9. Since nutrient regeneration is coupled to feeding, it seems impossible for the net effect of grazers on the entire phytoplankton assemblage ever to be an increasing function of grazer density. But grazing on colloidal or bacterial material may supply nutrients to algae. Also, zooplankton may excrete previously assimilated nutrients (*10*). Finally, chemical compounds released by zooplankton such as phosphatases (*11*) or chelators may supply otherwise unavailable nutrients. In six other experiments performed in this and another field site, fertilization effects were weaker than grazing effects; none were stronger.
10. For example, J. H. Martin, *Limnol. Oceanogr.* **13**, 63 (1968).
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12. The three equations were:

$$r(G) = \mu_1 + \mu_2\left[\frac{G}{K_1 + G}\right] + fG \quad (1)$$

$$b(G) = \mu_3 + \mu_4\left[\frac{G}{K_2 + G}\right] \quad (2)$$

$$d(G) = \mu_5 + fG \quad (3)$$

where: $r(G)$ is per capita rate of change of algal density as a function of grazer density (per day), $b(G)$ is algal per capita reproductive rate as a function of grazer density (per day), $d(G)$ is algal per capita mortality rate as a function of grazer density (per day), μ_i are growth rates (per day), K_i are half-saturation constants (individuals per liter), G is grazer density in individuals per liter, and f is proportional to filtering rate (per individual per day). These three equations were chosen because they provided an adequate fit to the data, not because they are expected for mechanistic, physiological reasons. However, analysis of a series of first-order nonlinear differential equations derived from first principles suggests they are approximately correct.
13. When Eq. 1 is fit to the data in Fig. 2a, a value of 0.281 (SE, 0.177) per day is obtained for μ_2. When Eq. 2 is fit to the data in Fig. 2b, a value of 0.387 (SE, 0.108) per day is obtained for μ_4. By a multivariate nonlinear regression analysis, these two estimates are not significantly different ($-2 \ln\Lambda = 0.09$, 1 d.f., $0.75 < P < 0.90$) (*14, 15*). These estimates do not significantly differ from the observed effect of nitrogen additions (0.41 per day).
14. The value Λ is the maximum likelihood ratio (*15*).
15. D. R. Cox and D. V. Hinkley, *Theoretical Statistics* (Chapman & Hall, London, 1974).
16. Density was determined from ten vertical hauls of an 80-μm mesh size Wisconsin zooplankton net. I assumed the capture efficiency was 100 percent; in the likely event that the efficiency was less than 100 percent, 19 individuals per liter is an underestimate.
17. The *D. pulex* density that half-saturates the fertilization effect is K. The data in Fig. 2a and Eq. 1 estimate K_1 as 4.02 (SE, 7.28) *D. pulex* per liter, and the data in Fig. 2b and Eq. 2 estimate K_2 as 0.162 (SE, 0.610) *D. pulex* per liter. Multivariate analysis finds these two estimates to be statistically different ($-2\ln\Lambda = 6.11$, d.f. = 1, $0.01 < P < 0.025$) (*14, 15*), casting doubt on the validity of the grazer exclosures for estimating K. The data in Fig. 2a do not depend on the growth of algae inside the grazer exclosures, so the larger of the two estimates may be more accurate.
18. Solving Eq. 1 at a density of 19 *D. pulex* per liter with the fitted parameter values reveals that this density of grazers increased phytoplankton reproductive rates 0.232 per day. Algae reproduced 30 percent faster than in the absence of grazers. With the slope of the line in Fig. 2c used to estimate the grazing effect, 19 *D. pulex* per liter increased phytoplankton mortality rate by 0.228 per day. The estimated fertilization effect (0.232 per day) is approximately equal to the estimated grazing effect (0.228 per day).
19. J. T. Lehman and C. D. Sandgren, *Limnol. Oceanogr.* **30**, 34 (1985).
20. Each datum in (a) and (b) represents the slope of a linear regression of ln (fluorescence) versus day for days 2 to 6, allowing for a lag in algal growth. Because removing a fraction of the volume inside the exclosure dilutes the remaining algae, a dilution factor of ln (a), where a is the fraction of the population remaining after dilution each time t, was added to the observed rates of change inside the grazer exclosures. Mortality rates in (c) were obtained from the differences of the corrected reproductive rates and the net rates of change.
21. It follows directly from the derivation of filtering rate (*22*) that the slope of $d(G)$ (or f in Eqs. 1 and 3) multiplied by the volume of fluid indicated on the horizontal axis (in this case 1000 ml) yields a filtering rate in the conventional units of milliliters per individual per day.
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24. I thank K. Larntz for his help with the statistical analyses. D. Tilman and J. P. Grover provided advice during all phases of this work, and they and D. McNaught, J. Shapiro, and D. Wright read and improved this report. Financial support was provided by the National Science Foundation Predoctoral Fellowship Program and the Dayton-Wilke Fund (to R.W.S.), NSF grant NSF/BSR 8114302, and Sea Grant NA82AA-D-00039, Project R/F-9 (to D. Tilman). This is Minnesota Sea Grant contribution 174.

