tal interpretation is peculiar to *M. montanus* remains to be determined. However, the ovarian response of the laboratory mouse suggests the potential of a more general mammalian response to chemical cues in the food resource.

EDWARD H. SANDERS*
PETE D. GARDNER
PATRICIA J. BERGER
NORMAN C. NEGUS

Departments of Biology and Chemistry, University of Utah, Salt Lake City 84112

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7. The plant fractions to be assayed were dissolved in a 1:4 mixture of ethanol and ether and mixed with ground Purina Laboratory (Rat) Chow; the solvent was allowed to evaporate. Control animals received Chow coated with solvent only. The assay animals, juvenile *Microtus montanus* (17 to 19 days old and weighing 13.0 to 14.5 g), were caged in pairs and given free access to the experimental or control diets. After a 7-day feeding period, the animals were killed and the uteri were removed and weighed.
8. A gas chromatograph (Perkin-Elmer 880) equipped with a flame ionization detector was used to separate components on a glass column (6 feet by 1/8 inch) packed with 3 percent OV-17 on Chromosorb AW-DMCS, 80/100 mesh. The column oven was programmed to increase from 80° to 200° C at a rate of 4° C per minute.
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12. Dry column chromatography was carried out on silica gel packed into nylon tubing 30 inches long and 0.5 inches in diameter. The chromatogram was cut into eight equal sections after being developed with 10 percent methanol in chloroform. The fourth section from the top, composed primarily of an intense purple band, was active.
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14. A dose-response curve was obtained by intraperitoneal injection of 4- to 5-week-old *Microtus montanus* with 6-MBOA in 0.5 ml of 5 percent propylene glycol for three consecutive days. Control animals received 5 percent propylene glycol injections only. The animals were killed 24 hours after the last injection and the uteri were removed and weighed.
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16. Juvenile *Microtus montanus* (aged 17 to 19 days, weighing 13.0 to 14.5 g) were ovariectomized or sham-operated on day 0 of the 7-day juvenile assay regime. The animals were then treated as in (*6*).
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20. The isolation and identification of 6-MBOA was supported by funds from the Dow Chemical Company and by University of Utah institutional funds. Other aspects of this study were supported by NSF grant DEB-79-21059.
* Present address: Utah Biomedical Test Laboratory, University of Utah Research Institute, 520 Wakara Way, Salt Lake City, Utah 84112.

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Chemical Triggering of Reproduction in *Microtus montanus*

Abstract. *In a replicated experiment, nonbreeding winter populations of* Microtus montanus *were given supplements of rolled oats coated with 6-methoxybenzoxazolinone, a naturally occurring plant derivative. After 3 weeks of this feeding regime, samples from the populations demonstrated a high incidence of pregnancy in females and testicular hypertrophy in males. Control populations receiving rolled oats coated only with the solvent showed no reproductive activity. These results demonstrate that the presence of 6-methoxybenzoxazolinone in the plant food resource acts as the ultimate cue to trigger reproductive effort in* Microtus montanus.

In 1977, we presented field experimental evidence that the montane vole (*Microtus montanus*) is cued to its reproductive effort by chemicals in the plant food resources (*1*). Subsequently, we were successful in isolating and identifying the active chemical component (*2*). Responses to 6-methoxybenzoxazolinone (6-MBOA) were directly comparable to those elicited from fresh grass supplements and laboratory experiments with crude extracts (*3*). However, the ultimate proof of the efficacy of 6-MBOA in triggering reproduction remained to be demonstrated in the field. We now present experimental proof that 6-MBOA is responsible for triggering reproduction in *Microtus montanus*.

