tion took place in contact with the modern brines. The relatively low SO_4^{2-} concentrations of most of these brines and the observation by Kinsman (*22*) that dolomitization does not occur in carbonate sabkhas composed mainly of calcite skeletal grains support our experimental results. Minor amounts of dissolved SO_4^{2-} strongly inhibit calcite dolomitization, whereas dolomitization of aragonite (the dominant $CaCO_3$ phase in most carbonate sabkhas) may still proceed at somewhat higher dissolved SO_4^{2-} concentrations. This explanation applies equally well to continental lacustrine environments, such as the Green River Formation, Wyoming, where large amounts of dolomitic mudstones are associated with both the oil shales and evaporites (*23*).

PAUL A. BAKER
MIRIAM KASTNER
Scripps Institution of Oceanography, University of California, San Diego, La Jolla 92093

References and Notes

1. K. A. Pisciotto and J. J. Mahoney, *Initial Rep. Deep Sea Drill. Proj.*, in press; I. Friedman and K. S. Murata, *Geochim. Cosmochim. Acta* **43**, 1357 (1979).
2. Lists of the occurrences of minerals in DSDP cores were made available by D. Hawkins of the DSDP Data Processing Group.
3. K. R. Kelts and J. A. McKenzie, *26th Int. Geol. Conf.* (1980) (Abstr.); see also *Initial Rep. Deep Sea Drill. Proj.*, in press.
4. J. M. Gieskes, in *Initial Rep. Deep Sea Drill. Proj.* **25**, 361 (1974).
5. Aragonite was prepared by the method of J. L. Wray and F. Daniels, *J. Am. Chem. Soc.* **79**, 2031 (1957).
6. "Protodolomites" and "dolomites" as used throughout this report were defined by A. M. Gaines [*J. Sediment. Petrol.* **47**, 543 (1977)] and J. R. Goldsmith and D. L. Graf [*J. Geol.* **66**, 678 (1958)].
7. M. Kastner, J. B. Keene, J. M. Gieskes, *Geochim. Cosmochim. Acta* **41**, 1041 (1977).
8. P. E. Rosenberg and H. D. Holland, *Science* **145**, 700 (1964).
9. H. E. Usdowski, *Die Genese von Dolomit in Sedimenten* (Springer, Berlin, 1967).
10. A. M. Gaines [in *Concepts and Models of Dolomitization*, D. H. Zenger *et al.*, Eds. (*Soc. Econ. Paleontol. Mineral. Spec. Publ. 28*) (Society of Economic Paleontologists and Mineralogists, Tulsa, 1980), p. 81] demonstrated in solutions of $I=2$ at 100°C that dolomite is stable at $Mg^{2+}/Ca^{2+} = 3$ to 7; furthermore, the rate of formation of dolomite was dependent on the value of Mg^{2+}/Ca^{2+} being a maximum at about 5; P. E. Rosenberg, D. M. Burt, H. D. Holland, *Geochim. Cosmochim. Acta* **31**, 391 (1967).
11. A. Katz and A. Matthews, *Geochim. Cosmochim. Acta* **41**, 297 (1977).
12. J. M. Gieskes, *Initial Rep. Deep Sea Drill. Proj.*, in press.
13. ———, *Soc. Econ. Paleontol. Mineral. Spec. Publ.*, in press.
14. P. A. Baker, M. Kastner, J. D. Byerlee, D. A. Lockner, *Mar. Geol.* **38**, 185 (1980).
15. B. H. Purser, Ed., *The Persian Gulf* (Springer, Berlin, 1973); J. E. Adams and M. L. Rhodes, *Bull. Am. Assoc. Pet. Geol.* **44**, 1912 (1960); W. Fisher and P. Rodda, *ibid.* **53**, 55 (1969).
16. K. J. Hsü and C. Siegenthaler, *Sedimentology* **12**, 11 (1969).
17. R. L. Folk and L. S. Land, *Bull. Am. Assoc. Pet. Geol.* **59**, 60 (1975); K. Badiozamani, *J. Sediment. Petrol.* **43**, 965 (1973); B. B. Hanshaw *et al.*, *Econ. Geol.* **66**, 710 (1971).
18. D. L. Langmuir, *Geochim. Cosmochim. Acta* **35**, 1023 (1971).
19. J. A. McKenzie, K. J. Hsü, J. F. Schneider, in *Concepts and Models of Dolomitization*, D. H. Zenger *et al.*, Eds. (Society of Economic Paleontologists and Mineralogists, Tulsa, 1980), p. 11.
20. G. P. Butler, *J. Sediment. Petrol.* **39**, 70 (1969).
21. P. Bush, in *The Persian Gulf*, B. H. Purser, Ed. (Springer, Berlin, 1973), p. 395.
22. D. J. J. Kinsman, *Bull. Am. Assoc. Pet. Geol.* **53**, 830 (1969).
23. H. P. Eugster and R. C. Surdam, *Geol. Soc. Am. Bull.* **85**, 1733 (1974); H. P. Eugster and L. A. Hardie, *ibid.* **86**, 319 (1975).
24. We thank R. Garrison, Y. Bentor, J. Gieskes, and D. Kent for helpful discussions and reviews of the manuscript and S. Morson-Baker for stylistic comments. We owe particular thanks to J. Gieskes, who aided us in many aspects of this research and supplied unpublished pore water data from the Gulf of California. Research was supported by Office of Naval Research contract USN/N00014-79-C-0152. Research in Table 2 was supported by NSF grant OCE78-09652. Research support was also provided by the Chevron Oil Field Research Company, La Habra, Calif.