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Stress-Induced Inhibition of Reproductive Functions: Role of Endogenous Corticotropin-Releasing Factor

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In the adult castrated male rat, exposure to inescapable, intermittent electroshocks inhibited the pulsatile pattern of luteinizing hormone release and markedly lowered its plasma concentrations. The central administration of the corticotropin-releasing factor (CRF) antagonist α-helical ovine CRF residues 9 to 41 reversed the inhibitory action of stress. Neither its peripheral injection, nor the intraventricular injection of the inactive CRF analog des-Glu[17] to Arg[35] ovine CRF was effective. These results suggest that endogenous CRF may mediate some deleterious effects of noxious stimuli on reproduction.

EXPOSURE TO STRESS IS ACCOMPANIED by disruption of reproductive functions in many species (*1–10*) including human beings (*11–17*), but the exact mechanisms that mediate these effects are not fully elucidated. Possible sites involved include (i) direct gonadal effects of the hormones secreted during stress [such as adrenocorticotropin (ACTH), steroids, catecholamines, and vasopressin] with subsequent alterations in sex steroid output (*8, 18–20*); (ii) a corticosteroid-mediated decrease in pituitary responsiveness to gonadotropin-releasing hormone (GnRH), resulting in decreased luteinizing hormone (LH) secretion (*21–23*); and (iii) a centrally mediated inhibition of GnRH release (*24*). While there is evidence that each of these mechanisms can indeed operate during stress and could interfere with normal pituitary and gonadal function, recent studies have additionally indicated that corticotropin-releasing factor (CRF), which is secreted by the brain during stress (*25*), will inhibit GnRH secretion into the hypophyseal portal circulation (*26*). These observations prompted us to investigate a possible central role of endogenous CRF in mediating stress-induced alterations in LH output in the rat.

We have studied castrated male rats because their pattern of pulsatile release is inhibited by stress (*5*). In the castrated rat, forced immobilization or a broken leg abol-

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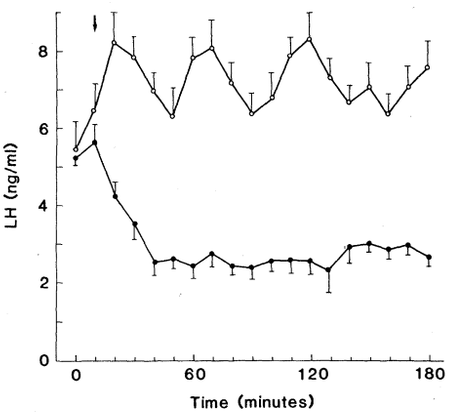

