

of collection) and opened sequentially for analysis of dissolved sulfate and methane over an 82-day period. The experimental results are summarized in Fig. 2 (22).

The methane concentration of the sediments remained at its original value until dissolved sulfate was totally exhausted at about 32 days. At this time methane production began and a maximum production rate of approximately $13 \mu\text{mole liter}^{-1} \text{ day}^{-1}$ was observed. The apparent drop-off in this production rate based on the methane concentration after 82 days was at least partially due to loss of methane through observable cracks in the lid of the 82-day jar caused by gas pressure buildup. The salinity and concentrations of other ions in this jar rose because of evaporation through the cracks. These problems were not encountered with the other jars.

The significant result of the jar experiment is that methane originally present in the salt marsh sediments neither increased nor decreased during sulfate reduction. To the extent that the jar experiment mimics nature (23) this suggests that sulfate reduction and methane production are mutually exclusive processes and that oxidation of methane by sulfate-reducing bacteria is not responsible for the observed distributions in Long Island Sound sediments. Instead, the observed coexistence of measurable concentrations of methane and sulfate ions may be accounted for by interdiffusion combined with limited production of methane in sulfate-free microenvironments as described in alternatives 2 and 4 above. Before a firm choice of hypotheses can be made, however, more data from both pore waters and laboratory experiments, especially combined with microbiological identifications, are needed.

CHRISTOPHER S. MARTENS*

ROBERT A. BERNER

Department of Geology and
Geophysics, Yale University,
New Haven, Connecticut 06520

References and Notes

1. F. A. Richards, in *Chemical Oceanography*, J. P. Riley and G. Skirrow, Eds. (Academic Press, New York, 1965), vol. 1, p. 611; in *Proceedings of the Second International Water Pollution Research Conference* (Pergamon, New York, 1965), p. 215.
2. L. P. Atkinson and F. A. Richards, *Deep-Sea Res.* **14**, 673 (1967).
3. K. O. Emery and D. Hoggan, *Am. Assoc. Pet. Geol. Bull.* **42**, 2174 (1958); D. F. Hammond, in *Natural Gases in Marine Sediments and Their Mode of Distribution*, I. R. Kaplan, Ed. (Plenum, New York, in press).
4. W. S. Reeburgh, *Limnol. Oceanogr.* **14**, 368 (1969).
5. A. Nissenbaum, B. J. Presley, I. R. Kaplan, *Geochim. Cosmochim. Acta* **36**, 1007 (1972).
6. G. E. Claypool and I. R. Kaplan, in *Natural Gases in Marine Sediments and Their Mode of Distribution*, I. R. Kaplan, Ed. (Plenum, New York, in press).
7. J. M. Brooks and W. M. Sackett, *J. Geophys. Res.* **78**, 5248 (1973).
8. R. S. Wolfe, *Adv. Microb. Physiol.* **6**, 107 (1971).
9. C. S. Martens, *Limnol. Oceanogr.*, in press.
10. J. W. Swinnerton, V. J. Linnenbom, C. H. Cheek, *Anal. Chem.* **34**, 483 (1962); *ibid.*, p. 1509.
11. W. S. Reeburgh, *Environ. Sci. Technol.* **2**, 140 (1968).
12. L. W. Winkler, *Ber. Dtsch. Chem. Ges.* **34**, 1408 (1901).
13. C. S. Martens and R. A. Berner, in preparation. Concentrations of dissolved N_2 and Ar as low as 2.0 and 0.1 ml liter⁻¹, respectively, were observed where methane was at saturation concentrations.
14. H. W. Feely and J. L. Kulp, *Am. Assoc. Pet. Geol. Bull.* **41**, 1802 (1957).
15. C. E. Zobell, *ibid.* **31**, 1709 (1947).
16. T. E. Cappenberg, *Hydrobiologia* **40**, 471 (1972).
17. J. B. Davis and H. F. Yarbrough, *Chem. Geol.* **1**, 137 (1966).
18. D. C. Thorstenson, *Geochim. Cosmochim. Acta* **34**, 745 (1970).
19. P. L. McCarty, in *Water Pollution Microbiology*, R. Mitchell, Ed. (Wiley-Interscience, New York, 1972).
20. J. D. Cline and F. A. Richards, *Limnol. Oceanogr.* **17**, 885 (1972).
21. W. G. Deuser, E. T. Degens, G. R. Harvey, *Science* **181**, 51 (1973).
22. Sulfide was measured in the jar experiment and was also present in the millimolar concentration range in the Long Island Sound sediment cores. No effects of variations in sulfide concentrations on methane production were observed.
23. The rate of sulfate reduction observed in the laboratory jar experiment, about 2.8×10^{-1} mole liter⁻¹ year⁻¹, approximates the highest rates calculated for marine sediments (M. Goldhaber and I. R. Kaplan, *J. Soil Sci.*, in press).
24. We thank M. Goldhaber and G. Claypool for helpful discussion and comments on the manuscript. A. Ruggiero skillfully aided with chemical analyses. M. Reed provided indispensable assistance with gravity coring operations. Financial support was provided by American Chemical Society Petroleum Research Fund grant 7002-AC2 and by National Science Foundation grant GA 30288X.