28 January 1981; revised 26 March 1981

Thyrotropin-Releasing Hormone Improves Cardiovascular Function in Experimental Endotoxic and Hemorrhagic Shock

Abstract. *Thyrotropin-releasing hormone significantly improved cardiovascular function when it was injected intravenously into conscious rats subjected to experimental endotoxic or hemorrhagic shock. Because thyrotropin-releasing hormone appears to be a "physiologic" opiate antagonist without effects on pain responsiveness, it may provide therapeutic benefits in the treatment of shock or acute hypotension.*

We have demonstrated that the opiate antagonist naloxone significantly improves cardiovascular performance and survival in experimental shock caused by endotoxemia (*1–3*), hemorrhage (*4, 5*), or spinal-cord injury (*6, 7*). This therapeutic action of naloxone in shock appears to be mediated by way of a blockade of endorphin action at opiate receptors within the central nervous system (*6*). However, receptor-level opiate antagonists such as naloxone may have the adverse effect of intensifying posttraumatic pain by inhibiting endorphin-mediated analgesia even as they improve the shock state. In contrast to naloxone, thyrotropin-releasing hormone (TRH) does not bind to opiate receptors, although it has selective activity in opposing many opiate-mediated effects (*8*). More specifically, TRH reverses behavioral and neuroendocrine changes produced by β-endorphin without altering the antinociceptive responses to either β-endorphin (*8*) or morphine (*9*). This ability of TRH to reverse the pharmacological and physiological effects of endorphins without modifying analgesia prompted us to examine its possible therapeutic utility in experimental shock.

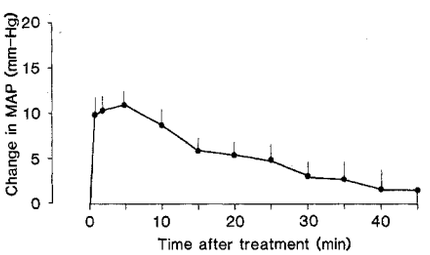

Fig. 1. The effects of intravenously administered TRH (2 mg/kg) on mean arterial pressure (MAP) in conscious, freely moving rats. Within seconds after the TRH injection there was a rapid increase in MAP. Vertical bars indicate the standard error of the mean, $N = 8$ rats; data are expressed as changes in MAP compared to values before treatment.

Tail-artery and external jugular vein cannulas were implanted in male Sprague-Dawley rats (250 to 300 g; Zivic-Miller Laboratories) as previously described (*1*). Twenty-four hours later, the arterial cannula was connected to a microtransducer (Narco Biosystems RP1500 attached to a Beckman physiograph, type R). This arrangement permitted continuous measurement of blood pressure and heart rate in awake, freely moving rats in their home cages.

