in a liquid scintillation spectrometer. Radioactivity was summed for each of the two amino acid peaks. The hydroxyproline radioactivity was corrected by a factor of 1.3 because 25 percent of the label of [3,4-^{3}H]proline is lost during conversion (*8*).

The mRNA fraction from *Rana pipiens* induced collagen synthesis in injected oocytes, as shown by the presence of labeled hydroxyproline in the protein hydrolyzate (Fig. 1). The ratio of corrected radioactivity in hydroxyproline to radioactivity in proline was 0.041. Injection of nuclear RNA of early neurulae (stage 14) also induced collagen synthesis in the oocytes, as revealed by the peak of labeled hydroxyproline (Fig. 2); in this case the incorporation ratio was 0.027. Injection of saline or nuclear RNA of early gastrulae (stage 10) (*4*) or of swimming larvae (stage 25) did not promote collagen synthesis, as shown by the absence of a radioactivity peak for hydroxyproline.

The elution patterns of labeled hydroxyproline varied somewhat, probably because of changes in degree of packing of the column. However, the labeled peaks were always identical with the optical density peaks of the added authentic proline and hydroxyproline. When the column fractions containing labeled hydroxyproline and proline were rechromatographed with authentic hydroxyproline and proline, the optical density and radioactive peaks coincided exactly. All experiments and control series were repeated three or four times with similar results.

Our most important result is that neurula nuclei contain RNA that promotes collagen synthesis in injected *Xenopus* oocytes, whereas activity of injected larval nuclear RNA cannot be detected. The absence of collagen synthesis after injection of larval nuclear RNA probably is not caused by a complete absence of the RNA that promotes collagen synthesis, but rather by the lower quantity of this RNA. This appears surprising because larvae synthesize much more collagen than do early neurulae (*3*). This synthesis can be explained by the greater conservation of collagen mRNA in the cytoplasm at the later developmental stage (*6*), even though transcription is restricted in the nuclei (*9*). Nuclear RNA from early gastrulae was inactive in promoting collagen synthesis, because synthesis of DNA-like RNA is just commencing again at this stage after being absent during cleavage (*10*).

J. W. ROLLINS*
R. A. FLICKINGER
Department of Biology, State University of New York, Buffalo 14214

References and Notes

1. J. B. Gurdon, C. S. Lane, H. R. Woodland, G. Marbaix, *Nature* **233**, 177 (1971); C. D. Lane, G. Marbaix, J. B. Gurdon, *J. Mol. Biol.* **61**, 73 (1971); V. A. Moar, J. B. Gurdon, C. D. Lane, G. Marbaix, *ibid.*, p. 61.
2. M. Mathews, *Biol. Rev. Cambridge Phil. Soc.* **42**, 499 (1967).
3. H. Green, B. Goldberg, M. Schwartz, D. Brown, *Develop. Biol.* **18**, 391 (1968); J. Klose and R. A. Flickinger, *Biochim. Biophys. Acta* **232**, 207 (1971).
4. W. Shumway, *Anat. Rec.* **78**, 139 (1940).
5. C. F. Graham, K. Arms, J. B. Gurdon, *Develop. Biol.* **14**, 349 (1966).
6. R. F. Greene and R. A. Flickinger, *Biochim. Biophys. Acta* **217**, 447 (1970).
7. S. Moore, H. Spackman, W. H. Stein, *Anal. Chem.* **30**, 1185 (1958).
8. D. J. Prockop, P. S. Ebert, B. M. Shapiro, *Arch. Biochem. Biophys.* **106**, 112 (1964).
9. W. C. Claycomb and C. A. Villee, *Exp. Cell Res.* **69**, 430 (1971).
10. D. Brown and E. Littna, *J. Mol. Biol.* **20**, 81 (1966).
11. Research supported by NIH grant GM 16236-03.
* Present address: Department of Pathology, New York University Medical School, New York 10016.

26 May 1972; revised 13 October 1972

Circadian Corticosteroid Periodicity: Critical Period for Abolition by Neonatal Injection of Corticosteroid

Abstract. *Circadian variation of corticosteroid concentrations in rat plasma is suppressed if corticosteroids are administered between days 2 to 4 of neonatal life, but not if they are given between days 12 to 14 of neonatal life. This indicates a critical period for the effect of corticosteroid administration on the central nervous system pathways regulating such periodicity. Circadian periodicity of corticosteriods is not affected by neonatal administration of testosterone or reserpine.*

Circadian periodicity of plasma corticosteroid concentration is absent at birth, and gradually appears as the organism matures (*1*). In the rat, such periodicity becomes established 21 to 25 days after birth, a time much later than the appearance of the capacity of the hypothalamic-pituitary-adrenal axis to respond to stress and steroid feedback (*2*). The appearance of cyclicity of luteinizing hormone release in the adult female rat is dependent on the concentration and nature of gonadal steroid present immediately after birth (*3*), and is also affected by neonatal administration of drugs (such as reserpine and chlorpromazine) presumed to affect neurotransmitter content in the central nervous system (*4*). The data in the present report indicate that the circadian periodicity of plasma corticosteroid concentration can also be suppressed following the administration of corticosteroids (but not reserpine) during a critical period after birth.