* Present address: Department of Geology and Curriculum in Marine Sciences, University of North Carolina, Chapel Hill 27514.

26 April 1974

Melatonin: Its Inhibition of Pineal Antigonadotrophic Activity in Male Hamsters

Abstract. *Exposure of male hamsters to short daily photoperiods (1 hour of light and 23 hours of darkness daily for 9 weeks led to total involution of the testes and accessory sex organs (seminal vesicles and coagulating glands). Pituitary levels of immunoreactive prolactin also decreased by about 60 percent after dark exposure. The inhibitory effects of darkness on the reproductive organs were prevented either by pinealectomy or by the subcutaneous implantation of a melatonin-beeswax pellet into the animals each week. Both pinealectomy and melatonin treatment also returned pituitary levels of prolactin toward normal. The results suggest that melatonin is not the pineal antigonadotrophic factor in the male golden hamster.*

A considerable amount of evidence suggests that *N*-acetyl-5-methoxytryptamine (melatonin) may be a pineal antigonadotrophic factor in the rat (1). In the golden hamster, however, melatonin has repeatedly failed to exhibit gonad-inhibiting activity (2). Yet, in both males and females of the species the pineal gland is known to be strongly suppressive to the reproductive system (3). However, melatonin may yet play some role in determining the ability of darkness to influence reproductive physiology in photosensitive species. The results of the present experiment show that melatonin acts in a heretofore unreported manner to prevent the pineal gland from inducing gonadal degeneration in dark-exposed male hamsters.

Forty young adult male hamsters (*Mesocricetus auratus*) (60 to 75 g) were purchased from Lakeview Hamster Colony, Newfield, N.J. Six of these were maintained in long daily periods of light (14 hours of light and 10 hours of darkness in each 24 hours) (LD 14:10) throughout the study. The remaining 34

hamsters were subdivided into four groups which were maintained in short daily light periods (1 hour of light and 23 hours of darkness in each 24 hours) (LD 1:23).

Of the four dark-exposed groups, one group received no further treatment, one group was pinealectomized by use of a standard procedure (4), one group received a weekly subcutaneous implant of a beeswax pellet (25 mg), and the final group received weekly a beeswax pellet that contained 1 mg of melatonin (5). Administration of melatonin in this manner has been found to produce endocrine effects (6). Nine weeks after the onset of the experiment the hamsters were decapitated and trunk blood was collected in heparinized tubes. Body, testicular, accessory organ (seminal vesicles and coagulating glands), and anterior pituitary gland weights were recorded. Pituitaries and plasma samples were analyzed by radioimmunoassay for prolactin by using an established technique (7). Data were statistically analyzed on a Programma

Table 1. Mean body and anterior pituitary gland weights and mean plasma prolactin levels in adult male hamsters. Prolactin levels are expressed as micrograms of standard hamster anterior pituitary (SHAP) according to the description of Donofrio *et al.* (7). BW, body weight.