In control rats, which were not subjected to shock, the cardiovascular effects of intravenous TRH alone were determined. After injecting the TRH (2 mg/kg; Beckman Labs.) we flushed the cannula with 0.2 ml of saline to ensure complete drug delivery. Cardiovascular variables were monitored for 45 minutes. The administration of TRH caused a significant increase in mean arterial pressure (MAP) of 11.1 ± 1.5 mm-Hg ($N = 10$, $t = 7.25$, $P < .001$; Student's t-test), with peak effects observed at 5 minutes after injection and persisting for 30 minutes (Fig. 1). Heart rate and pulse pressure were also significantly increased by this dose of TRH in all experiments (data not shown).

A second group of rats was subjected to endotoxic shock by the intravenous administration of *Escherichia coli* lipopolysaccharide endotoxin (15 mg/kg; Difco, control No. 654109) (*1*). In these animals, MAP fell precipitously at varying times over the next hour to 67 to 70 mm-Hg (*1*), which was approximately 26 percent (24 mm-Hg) below baseline levels of 92.7 ± 1.9 mm-Hg ($N = 22$). These rats, randomly divided into two groups, were then immediately injected

0036-8075/81/0710-0216$00.50/0

intravenously with either TRH (2 mg/kg, $N = 13$) or an equal volume of saline (1.0 ml/kg, $N = 11$); after each injection the cannula was flushed with 0.2 ml of saline. In contrast to the saline treatment, which did not improve arterial pressure, TRH-injected animals showed a rapid increase in MAP, which averaged 20 to 25 mm-Hg during the first 30 minutes after treatment (Fig. 2, top). Area scores (Fig. 2), computed by triangulation (*10*), reveal the significant differences between the two treatment groups ($t = 5.66$, $P < .001$).

A third group of rats was subjected to hemorrhagic shock according to our previous experimental design (*4*). In a total of 27 rats, blood was withdrawn from the indwelling external jugular vein cannula; MAP fell from 96.5 ± 1.6 mm-Hg to 37.2 ± 1.0 mm-Hg where it was maintained for 20 minutes. The volume of blood removed, totaling 2.5 ± 0.1 ml per 100 g of body weight, approximated 45 percent of the estimated total blood volume. Following the 20 minutes of controlled oligemia, the animals were injected intravenously with either TRH (2 mg/kg, $N = 15$) or an equal volume of saline (1.0 ml/kg, $N = 12$), with the cannula being flushed with 0.2 ml of saline. Shed blood was not returned. As shown in Fig. 2 (bottom), the TRH-treated rats experienced a rapid improvement in MAP which was 20 to 25 mm-Hg higher than in the saline-control rats for the first 15 minutes after injection. The duration of this effect was about 30 minutes, approximately equivalent to the duration of the pressor response to TRH in normotensive rats not subjected to shock (Fig. 1). In this model, the saline-treated rats also experienced an increase in MAP, a consequence of the fluid volume infused. Nevertheless, area scores were significantly greater in TRH-treated animals ($t = 2.98$, $P < .01$).

The magnitude of the pressor response after the intravenous administration of TRH (2 mg/kg) was twice as great in rats subjected to shock hypotension than in normotensive control animals (compare Figs. 1 and 2). Because the volume of blood that is available to perfuse critical organs is functionally reduced after endotoxic or hemorrhagic shock, it appears likely that the magnified pressor response in these animals is a consequence of the increased concentration of the TRH that was administered on a fixed body-weight basis.

The results of these studies show that the "physiologic" opiate-antagonist TRH, like the opiate-receptor antagonist naloxone (*1–7*), significantly improves the hypotension associated with experimental endotoxic and hemorrhagic shock. However, the precise mechanisms by which these two substances affect arterial pressure are distinctly different. For example, naloxone, which functions as a receptor-level competitive antagonist of endorphins, has no significant effect on blood pressure in normotensive rats that have not been subjected to shock (*1*). This finding indicates a selective, indirect action of naloxone in reversing an endorphin-mediated shock hypotension rather than a direct cardiovascular response to this opiate antagonist. By contrast, intravenous (Fig. 1) or intracerebroventricular TRH (*11*) produces an elevation of arterial pressure in normotensive rats, thus providing evidence for a direct cardiovascular action at central or peripheral TRH effector sites.

