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.

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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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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

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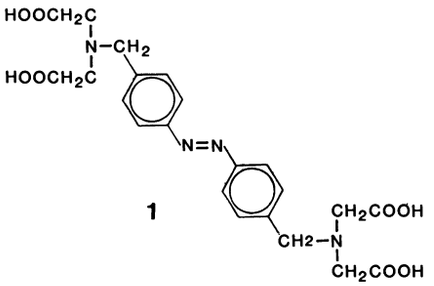

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Fig. 1. Structure of 4,4′-bis(α-iminodiacetic acid)azotoluene (**1**).

find application in solar energy conversion technology.

The photochromic compound, *trans*-4,4′-bis(α-iminodiacetic acid)azotoluene (*trans*-**1**) (Fig. 1), was prepared by refluxing iminodiacetic acid (4×10^{-3} mole) with 4,4′-bis(α-bromomethyl)azobenzene (10^{-3} mole) and 0.5 g of calcium chloride in a mixture of 20 ml of dimethylsulfoxide and 30 ml of dioxane for 18 hours, with stirring. The precipitate was removed by centrifugation, and the supernatant was evaporated to near dryness in vacuo. After extraction with ethyl ether (to remove the remaining dimethylsulfoxide), the residue was dissolved in methanol, filtered, and concentrated to a small volume, with resulting crystallization of 180 mg of *trans*-**1** (melting point, 208° to 209°C). Analysis for C, H, and N showed the compound to be pure. It had an absorption peak at 322 nm with a molar extinction coefficient of 19,700. Exposure to light of 320 nm (Spectroline B-100) converted approximately 80 percent of the *trans* isomer to *cis*-**1**, with an accompanying decrease in the 322-nm peak. Exposure of *cis*-**1** to a photoflood lamp yielded an equilibrium mixture of about 80 percent *trans*-**1** and 20 percent *cis*-**1**. For the experiment described below, we used ultraviolet spectrophotometry and nuclear magnetic resonance (NMR) with a Bruker WM-300 (300 MHz) Fourier transform spectrometer to determine the concentrations of *cis* and *trans* isomers in solutions exposed to light. With respect to NMR, (D_2O) *trans* isomer: δ7.99(d, 4H), 7.76(d, 4H); *cis* isomer: δ7.47(d, 4H), 6.97(d, 4H). The ratio of the two isomers in solution is given by NMR integration. These determinations were correlated with the absorbance at 322 nm.

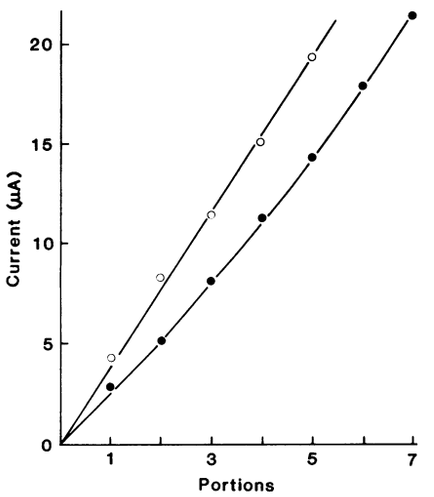

Fig. 2. The variation of the diffusion current (in microamperes) with the addition of portions of Zn^{2+} to 10 ml of 0.1*M* $NaNO_3$ solution that was 0.01 m*M* with respect to **1** either as pure *trans* isomer or as a mixture of *cis* and *trans* isomers. (Each portion of Zn^{2+} solution was 20 μl of 1 m*M* $ZnSO_4$. Therefore, in the absence of binding, the Zn^{2+} concentration after the first addition was, within 1 percent, $2 \times 10^{-6}M$ and, after five additions, $10^{-5}M$.) The graph shows the currents in the absence of **1** (○) and in the presence of *cis*-**1** (●). In the presence of *trans*-**1**, the data coincide with upper curve (○).

In our experiments, the phototransformations were achieved to varying extents in each set of experiments, but the concentrations of the two forms were estimated by comparing the ultraviolet spectra of the pure *trans* form with that of the mixtures. The equilibrium mixtures were stable for many days in the dark and remained unchanged in the spectrophotometer. All polarographic experiments described below were carried out in a darkened room.