Fig. 1. Effect of electroshocks on basal plasma LH in castrated male rats. The rats were placed in individual shockers and exposed to footshocks for 3 hours. The arrow indicates the onset of the electroshocks. The shocks (2 mA, 2-second duration) were delivered randomly on an average of four per minute to the grid floors of plexiglass chambers (30 by 126 by 30 cm) by a Colbourn shocker. Blood samples (0.3 ml) were obtained every 10 minutes and replaced with an equal volume of lactated Ringer or Plasmanate (plasma protein fraction, 5 percent; Cuttler Biological, Berkeley, CA). Open circles, control rats; closed circles, shocked rats. Each point represents the mean ± SEM of six to eight rats. Plasma LH was measured by radioimmunoassay (intra- and inter-coefficients of variation, 5.2 and 9.8 percent, respectively). Data were analyzed by one- and two-way analyses of variance. For reasons of clarity, levels of statistical significance are not indicated on figures. In this experiment, plasma LH concentrations of stressed rats were significantly ($P \leq 0.01$) different from control animals at all times.

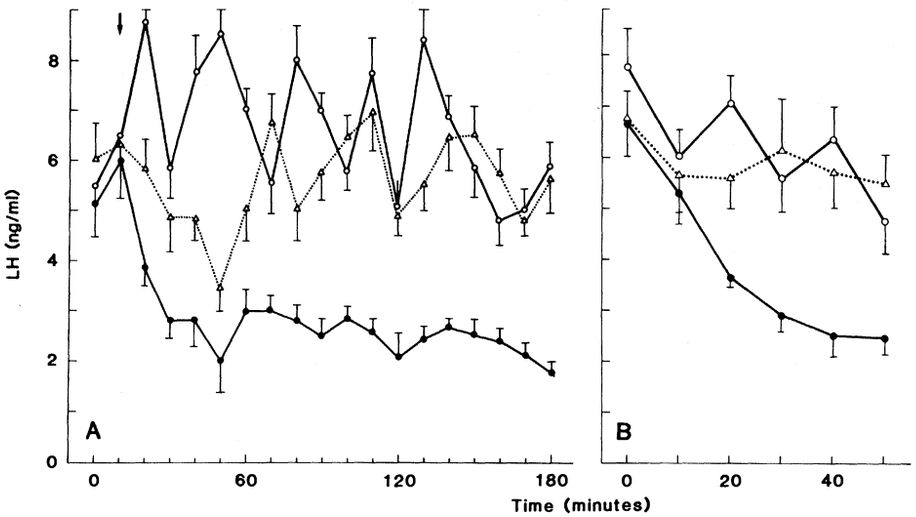

Fig. 2. Reversal of shock-induced inhibition of LH release by the CRF antagonist α-helical oCRF residues 9 to 41 per kilogram of body weight. The antagonist was administered in a volume of 10 μl in the lateral ventricle of the brain 5 minutes (A) or 45 minutes (B) before the onset of the shock period. Open circles, control rats; closed circles, shocked rats; triangles, shocked rats receiving 100 μg of α-helical oCRF residues 9 to 41. Each point represents the mean ± SEM of six to eight rats. For reasons of clarity, the effect of the antagonist alone is not shown. Plasma LH values of stressed rats were significantly ($P \leq 0.01$) different from control animals at all times. (A) Plasma LH values of stressed rats receiving the CRF antagonist were different ($P \leq 0.01$) from control animals only at 40 and 50 minutes. (B) There was no statistical difference ($P \geq 0.05$) between control rats and stressed rats receiving the CRF antagonist.

ished the pattern of pulsatile LH release (*5*). These stresses, however, as well as exposure to ether vapors, cold, forced exercise, or surgery (*2, 9, 10*), are often difficult to use in a procedure that requires frequent bleedings; additionally they may not be qualitatively homogenous over prolonged periods of time. Therefore, we have chosen exposure to inescapable, intermittent electroshocks as a reliable, convenient, and quantitatively controllable stressful stimulus, which can be applied to freely moving rats bearing venous catheters. The animals were castrated 7 to 10 days before the experiment and had permanent cannulas implanted in the lateral ventricle of the brain (*27*) and indwelling catheters implanted in the jugular vein (*28*) to allow serial blood sampling. The CRF antagonist α-helical ovine CRF (oCRF) residues 9 to 41 (*29*) and the inactive CRF analog, des-Glu^{17} to Arg^{35}-oCRF, were synthetized by solid-phase methodology (*30*), and dissolved in water containing 0.01 percent ascorbic acid. The CRF antiserum was raised against oCRF (*31*), but contains antibodies that react with rat CRF (rCRF) (*28*).