N	Body weight (g)	Anterior pituitary		Plasma prolactin (μ g SHAP/ml)
		Weight (mg)	Ratio (mg/100 g BW)	
6	138 \pm 6	LD 14:10, intact 2.89 \pm 0.16	2.09 \pm 0.05	0.91 \pm 0.12
6	150 \pm 10	LD 1:23, intact 2.74 \pm 0.22	1.82 \pm 0.19	0.79 \pm 0.09
7	136 \pm 4	LD 1:23, pinealectomized 2.54 \pm 0.19	1.86 \pm 0.11	0.78 \pm 0.12
7	146 \pm 5	LD 1:23, wax implants 2.67 \pm 0.16	1.82 \pm 0.14	0.80 \pm 0.05
14	142 \pm 8	LD 1:23, melatonin implants 2.72 \pm 0.06	1.91 \pm 0.10	1.23 \pm 0.07*

* $P < .02$ compared to all other groups.

101 computer with the use of an analysis of variance.

Body and anterior pituitary gland weights did not differ among the five experimental groups (Table 1). Exposure of hamsters to LD 1:23 led to the expected involution of the testes and accessory organs, a response that was prevented completely by pinealectomy (Fig. 1). The weekly implantation of beeswax pellets had no effect on the

degenerative responses of the reproductive organs of hamsters exposed to LD 1:23. However, melatonin-implanted hamsters possessed reproductive organs which were similar in size to those of hamsters kept in LD 14:10. That is, like pinealectomy, melatonin administration prevented gonadal and accessory organ involution in hamsters exposed to short daily photoperiods.

Both the concentration and the con-

tent of pituitary prolactin were dramatically reduced in hamsters exposed to LD 1:23 (Fig. 2). Pinealectomy and melatonin treatment prevented the fall in pituitary prolactin concentration induced by dark exposure; however, total pituitary prolactin levels still remained slightly depressed. Prolactin titers were altered (elevated) only in the melatonin-treated hamsters (Table 1).

The prevention of gonadal and accessory organ involution by pinealectomy in dark-exposed hamsters is well documented (3). Likewise, the prevention of the marked fall in pituitary prolactin levels by pineal removal in dark-exposed hamsters confirms a previous finding (7, 8). Conversely, the results obtained with melatonin were not anticipated. Melatonin prevented the drop in gonadal and accessory organ weights after LD 1:23 and also almost totally overcame the inhibitory effect of darkness on pituitary prolactin levels. Furthermore, melatonin caused a significant rise in plasma prolactin levels; this latter finding is consistent with observations in the rat (9).