Newborn Sprague-Dawley rats were identified as to litter source and housed in a light-regulated, temperature-controlled room. Animals were weaned and separated according to sex at 21 days of age, and were handled daily from the time of weaning. Experimental groups consisted of controls and animals injected subcutaneously with equal volumes of saline, corticosterone, testosterone, hydrocortisone, or dexamethasone. The drug dosages employed and the ages of animals at the time of injection are indicated in Fig. 1. For each category, groups of six animals (usually three of each sex), 30 days of age, were decapitated at 4-hour intervals during a 24-hour period, and trunk blood was collected for plasma corticosteroid determination (*5*). Assays were performed in duplicate on samples from individual animals. The animals chosen for each sampling time represented a cross section of the litters available in a particular study. Data from this laboratory (*6*) and elsewhere (*7*) indicated plasma corticosteroid concentrations and patterns are similar in male and female animals of this age, so that results for both sexes were combined.

Dexamethasone or hydrocortisone, given 2 to 4 days after birth, virtually suppressed the circadian periodicity of plasma corticosteroid concentrations at 30 days of age; hydrocortisone had the greater effect. (The mean extent of circadian variation of plasma corticosteroids was 8.2 μg/100 ml in the group receiving dexamethasone, 2.7 μg/100 ml in the hydrocortisone-treated group, 22.8 μg/100 ml in controls, and 21.7 μg/100 ml in those receiving saline.) A similar dose of dexamethasone administered 12 to 14 days after birth had no effect on circadian variation. Corticosterone, testosterone, or reserpine, given 2 to 4 days after birth, was also

Table 1. Effect of different neonatal treatment regimens on body and adrenal weights of rats at 30 days of age. Dosages and administration times are given in Figs. 1 and 2. Abbreviations: Dex., dexamethasone phosphate; S.E., standard error.

Treatment	Number of animals		Body weight (g) (mean ± S.E.)		Adrenal weight (mg per 100 g of body weight) (mean ± S.E.)	
	M	F	Male	Female	Male	Female
Control	40	38	80.79 ± 1.99	71.73 ± 2.09	21.91 ± 0.84*	23.03 ± 0.83
Saline	15	7	71.85 ± 4.45	63.48 ± 2.19	28.26 ± 2.38	26.97 ± 2.96
Reserpine	26	11	74.42 ± 3.34	58.09 ± 5.79	26.93 ± 1.29	30.96 ± 1.96
Testosterone	25	17	64.61 ± 3.69	64.27 ± 4.69	32.16 ± 1.92	27.00 ± 1.79
Dex. (day 2–4)	22	20	67.11 ± 2.06	64.34 ± 1.74	29.88 ± 2.09	36.00 ± 2.41†
Dex. (day 12–14)	21	21	73.07 ± 2.47	64.75 ± 1.99	33.99 ± 1.24†	33.23 ± 2.10
Corticosterone	19	23	78.95 ± 3.43	71.52 ± 2.13	29.12 ± 1.69	29.58 ± 1.49
Hydrocortisone	22	19	55.02 ± 3.18†	49.24 ± 2.85†	31.05 ± 1.15	30.91 ± 1.80

* $P < .01$ compared to saline-injected animals. † $P < .05$ compared to saline-injected animals.

ineffective in altering circadian periodicity. (Corticosterone, the naturally occurring corticosteroid in the rat, may have been ineffective because of lessened absorption from the oil suspension in which it was injected. In contrast, dexamethasone was given in aqueous solution and hydrocortisone was suspended in polysorbate and sodium carboxymethyl cellulose.) After reserpine administration, corticosteroid concentrations at both 8 a.m. and 8 p.m. were elevated, but not significantly ($P > .05$). In this regard, others (*8*) have reported reserpine-induced hypersecretion of adrenocorticotropic hormone (ACTH) in adult animals receiving a single dose of reserpine.

Varying effects of drug administration on body and adrenal weight were noted (Table 1). Although either dexamethasone or hydrocortisone, given in the neonatal period, markedly reduced the amplitude of circadian variation of plasma corticosteroid concentration, only the latter was associated with a significant decrease in body weight of male and female animals. Saline administration was associated with a significant increase in male adrenal weight. Although none of the increments in adrenal weight after steroid or reserpine treatment were significant ($P > .01$), in no instance were decrements in adrenal weight observed.