Replicated field experiments were accomplished in the winters of 1978–79 and 1979–80, in the salt grass meadows (*Distichlis stricta*) at Timpie Springs, Utah. Previous triggering experiments had been performed at the identical site (*1*). Experimental and control plots were selected as described (*1, 4*). Before each experiment, the *Microtus* population was sampled by livetrapping (Table 1); these samples demonstrated that the populations were reproductively inactive. We then provided supplemental food in the experimental and control plots. In the experimental plots, the supplemental food consisted of rolled oats coated with 6-MBOA (40 μg per gram of oats) (*5*). The supplemental food for the control plot was rolled oats coated with solvent only. Thus, the only difference between the supplemental treatments in the two plots was the addition of 6-MBOA in the experimental plot. Oats were distributed in each plot at the rate of 1 kg every 7 days for 3 weeks. Petri dishes containing the oats were placed beside runways; the food was protected from weather by galvanized metal shelters (*6*).

When the supplemental feeding period was terminated, both areas were sampled by livetrapping, and the animals were killed and examined for reproductive activity (*7*). In the winter of 1978–79, the experimental regime was initiated on 13 January 1979 and terminated on 11 February. In the winter of 1979–80, a similar experiment was initiated on 24 November 1979 and terminated on 17 December. The rationale for the earlier timing of the second experiment was to determine whether there was a period before the winter solstice (22 December) when the population would be unresponsive to 6-MBOA. The two experimental regimes produced essentially identical results (Table 1).

In both experiments, a large propor-

Table 1. Reproductive responses of female and male *Microtus montanus* to supplements of 6-MBOA coated on rolled oats (E) during January and February 1979 (experiment 1) and November and December 1979 (experiment 2), compared with responses of control animals (C) that received supplements of rolled oats coated only with the solvent.

Date	Sex	Treatment	*N*	Proportion pregnant	Weight of uterus or testes (mg)
		Experiment 1			
4 December 1978	♀	Presample	6	0.0	4.6 ± 1.5
11 February 1979	♀	E	11	0.6	58.6 ± 8.0*
11 February 1979	♀	C	11	0.0	32.7 ± 18.2
11 February 1979	♂	E	11		231.6 ± 55.8
11 February 1979	♂	C	8		139.2 ± 76.6
		Experiment 2			
29 October 1979	♀	Presample	8	0.0	10.6 ± 5.4
18 December 1979	♀	E	7	0.86	40.1 ± 8.7*
18 December 1979	♀	C	22	0.0	14.2 ± 7.9
18 December 1979	♂	E	9		137.2 ± 35.1
18 December 1979	♂	C	10		63.2 ± 57.2

*Includes only the uterine weights of animals not visibly pregnant.

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tion of the females in the experimental plots were in early pregnancy at the termination of the regimes. None of the females in the control plots were pregnant, nor were there large follicles in the ovaries. The mean testicular weight of experimental males was considerably greater than that of control males (Table 1). There was no apparent inhibition of response in the experiment that preceded the winter solstice.

These results demonstrate that 6-MBOA elicits a rapid commitment to reproductive effort in both male and female *Microtus montanus*. The significance of this response can now be placed in ecological perspective. 2,4-Dihydroxy-7-methoxy-2*H*-1,4-benzoxazin-3-(4*H*)-one (DIMBOA), the precursor of 6-MBOA, is particularly abundant as the glucoside in vegetatively growing young seedlings (*8*). Injury of plant tissue by a predator releases enzymes that facilitate a rapid conversion of DIMBOA to 6-MBOA. When *Microtus* eats young, actively growing plants, it ingests 6-MBOA. This chemical, therefore, represents a reliable cue that the vegetative growing season has begun. Timing its reproductive effort to the 6-MBOA cue permits this short-lived herbivore to produce offspring at the optimal time for survival of the young, even in fluctuating environments. In essence, the cue ensures that an abundant vegetative food resource will be available in the near future and is an indicator of spring growing conditions as well as of drought termination.

Many mammalian species appear to utilize changes in the photoperiod as cues to initiate or cease reproductive efforts (*9*). Although photoperiod changes are sufficient cues in highly predictable environments and for species with longevities of several years, *Microtus montanus* typically lives less than 1 year and has only one breeding season in which to make a reproductive effort. Being strict herbivores in montane grasslands, these animals use a food resource whose abundance is temporally uncertain; that is, vegetative growth might begin in a montane meadow during April in one year and during late June in another, depending on snowmelt and other climatic conditions. Thus, photoperiod is of little predictive value, whereas the presence of 6-MBOA in plants affords close tracking of the food resource and optimal timing of reproductive effort. Field observations have confirmed that *Microtus montanus* populations synchronize reproductive effort with the onset of vegetative growth, including cessation of reproduction during drought and immediate resumption after rainfall-induced vegetative growth (*10*).