We suggest that TRH appears to antagonize the cardiovascular as well as other effects of endogenous opiates through opposing physiological systems utilizing different receptors (*8*), possibly in a manner analogous to the antagonistic interaction between epinephrine and histamine (*12*). These direct cardiovascular actions of TRH appear to be independent of its hypothalamic role in regulating pituitary-thyroid function, since very low doses of TRH, TRH metabolites, and synthetic analogs produce autonomic changes at central sites without affecting pituitary-thyroid activity (*13*). Furthermore, TRH has been shown to antagonize the nonanalgesic effects of β-endorphin in hypophysectomized animals with atrophic thyroid glands (*8*). However, with the parenteral dose of TRH used in the present studies, activation of the pituitary-thyroid axis could result in a much more delayed elaboration of thyroid hormones (*14*) which could, in turn, produce direct cardiotonic effects of their own (*15*).

There is evidence that the rapid pressor effects of TRH are centrally mediated and are only partially explained by an activation of peripheral sympathetic responses (*16*). By contrast, many other pressor substances, such as angiotensin and sympathomimetic amines, are known to act at peripheral sites to transiently elevate blood pressure via increased peripheral resistance (*17*). However, the therapeutic utility of these substances in treating shock is limited by rapid tachyphylaxis as well as their lack of ability to improve tissue perfusion (*17*). Although partial tachyphylaxis to the pressor effects of TRH may occur (*18*), a single injection of this substance

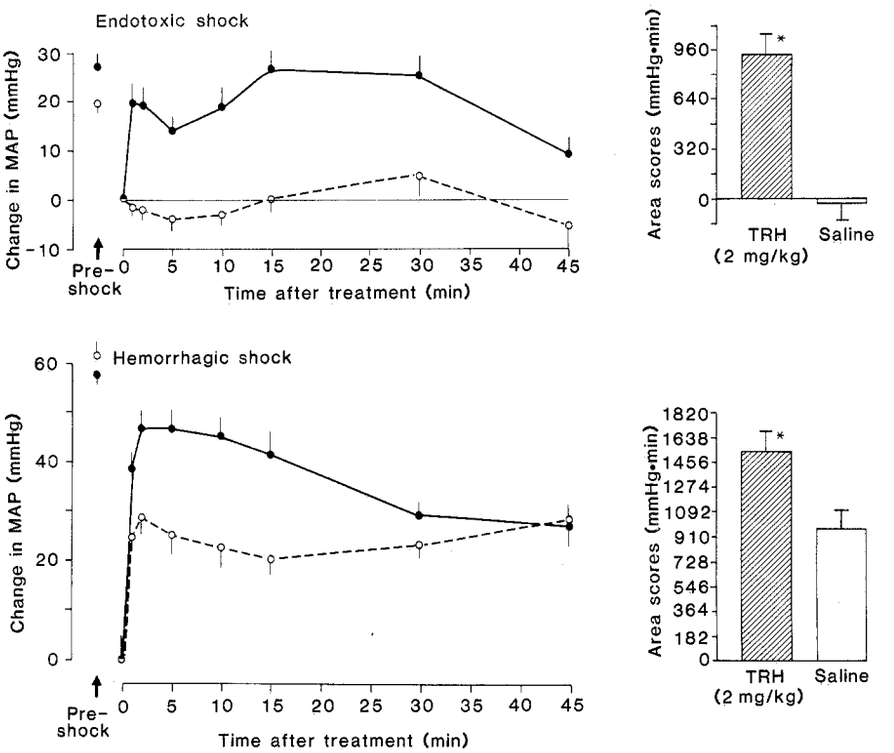

Fig. 2. Rats received intravenous injections of either TRH (2 mg/kg; solid lines) or an equal volume of saline (dashed lines) after they were subjected to endotoxic (top) or hemorrhagic (bottom) shock hypotension. The data are expressed as changes in MAP prior to shock (*Pre-shock*) and at various times after drug treatment at time 0. Vertical bars indicate the standard error of the mean; populations of rats and absolute MAP values are defined in the text. A significant increase in MAP was observed when comparing time integrated area responses from TRH-treated rats with saline controls (histograms on right; asterisks indicate $P < .01$).

produces prolonged cardiovascular effects in shock (Fig. 2). Thus, the centrally mediated cardiovascular effects of TRH, like those of naloxone, should also result in improved tissue perfusion and survival in shock states (*18*).

