injected into each bottle, the bottles were incubated in flowing seawater in direct sunlight for 6 hours either before or after local noon, and the reaction was terminated by adding 0.1 ml of 50 percent trichloroacetic acid (TCA) to each bottle. Controls with seawater alone and controls with TCA added were also incubated for each station (sampling location). Both controls showed no ethylene production. The gas phase was assayed with a Porapak R column (2.7 m by 0.3 cm) at 55°C on a Perkin-Elmer (model 226) gas chromatograph, and moles of ethylene were converted to moles of ammonia by using a factor of 1.5. This factor is based on theory and can range from 1.4 to 1.8 (*6*). Data on nitrogen fixation by *Dichothrix* remains to be verified by the ^{15}N reference method. Nitrogen fixation is light-dependent and daily fixation was calculated on the basis of a 12-hour light day.

The average nitrogen fixation in May at six stations in the western Sargasso Sea was 0.040 μg of N_2 per microgram of cellular nitrogen per day, and in July in the Gulf Stream south of Cape Cod it was 0.012 μg of N_2 per microgram of cellular nitrogen per day (Table 1). The variation in nitrogen fixation rates was high for replicate samples. A similar high variation was noted when the ^{15}N technique was used for measuring nitrogen fixation by *Oscillatoria* in the Atlantic and Indian oceans; this was apparently due in part to differences in the physiological states of the colonies tested (*3*). Physiological differences, such as the age of a colony and the presence of heterocysts, are probable causes of the variation observed in *Dichothrix* nitrogen fixation rates. In May the nutrient concentrations were determined at each station (*7*), the concentrations of NO_2, NO_3, NH_4, and PO_4 averaged (in 10^{-6} gram atom per liter) 0.06, 0.22, 2.41, and 0.18, respectively, and there was little variation from station to station. The ammonia concentration was relatively high, although not high enough to inhibit nitrogen fixation, supporting the view that it is unlikely that concentrations of combined nitrogen compounds in the open ocean are ever high enough to inhibit nitrogen fixation (*5*).

Cellular carbon/nitrogen ratios averaged 14.1 in May and 10.1 in July, and both values are typical of nitrogen-starved cells. The cellular carbon and nitrogen concentrations were determined with a Perkin-Elmer (model 260) elemental analyzer. The C/N ratio of a healthy mixed phytoplankton population is about 3, while that of a nitrogen-starved one rises to about 4.7 (*8*). Typical blue-green algae have a C/N ratio of about 6.2 (*9*), and it would be expected to increase as cells become deficient in nitrogen.

Nitrogen fixation per square meter of sea surface can be calculated by using the data for the *Dichothrix* standing crop, the dry weight of *Dichothrix* per milligram dry weight of *Sargassum*, and measurements of the *Sargassum* standing crop (*10*). The wet weight of the *Sargassum* standing crop was converted to dry weight by using the factor 5.9, which was obtained in previous work on the western Sargasso Sea (*11*). In May the average nitrogen fixation (N_2) was 0.175 $\mu g\ m^{-2}\ day^{-1}$ for all stations; the highest average rates (0.225 $\mu g\ m^{-2}\ day^{-1}$) were at the three stations north of 26°N and a lower average value (0.099 $\mu g\ m^{-2}\ day^{-1}$) was obtained for the three southerly stations. In July the average nitrogen fixed in the Gulf Stream south of Cape Cod was 0.260 $\mu g\ m^{-2}\ day^{-1}$, which compares favorably with the three northerly stations in May. It is possible that higher nitrogen fixation rates occur. Data for the *Dichothrix* standing crop are available for various areas of the western North Atlantic Ocean for October 1971 (Table 2); when the average nitrogen fixation rate for May and July (0.0832 μg of N_2 per milligram dry weight per day) is applied, rates as high as 10.8 $\mu g\ m^{-2}\ day^{-1}$ are extrapolated. However, even rates of this magnitude are low compared with the contribution of fixed nitrogen from *Oscillatoria*, which is about 100 to 200 μg per cubic meter per day in the tropical Atlantic (*12*); the largest *Oscillatoria* populations occur at depths of about 15 to 25 m (*13*).

It is possible that nitrogen fixation may affect the pelagic *Sargassum* community, which is composed of well over 100 organisms (*14*), by extracellular release of combined nitrogen and uptake by *Sargassum* or phytoplankton (*15*) (or by the grazing of herbivorous organisms on the *Dichothrix*). This effect will be magnified in dense concentrations of *Sargassum*, such as in rafts or windrows.