Control animals showed a typical pattern of periodic LH pulses (Fig. 1), with values ranging from 4.8 to 8.8 ng/ml over a 180-minute period. These pulses were abolished during exposure to footshocks; furthermore, plasma LH [but not follicle-stimulating hormone (FSH)] concentrations of stressed rats significantly ($P \leq 0.01$) decreased within 5 to 10 minutes after the onset of stress and remained depressed for the entire duration of the experiment (range, 1.8 to 3.0 ng/ml). α-Helical oCRF residues 9 to 41 [~15 nmol (100 μg)] were administered into the lateral ventricle of the brain 5 minutes before the onset of shocks.

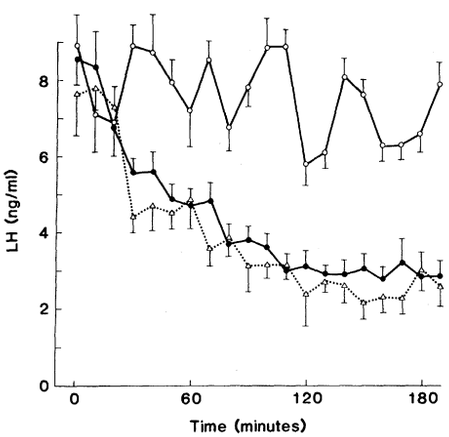

Fig. 3. Effect of the peripheral (intrajugular) administration of 0.5 mg of α-helical oCRF residues 9 to 41 per kilogram of body weight in castrated rats exposed to electroshocks. Open circles, control rats; closed circles, shocked rats; triangles, shocked rats receiving antagonist in the jugular vein. Each point represents the mean ± SEM of six to eight rats. Plasma LH of stressed rats (with or without the CRF antagonist) was statistically different ($P \leq 0.01$) from that of control animals at all times.

Although this treatment did not significantly alter LH secretion, it markedly reversed the inhibitory effect of stress (Fig. 2A). Lower doses of the antagonist did not show any consistent effect. Plasma LH values of stressed animals receiving the antagonist, after an initial decrease that may have reflected the time required for distribution of α-helical oCRF residues 9 to 41, returned to higher concentrations and showed evidence of a pulsatile pattern that was statistically indistinguishable from that of control rats. For the final 2 hours, the mean range of plasma LH values (4.8 to 7.1 ng/ml) and their pulsatile fluctuations in the stressed rats treated with CRF antagonist were comparable to the LH secretion observed in control animals. In another experiment, antagonist administered 40 minutes before the onset of stress reversed the inhibitory action of the electroshocks at all times (Fig. 2B). These results suggest that endogenous CRF may act centrally to mediate stress-induced inhibition of LH release.

Two control experiments were designed to assess the specificity of the central administration of α-helical oCRF residues 9 to 41. In the first, ~15 nmol (100 μg) of the inactive CRF analog des-Glu^{17} to Arg^{35}-oCRF were injected into the lateral ventricle of rats exposed to electroshocks, but did not reverse the effect of stress. In the second, ~75 nmol (0.5 mg) of the CRF antagonist per kilogram of body weight was injected peripherally 40 minutes before the onset of stress. This treatment did not significantly alter the inhibitory action of the electroshocks on plasma LH concentration (Fig. 3).

Measurement of circulating ACTH and corticosterone levels in the cycling female rats has indicated an increased secretion of these two hormones (*32*) at the time of the peak of plasma LH concentrations observed during the so-called critical period of proestrus. Additionally, the indirect measurement of the net ACTH-releasing activity present in hypothalamic extracts has also suggested that the brain concentrations of CRF or other ACTH secretagogues may exhibit changes related to the estrous cycle (*33*). It is therefore probable that CRF regulates some of the events that control normal cyclicity in the female rat, a hypothesis we tested with the use of CRF antagonists.