Depending on the dosage, naloxone may either diminish or enhance clinical pain in humans (*19*). Although some evidence suggests that naloxone may also effectively reverse shock in humans (*20*), it has not been established whether clinical pain in such states would be altered by this opiate antagonist at the doses required for the therapeutic effect. In animal experiments TRH has been shown to be devoid of effects on nociceptive latencies (*8*), and in the experiments described here TRH appears to be as effective as naloxone in improving the cardiovascular pathophysiology in experimental shock. Collectively, these findings suggest that TRH or TRH metabolites and analogs may be useful therapeutically for shock or acute hypotension, acting in a manner similar to naloxone but without intensifying pain perception.

JOHN W. HOLADAY
ROBERT J. D'AMATO
Department of Medical Neurosciences, Walter Reed Army Institute of Research, Washington, D.C. 20012
ALAN I. FADEN
Department of Neurology, Uniformed Services University of the Health Sciences, Bethesda, Maryland 20014

References and Notes

1. J. W. Holaday and A. I. Faden, *Nature (London)* **275**, 450 (1978); A. I. Faden and J. W. Holaday, *J. Pharmacol. Exp. Ther.* **212**, 441 (1980).
2. D. G. Reynolds, N. J. Gurll, T. Vargish, R. Lechner, A. I. Faden, J. W. Holaday, *Circ. Shock* **7**, 39 (1980).
3. J. W. Holaday and A. I. Faden, in *Neurosecretion and Brain Peptides*, G. Martin, S. Reichlin, K. Bick, Eds. (Raven, New York, 1981), p. 421.
4. A. I. Faden and J. W. Holaday, *Science* **205**, 317 (1979).
5. T. Vargish, D. G. Reynolds, N. J. Gurll, R. B. Lechner, J. W. Holaday, A. I. Faden, *Circ. Shock* **7**, 31 (1980).
6. J. W. Holaday and A. I. Faden, *Brain Res.* **189**, 295 (1980); A. I. Faden, T. P. Jacobs, J. W. Holaday, *J. Autonom. Nerv. Sys.* **2**, 295 (1980).
7. A. I. Faden, T. P. Jacobs, J. W. Holaday, *Science* **211**, 493 (1981).
8. J. W. Holaday, C. H. Li, H. H. Loh, *Soc. Neurosci. Abstr.* **3**, 347 (1977); J. W. Holaday, L. F. Tseng, H. H. Loh, C. H. Li, *Life Sci.* **22**, 1537 (1978); Y. Taché, M. Lis, R. Collu, *ibid.* **21**, 841 (1977).
9. A. Horita, M. A. Carino, R. M. Chesnut, *Psychopharmacologia* **49**, 57 (1976).
10. J. W. Holaday, P. Y. Law, L. F. Tseng, H. H. Loh, C. H. Li, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 4628 (1977).
11. J. S. Beale, R. P. White, S. P. Huang, *Neuropharmacology* **16**, 499 (1977); F. Koivusalo, I. Paakkari, J. Leppaluoto, H. Karppanen, *Acta Physiol. Scand.* **106**, 83 (1979).
12. F. H. Meyers, E. Jawetz, A. Goldfien, *Review of Medical Pharmacology* (Lange Medical Publications, Los Altos, Calif., ed. 4, 1974), pp. 78–92.
13. G. G. Yarbrough, *Prog. Neurobiol.* **12**, 291 (1979); R. D. Myers, G. Metcalf, J. C. Rice, *Brain Res.* **126**, 105 (1977).
14. At least 1 hour is required after TRH administration to rats for increases in triiodothyronine to become measurable; thyroxine concentrations failed to change over 120 minutes after TRH challenge [B. M. Lifschitz, C. R. Defesi, M. I. Surks, *Endocrinology* **102**, 1775 (1978)].
15. H. M. Goodman and L. V. Middlesworth, in *Medical Physiology*, V. B. Mountcastle, Ed. (Mosby, St. Louis, ed. 13, 1974), p. 1632.
16. A. Horita, M. A. Carino, R. E. Weitzman, in *Catecholamines: Basic and Clinical Frontiers*, E. Usdin, I. Kopin, J. Barchas, Eds. (Pergamon, New York, 1979), p. 1140.
17. I. R. Innes and M. Nickerson, in *The Pharmacological Basis of Therapeutics*, L. S. Goodman and A. Gilman, Eds. (Macmillan, New York, ed. 5, 1975), p. 478; W. W. Douglas, in *ibid.*, p. 663.
18. J. W. Holaday, B. A. Ruvio, A. I. Faden, in preparation.
19. J. D. Levine, N. C. Gordon, H. L. Fields, *Nature (London)* **278**, 740 (1979).
20. M. Tiengo, *Lancet* **1980-I**, 690 (1980).
21. We thank B. Ruvio, T. Martin, J. Kenner, and C. Johnson for technical assistance and P. Conners and A. Trees for preparation of the manuscript.