EDWARD J. CARPENTER
Woods Hole Oceanographic Institution, Woods Hole, Massachusetts 02543

References and Notes

1. A. C. Redfield, *Amer. Sci.* **46**, 205 (1958).
2. R. C. Dugdale, D. W. Menzel, J. H. Ryther, *Deep-Sea Res.* **7**, 298 (1961); J. S. Bunt, K. E. Cooksey, M. A. Heeb, C. C. Lee, B. F. Taylor, *Nature* **227**, 1163 (1970).
→ R. C. Dugdale, J. J. Goering J. H. Ryther, *Limnol. Oceanogr.* **9**, 507 (1964).
4. H. J. van Gorkom and M. Donze, *Nature* **234**, 231 (1971).
5. W. D. P. Stewart, in *Fertility of the Sea*, J. D. Costlow, Ed. (Gordon & Breach, New York, 1971), p. 437.
→ W. D. P. Stewart, G. P. Fitzgerald, R. H. Burris, *Arch. Mikrobiol.* **62**, 336 (1968).
7. J. D. H. Strickland and T. R. Parsons, *Bull. Fish. Res. Bd. Can. 122* (1968).
→ N. J. Antia, C. D. McAllister, T. R. Parsons, K. Stephens, J. D. H. Strickland, *Limnol. Oceanogr.* **8**, 166 (1963).
9. A. P. Vinogradov, *The Elementary Chemical*

Composition of Marine Organisms (Sears Foundation for Marine Research, Yale University, New Haven, Conn., translated by J. Efron and J. K. Setlow, 1953).
10. A. E. Parr, *Bull. Bingham Oceanogr. Collect. Yale Univ.* **7**, 1 (1939).
11. E. J. Carpenter, unpublished results.
12. J. J. Goering, R. C. Dugdale, D. W. Menzel, *Limnol. Oceanogr.* **11**, 614 (1966).
13. R. R. L. Guillard and W. F. Blankley, personal communication.
14. J. K. Dooley, *Contrib. Mar. Sci.* **16**, 1 (1972).
15. K. Jones and W. D. P. Stewart, *J. Mar. Biol. Ass. U.K.*, **49**, 475 (1969); *ibid.*, p. 701.
16. I thank S. Smith, J. P. Clarner, and N. Corwin for assistance; and R. R. L. Guillard, S. W. Watson, and R. S. Scheltema for comments on the manuscript. Gratitude is also expressed to W. J. Woelkerling for confirming the identification of *D. fucicola*. Contribution No. 2922 from the Woods Hole Oceanographic Institution.

25 August 1972; revised 16 October 1972

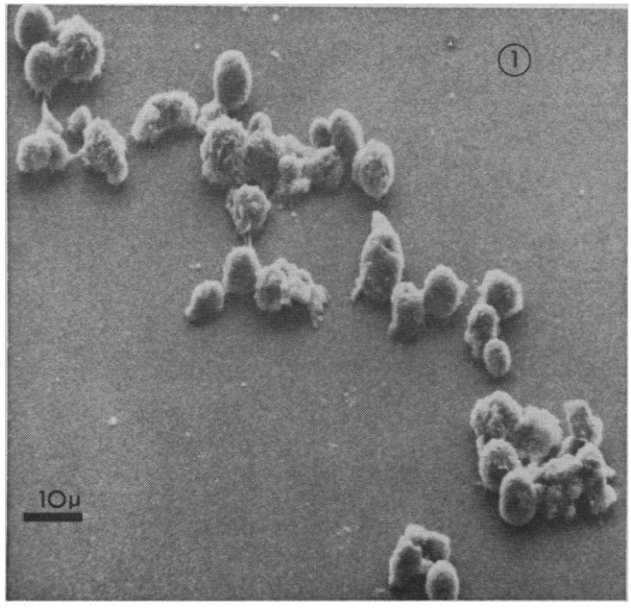
Dispersal of Rabbit Lung into Individual Viable Cells: A New Model for the Study of Lung Metabolism

Abstract. *This new enzymatic method disperses rabbit lung into morphologically intact, viable individual cells. The scattered cells constitute more than 50 percent of the original tissue. At least 90 percent of the cells exclude trypan blue from the nucleus. The dispersed lung cells consumed 6.2 microliters of oxygen per hour per milligram, dry weight. They incorporated [1-^{14}C]palmitate into lecithin.*

Evaluation of the individual metabolic characteristics of each of the many morphologically distinct types of cells that occur in mammalian lungs requires a method to disperse them into a mixed population of individual viable cells (IVC) prior to their isolation into homogeneous groups. Dispersal of other tissues into IVC has been accomplished with mechanical, enzymatic, chemical, antibody-mediated, and combined methods (*1*), but thus far no satisfactory method for the dispersal of mammalian lung has been available. This report describes a method for the enzymatic dispersal of rabbit lung into IVC that are morphologically intact, exclude a vital dye, consume oxygen, and incorporate palmitate into lecithin.