Heretofore, it frequently had been

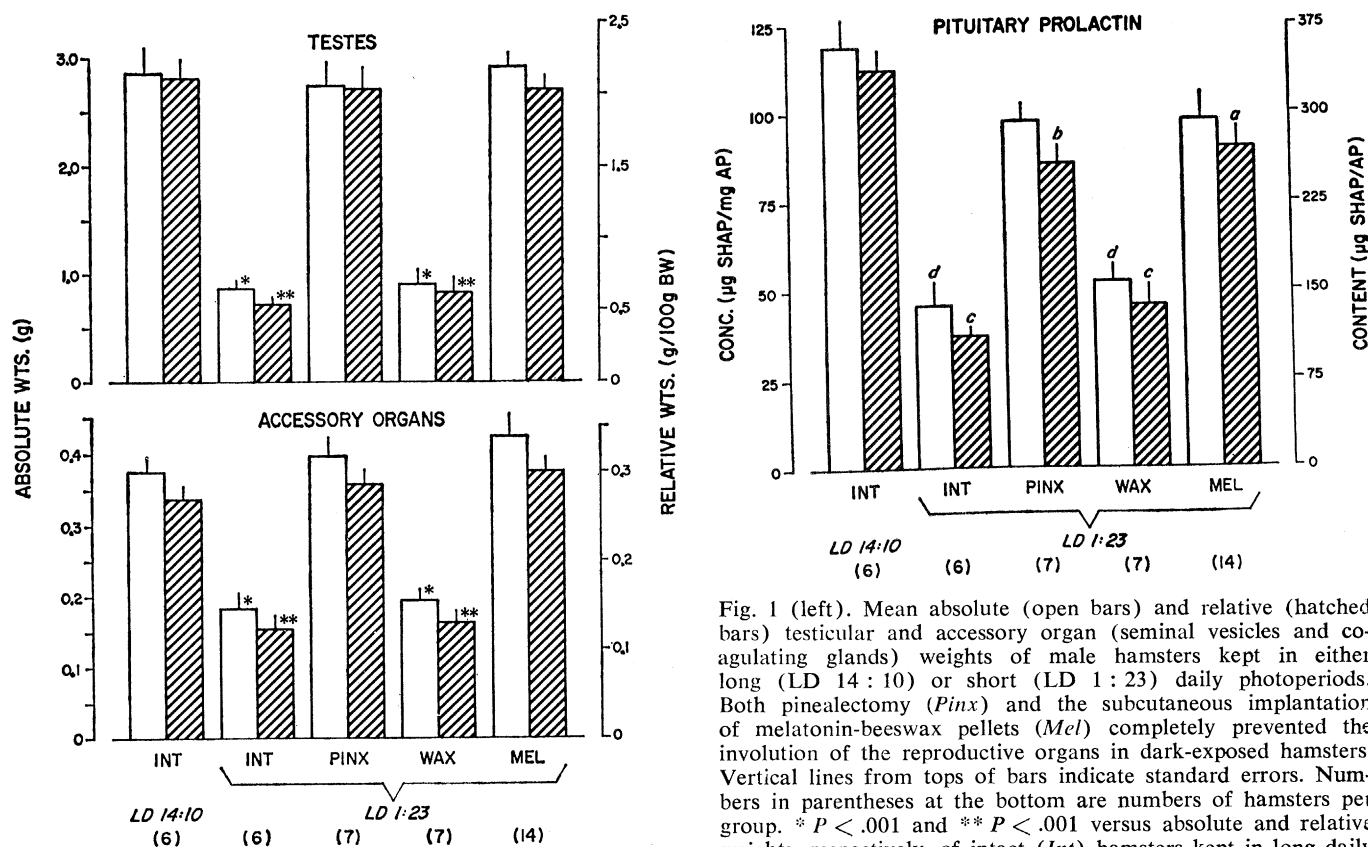


Fig. 1 (left). Mean absolute (open bars) and relative (hatched bars) testicular and accessory organ (seminal vesicles and coagulating glands) weights of male hamsters kept in either long (LD 14:10) or short (LD 1:23) daily photoperiods. Both pinealectomy (Pinx) and the subcutaneous implantation of melatonin-beeswax pellets (Mel) completely prevented the involution of the reproductive organs in dark-exposed hamsters. Vertical lines from tops of bars indicate standard errors. Numbers in parentheses at the bottom are numbers of hamsters per group. * $P < .001$ and ** $P < .001$ versus absolute and relative weights, respectively, of intact (Int) hamsters kept in long daily photoperiods. Fig. 2 (right). Concentration (open bars) and content (hatched bars) of prolactin in pituitary glands of male hamsters kept in either long (LD 14:10) or short (LD 1:23) daily photoperiods. (a) $P < .05$, (b) $P < .01$, and (c) $P < .001$ versus prolactin content in intact (Int) hamsters kept in long photoperiods; (d) $P < .001$ versus prolactin concentration in intact hamsters kept in long daily photoperiods. Prolactin levels are expressed as micrograms of standard hamster anterior pituitary (SHAP) according to the description of Donofrio *et al.* (7).