29 January 1981; revised 6 April 1981

Epidermal Growth Factor Enhances Viral Transformation of Granulosa Cells

Abstract. *Kirsten sarcoma virus produced a low incidence of transient morphological transformation in primary cultures of rat ovarian granulosa cells. In the presence of epidermal growth factor, the incidence of transient transformation increased severalfold and two continuous cell lines were established. Epidermal growth factor, a naturally occurring polypeptide hormone, appears to act here as a tumor promoter in the retrovirus-induced transformation of a mesodermally derived epithelium.*

Kirsten murine sarcoma virus (Ki-MSV) produces stable morphological transformation of several connective tissue cell types in culture, and such transformed lines form sarcomas when injected into sublethally irradiated rats (*1*, *2*). An epithelial tissue of mesodermal origin, the adrenal cortex, is susceptible to transformation by Ki-MSV, and differentiated carcinomas result as well as sarcomas (*3*). We attempted to extend these observations by infecting rat ovarian granulosa cells, another steroid-secreting epithelial cell type of mesodermal origin, with Ki-MSV.

Transformation foci appeared in all cultures within 1 week of infection (*4*). Without epidermal growth factor (EGF), very few foci were produced, usually fewer than ten per dish. The same virus preparations, used here at 1.0 ml per culture, contained between 2.3×10^4 and 2.8×10^6 focus-forming units per milliliter when assayed on comparable NRK (normal rat kidney) cultures (*3*). Unlike Ki-MSV–induced foci in cultures of adrenocortical cells or fibroblasts (*3*), foci in granulosa cell cultures invariably reverted to normal morphology within 3 weeks (Fig. 1). In no case did a stably transformed cell line result.

Addition of EGF to cultures at the

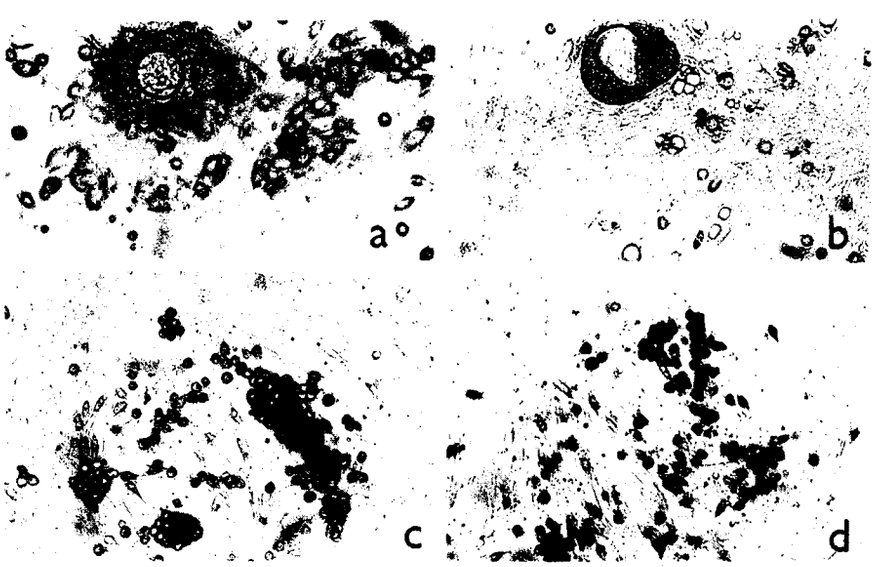

Fig. 1. Various stages of morphological transformation of Ki-MSV–infected granulosa cells. (a) Morphologically transformed cumulus oophorus cells surrounding an oocyte 6 days after infection (×50). (b) The same focus 58 days later. The transformed morphology has been lost. The degenerating oocyte is still prominent (×50). (c) A focus of transformation 8 days after infection. The cells are rounded and form clumps and are retracting from the substratum (×40). (d) The same area 6 days later. The focus is reverting (×40).

0036-8075/81/0710-0218$00.50/0