New Zealand white male rabbits (average weight, 1.7 kg) were anesthetized intravenously with sodium pentobarbital. After tracheostomy, the lungs were artificially ventilated with air, the chest was opened, and a catheter inserted through the right ventricle into the pulmonary artery was secured with ligatures. The left atrium was excised to reduce outflow obstruction to the perfusate and minimize formation of pulmonary edema. The lungs were perfused at 38°C in situ with 100 to 200 ml of Krebs (*2*) serum substitute solution III (KSSS), which is low in bicarbonate and high in phosphate, plus 2 percent fetal calf serum at *p*H 7.4. Perfusions done at flows of 60 to 70 ml/min and at a pressure of less than 20 cm-H_2O removed most of the blood cellular elements. The lungs were excised, weighed, and filled through the trachea with 80 to 100 ml of iced KSSS without calcium or magnesium but with 2 percent fetal calf serum. The solution contained, per milliliter, 1 mg of crude collagenase, 2 mg of Pronase, 0.5 mg of chymopapain, 10 units of elastase, 0.03 mg of deoxyribonuclease to reduce agglutination of cells, and 0.005 ml of crude catalase (*3*). The lungs were cut into approximately 1-cm cubes and then into slices 1 mm thick. These were incubated in the above enzyme mixture at 38°C for 60 minutes with gentle agitation at 90 cycles per minute in a Dubnoff shaker. A *p*H stat delivering 0.154*M* NaOH maintained the *p*H at 7.4. An oxygen stat delivered 0.088*M* H_2O_2 to maintain the partial pressure of oxygen (P_{O_2}) at 130 ± 10 torr (*4*).

To reduce mechanical and chemical damage to the loose cells from continued shaking in the enzyme mixture, the IVC were filtered through polyester mesh at intervals of 10, 20, 30, 45, and 60 minutes while the undispersed tissue residuum was returned to the enzyme mixture in the dispersal flask.



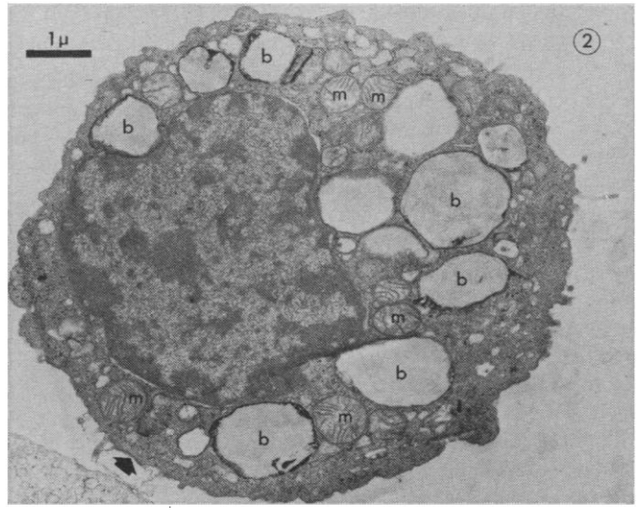


Fig. 1 (left). Scanning electron micrograph of air-dried preparation of individual dispersed lung cells coated with 30 percent fetal calf serum, which shows that the individual cells tend to be spherical or ovoid with irregular processes extending from some of the cells. Specific morphologic cell types were not identified by this method (× 800). Fig. 2 (right). Transmission electron micrograph of a granular pneumonocyte or type II alveolar cell which shows good preservation of the cytoplasmic organelles including mitochondria (*m*). The "lamellar bodies" (*b*) have lost the typical lamellate character which usually distinguishes this cell so that the electron-dense material is now located at the periphery of the cytosome thought to contain surfactant (*5*). This change occurs after perfusion to remove blood cellular elements. A small portion of the membrane of another cell remains attached (arrow) (× 9260).