Our results suggest that endogenous CRF at least partially mediates stress-induced inhibition of LH release in the rat. The most probable hypothesis is that CRF, which is released during stress (*25*), acts within the brain to inhibit GnRH secretion into the portal circulation (*26*). The possibility that an increased release of endogenous CRF might also modulate stress-induced disrup-

tion of reproductive functions such as blockade of ovulation in the rat (*34*), dysmenorrhea in women (*11–15*), or altered androgen secretion in males (*16, 17*) may yield new insights into the causes of infertility during exposure to stressful circumstances. Additionally, in view of the anorexic (*34*) and antireproductive (*24*) effects of CRF, it is tempting to consider a pathogenic role of CRF in anorexia nervosa—a disorder often associated with activation of the hypothalamic-pituitary-adrenal axis (*35*).

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Obesity, Overeating, and Rapid Gastric Emptying in Rats with Ventromedial Hypothalamic Lesions

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Measurements confirm the quantitative theoretical prediction that the autonomic nonendocrine abnormality of rapid daytime gastric emptying is the major primary cause of the obesity resulting from ventromedial hypothalamic lesions in rats. Therapy for obesity could include slowing of stomach emptying.

DESTRUCTION OF THE VENTROMEDIAL region (*1*) of the hypothalamus (VMH) in rats causes a syndrome characterized by a dynamic phase of increasing obesity, usually accompanied by hyperphagia. This is followed by a phase of static obesity when the total intake of food per day is more normal. During dynamic and static phases, the rat takes meals abnormally frequently during the light period of the day-night cycle (*2*).

Obesity and hyperphagia brought about by destruction of the VMH cannot be attributed to a deficit in satiety mechanisms. VMH lesions do not disrupt either the immediate satiating effects of food (*3*) or the parenteral satiety generated by the use of absorbed food carbohydrate for energy (*4, 5*). The VMH therefore is not the satiety center, mediating normal inhibition of feeding; yet the VMH receives information from the viscera (*6*) and contains neurons whose firing responds to their own glucose metabolism (*7*). Since these afferents do not directly inhibit feeding behavior, their function is presumably to modulate autonomic and perhaps hypophyseal outputs affecting the processing of food (*8*).

We have now found that VMH lesions disrupt autonomic output controlling the stomach and increase the normally slow daylight rate of gastric emptying of regular diet. We propose that the abbreviated satiating effect of food resulting from such gastric acceleration either is the sole primary cause of the obesity or is at least a quantitatively major primary cause along with other autonomic defects, such as insulin hypersecretion (*9*) and possibly reductions in fat mobilization (*10*) and thermogenic capacity (*11*). That is, the VMH syndrome is neither metabolic nor behavioral, and pair-feeding experiments (*12*) cannot be satisfactorily interpreted.

The experiments described here originated from our theory that eating motivation is physiologically controlled by the flow of readily used energy from absorption (*5, 13*). This theory provided the basis for quantitative explanations of feeding patterns and changes in body composition that were modeled by computer (*14*). The calculations showed that variations in the rate of gastric

Table 1. Rates of gastric emptying during early daylight in ventromedial hypothalamic and control rats (n = 6 to 8).

Stage	Gastric emptying rate (g hour^{-1})				Difference*
	VMH lesions		Sham-operated		
	Mean	SD	Mean	SD	
Four hours after lesions	0.54	0.04	0.42	0.09	$P < 0.01$
One week after lesions	0.69	0.08	0.43	0.03	$P < 0.01$
Dynamic obese phase	0.74	0.09	0.45	0.09	$P < 0.02$
Static obese phase	0.62	0.14	0.43	0.08	$P < 0.01$

*Calculated (t test) between VMH and sham-operated groups.

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