suggested that the antigonadotrophic effect of the pineal gland was due to an increased synthesis and release of melatonin by the gland (1). Whereas this may be true for some species, it does not seem to be true for the hamster, where melatonin treatment had the same effect as pinealectomy. There are several possible explanations for the results. Possibly melatonin inhibited the synthesis or the release, or both, of the true pineal antigonadotrophic factor. Other substances, especially polypeptides, are considered by many workers to be the pineal factors which account for the antigonadotrophic activity of the gland (10). Furthermore, several workers have suggested that the indoleamines (including melatonin) within the pineal may be concerned with the release of other substances by the gland (11). A second explanation for the present results is that melatonin may have rendered the hypothalamo-pituitary axis resistant to inhibition by the pineal antigonadotrophic factor. Finally, melatonin may be directly stimulatory to the gonads in the hamster. Regardless of the mechanisms involved, the results of this study suggest that melatonin is not the pineal antigonadotrophic factor in the golden hamster.

R. J. REITER, M. K. VAUGHAN
D. E. BLASK, L. Y. JOHNSON

Department of Anatomy, University of
Texas Health Science Center at
San Antonio, San Antonio 78284

References and Notes

1. R. J. Wurtman, J. Axelrod, D. E. Kelly, *The Pineal* (Academic Press, New York, 1968); F. Fraschini, in *Progress in Endocrinology*, C. Gual, Ed. (Excerpta Medica, Amsterdam, 1969), pp. 637-644; I. A. Kamberi, R. S. Mical, J. C. Porter, *Endocrinology* **87**, 1 (1970).
2. R. J. Reiter, in *Progress in Endocrinology*, C. Gual, Ed. (Excerpta Medica, Amsterdam, 1969), pp. 631-636; in *Handbook of Physiology*, E. Knobil and W. H. Sawyer, Eds. (American Physiological Society, Washington, D.C., in press).
3. R. A. Hoffman and R. J. Reiter, *Science* **148**, 1609 (1965); R. J. Reiter and R. J. Hester, *Endocrinology* **79**, 1168 (1966); R. J. Reiter, *Annu. Rev. Physiol.* **35**, 305 (1973).
4. R. A. Hoffman and R. J. Reiter, *Anat. Rec.* **153**, 19 (1965).
5. The melatonin-beeswax pellets were prepared in the following manner. A determined amount of beeswax was melted in a beaker on a hot plate. After its removal from the heat, as it began to solidify, a measured amount of crystalline melatonin (Regis Chemical Co., Chicago) was added and the mixture was kneaded to ensure thorough mixing. The semisolid mixture was then formed into disks (pellets) which weighed about 25 mg. The final concentration was 1 mg of melatonin in 24 mg of beeswax. Beeswax disks that did not contain melatonin were used for the control implantations. All pellets were implanted subcutaneously once per week while the hamsters were anesthetized with ether. The pellets were prepared fresh immediately before their implantation.
6. C. C. Rust and R. K. Meyer, *Science* **165**, 921 (1969); S. Sorrentino, Jr., R. J. Reiter, D. S. Schalch, *J. Endocrinol.* **51**, 213 (1971).
7. R. J. Donofrio, R. J. Reiter, S. Sorrentino, Jr., D. E. Blask, J. A. Talbot, *Neuroendocrinology* **13**, 79 (1973/74).
8. R. J. Reiter and L. Y. Johnson, *Horm. Res.*, in press.
9. I. A. Kamberi, R. S. Mical, J. C. Porter, *Endocrinology* **88**, 1288 (1971).
10. S. M. Milcu, S. Pavel, C. Neacsu, *ibid.* **72**, 563 (1963); L. Thieblot and M. Menigot, *J. Neuro-Visc. Relat. Suppl.* **10**, 153 (1971); A. Moszkowska and I. Ebels, *ibid.*, p. 160; B. Benson, M. J. Matthews, A. E. Rodin, *Acta Endocrinol.* **69**, 257 (1972); A. Moszkowska, A. Scemama, M. N. Lombard, M. Héry, *J. Neural Trans.* **34**, 11 (1973); I. Ebels, B. Benson, M. J. Matthews, *Anal. Biochem.* **56**, 546 (1973); S. Pavel, M. Petrescu, N. Vicolanu, *Neuroendocrinology* **11**, 370 (1973).
11. W. B. Quay, *Pharmacol. Rev.* **17**, 321 (1965); *J. Neuro-Visc. Relat. Suppl.* **9**, 212 (1969); S. Pavel, *Nature (Lond.)* **246**, 183 (1973).
12. Supported in part by PHS grant HD-06523. R.J.R. is a PHS career development awardee, HD-42398.