This cueing mechanism may prove to be common among herbivorous mammals inhabiting highly unpredictable environments. Observational data on rabbits, kangaroos, other microtine rodents and desert rodents (*11*) suggest that these species may also respond to cues from their vegetative food resources. Our investigations of the physiological pathway of 6-MBOA in *Microtus* indicate that the site of action of 6-MBOA is high in the neuroendocrine circuitry. Accordingly, 6-MBOA may elicit reproductive responses in a wide spectrum of vertebrate systems.

The population consequences of this reproductive cueing mechanism await further study. However, our present knowledge of differential age at first reproduction and fitness of *Microtus montanus* cohorts (*10*) indicates that yearly differences in the time of appearance of 6-MBOA in the food resource may exert large effects on population dynamics. Thus, 6-MBOA may have a role in the legendary multiannual population fluctuations of microtine rodents.

PATRICIA J. BERGER
NORMAN C. NEGUS
EDWARD H. SANDERS*
PETE D. GARDNER
Departments of Biology and *Chemistry, University of Utah, Salt Lake City 84112*

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4. Two plots, approximately 400 m^2 in area, were selected. Both areas were vegetated by dense, almost pure, stands of salt grass, and were identical in soil, moisture, and topographic conditions. The experimental plot was an island separated by water from the mainland.
5. The 6-MBOA was dissolved in a mixture of ethanol and ether (1:4) and coated on rolled oats; the solvents were then evaporated.
6. An approximate estimate of consumption based on the density of animals is 3 to 4 g of oats per animal per day.
7. In the males, testes were removed and weighed. In the females, the uteri were removed, weighed, and examined for visible signs of pregnancy. If pregnancy was not obvious, the ovaries were removed and sectioned. The presence of newly forming corpora lutea was equated with early pregnancy, large follicles indicated a cycling ovary, and the presence of only a few small follicles was interpreted as reproductive quiescence.
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* Present address: Utah Biomedical Test Laboratory, University of Utah Research Institute, 520 Wakara Way, Salt Lake City 84112.

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Photoregulated Ion Binding

Abstract. *A photoregulated chelating agent has been synthesized. It is a photochromic azobenzene compound containing two iminodiacetic acid groups and can exist as* cis *and* trans *stereoisomers. The planar* trans *isomer does not bind zinc ions. On exposure to light of 320 nanometers, the* trans *isomer is converted to a nonplanar* cis *isomer, which, because of cooperativity between the two iminodiacetic acid groups, binds zinc ions with the value of the binding constant estimated to be* $1.1 \times 10^5 \pm 0.2 \times 10^5$ *liters per mole at a ratio of one molecule of chelating agent to one zinc ion. The interconversion of the* cis *and* trans *isomers is reversible, suggesting possible application of this class of compounds as photoresponsive ion pumps.*

Photochromic compounds (*1*) function as photoregulators in such naturally occurring photoresponsive systems as the retina of the eye (rhodopsin), the purple membrane of halophilic bacteria (bacteriorhodopsin), and the phytochrome systems of plants (*2*). Common to all of these systems is a mechanism by which a light-induced change in the configuration of a low molecular weight photochromic compound induces a conformational change in a macromolecule. In model systems constructed to mimic the behavior of these naturally occurring processes, enzyme activity (*3*) or ion fluxes through a membrane (*4*) are modulated by a photochromic effector.

In this report, we describe a photochromic compound that can be induced to bind or to release an ion when stimulated by light of appropriate wavelengths. Although we know of no analogous low molecular weight compounds in nature, a study of molecules of this kind may provide information about macromolecular ionophores that are regulated, directly or indirectly, by light (*5*). Moreover, compounds of this type might be useful as photoswitching devices in biological experiments (*6*) and could also