Others (*9*) have reported that animals given corticosteroids shortly after birth weighed less at 30 to 44 days of age than did controls, whereas basal morning concentrations of plasma corticosteroids were not significantly altered. Adrenal weights were increased in treated male animals killed at 214 to 419 days of age (*9*). Neonatal testosterone treatment (*10*) is associated with an increase in adrenal weight in female (but not male) animals at 5 months of age, and with normal basal plasma corticosteroid concentrations.

Other reports have indicated that neonatal corticosteroid administration has a profound effect on the chemistry and development of the central nervous system. Vernadakis and Woodbury (*11*) demonstrated a biphasic effect on the central nervous system; delayed maturation, decreased brain excitability, decreased protein synthesis, and increased ratio of intracellular to extracellular K^+ resulted when cortisol was administered 1 to 7 days after birth, and opposite effects were obtained when cortisol was given later than 8 days after birth. A single 1-mg dose of cortisol (*12*) on day 1 was associated with delayed ontogeny of cerebral cortical dendritic spines, decreased spontaneous locomotor activity, and decreased brain cholesterol concentrations in animals 30 to 44 days of age.

A primary role for the central nervous system in the regulation of the normal periodicity of ACTH and cortisol concentrations in plasma is accepted (*6*). In adult animals, circadian periodicity of corticosteroid concentrations was abolished after fornix transection, production of hypothalamic or septal lesions, or administration of drugs known to effect the concentration of several postulated neurotransmitters in the central nervous system (*13*). None of these procedures had any effect on other aspects of the hypothalamic-

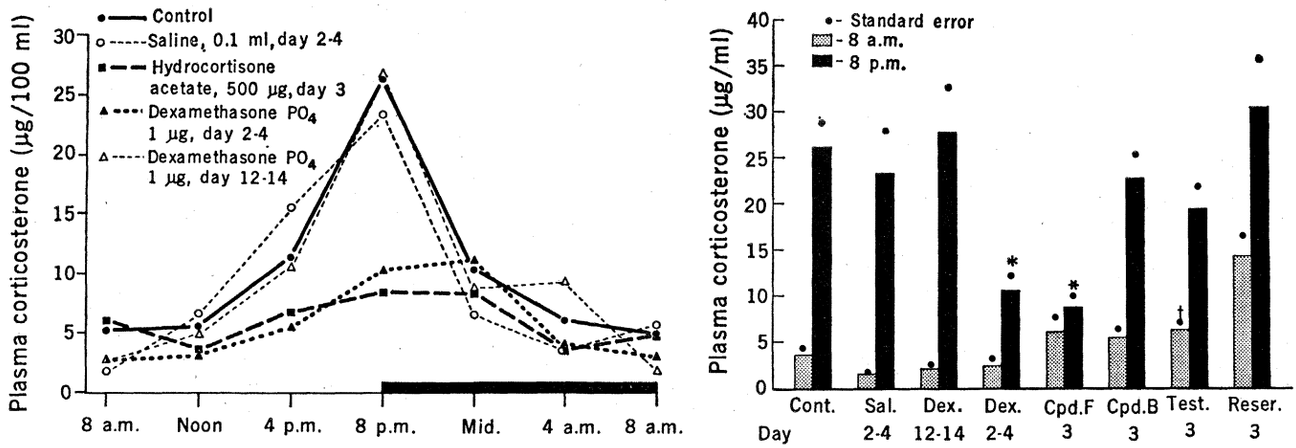

Fig. 1 (left). Effect of neonatal administration of corticosteroids on the circadian periodicity of plasma corticosteroid concentrations in rats 30 days of age ($N = 6$ at each point). Black horizontal bar indicates lights off. Fig. 2 (right). Extent of circadian variation of plasma corticosteroid concentrations in rats 30 days of age after various treatments. Abbreviations are *Cont.*, control; *Sal.*, saline, 0.1 ml; *Dex.*, dexamethasone phosphate, 1 µg; *Cpd.F*, hydrocortisone acetate, 500 µg; *Cpd.B*, corticosterone in oil, 1000 µg; *Test.*, testosterone propionate, 400 µg; *Reser.*, reserpine, 10 µg. Asterisks indicate $P < .01$ compared to 8 p.m. value for saline-treated animals; dagger indicates $P < .05$ compared to 8 a.m. value for saline-treated animals. None of the other values indicated were significantly different ($P > .05$) from those noted for saline-treated animals.

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