3 May 1974

Rod Origin of Prolonged Afterimages

Abstract. *Afterimages fade against any unchanging background but generally reappear if the background changes suddenly. Under some conditions, however, a change of background color fails to revive a faded afterimage. This happens only if the interchanged backgrounds equally stimulate the rod receptors. It follows that afterimages seen under these conditions are generated by rods.*

After exposure to an intense light, strong enough to bleach most of the visual pigment, an afterimage may be seen for half an hour or more. The appearance of the afterimage depends on the brightness and color of the background against which it is observed. In the dark, or when the eyes are closed, it glows dimly against the dark surround (the positive afterimage); against a uniform bright background it appears dark (the negative afterimage). But an afterimage does not long remain visible against any steady background. Instead, an initially crisp image fades progressively, and totally disappears within a few seconds. After it has faded, any sudden change in background intensity will revive it: dimming the background discloses a positive afterimage; making it brighter creates a negative afterimage (1). These afterimages are not generated in the brain but originate within the eye itself. If only one eye is bleached, it is the intensity of the background seen by that eye which determines the appearance of the resulting afterimage, so that once the afterimage has faded, changes of background intensity that are seen only by the unbleached eye are powerless to revive it (2). If an eye is bleached while temporarily blinded by pressure on the eyeball, the brain can contain no record of the bleaching exposure, yet an afterimage may appear when the pressure is released (3). This afterimage must be generated within the eye.

The normal eye contains two classes of receptor that might generate an afterimage: the cones, which are the receptors of daylight and color vision, and the rods, which permit vision in dim light. After exposure to an intense

bleaching light, cones recover their sensitivity in about 5 minutes, rods much more slowly. Rods allow no color discrimination and are relatively insensitive to long-wave light, so that for rods, a dim blue and a bright red may be indistinguishable. We show that after bleaching, once the cones have had time to recover their sensitivity, differently colored backgrounds which are indistinguishable by rods but very different for cones may be interchanged without reviving the afterimage. This afterimage must therefore be generated by rods alone (4).

With a 30 second exposure to a white light of 2.2 million trolands we bleached nearly all the visual pigment in a circular region of retina subtending 10° and centered 10° above the line of sight. The observer then directed his gaze at a small red spot near the bottom of a large (21°) red (620 nm) circular field, the "conditioning" background (5). The afterimage of the bleaching light appeared as a dark disk in the center of the field, but if fixation was maintained for a few seconds it gradually faded. After it had faded the observer depressed a lever which abruptly replaced the red conditioning background with light of a different wavelength; this substitution usually revived the afterimage. After viewing the afterimage against the substituted background for about half a second, he restored the red conditioning background and allowed the afterimage to fade once again. He then adjusted the intensity of the substituted background light, by means of a graded neutral filter, and again depressed the lever to observe the afterimage against the substituted background at its new intensity. The observer's task was to