The binding of Zn^{2+} to **1** was studied by measuring the concentrations of free Zn^{2+} after addition to a solution containing a known amount of **1**. We measured the free Zn ions using a polarograph (Metrohm Polarecord, model E 261) with a rapid-dropping mercury electrode attachment. The half-wave potential for the reduction of Zn ions was −0.8 V, and the diffusion current associated with this potential was directly proportional to the concentration of Zn ions in solution. Microliter quantities of the Zn^{2+}-containing solution were added to a 0.1*M* $NaNO_3$ (supporting electrolyte) solution; the diffusion current, measured after each addition, was directly proportional to the Zn ion concentration, as shown in the upper curve of Fig. 2, which represents a typical experiment. Overlapping results were obtained for solutions of **1** in the *trans* conformation. However, solutions containing *cis*-**1** gave significantly smaller currents (Fig. 2, lower curve), indicating binding of Zn ions. Since bound ions do not contribute to the diffusion current, they can be detected as the difference between the currents expected for a particular addition of Zn ions and the observed currents (the difference between the two curves of Fig. 2). The Zn ions bound only to the *cis* form. When this form was converted back to the *trans* form, bound ions were released, and the measured current increased.

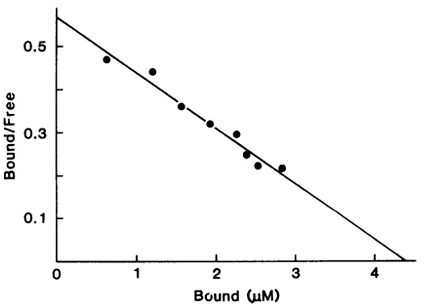

Fig. 3. A plot of data according to Eq. 5 for Zn^{2+} binding to *cis*-**1**. The ratio of bound to free Zn^{2+} is plotted against the concentration of bound Zn^{2+} (in micromoles per liter). The equilibrium binding constant is $1.3 \times 10^5 M^{-1}$, as determined by the slope of the curve. The experimental conditions are the same as those described in the legend to Fig. 2.

In the experiment plotted in Fig. 3, the Zn^{2+} binding was determined as before, and the concentration of the *cis* form was determined spectrophotometrically at the same time (see above). This permitted a detailed analysis of the stoichiometry of the binding. From a comparison of the number of Zn ions bound to the concentration of the *cis* compound present and a study of the various possible combinations, it appears that each *cis*-**1** molecule binds one Zn ion in the following equilibrium

$$\mathbf{1} + Zn \rightleftharpoons \mathbf{1} \cdot Zn \qquad (1)$$

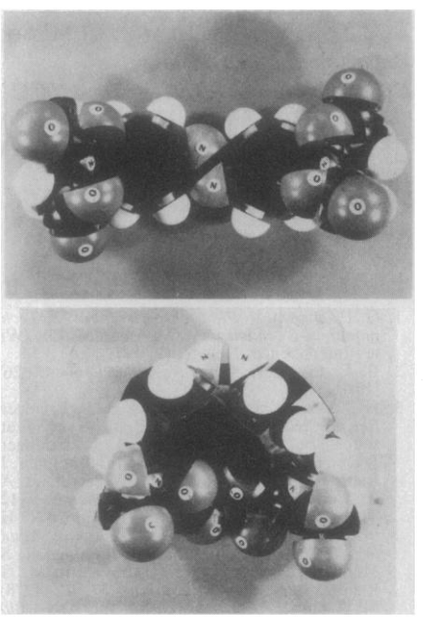

Fig. 4. Models of (top) *trans*-**1** and (bottom) *cis*-**1**.

If **1** is free **1**, F is free Zn^{2+}, B is bound Zn^{2+}, and K is the binding constant

$$K = [\mathbf{1} \cdot Zn]/[\mathbf{1}]\,[Zn] = B/[\mathbf{1}]\,F \quad (2)$$

Since the bound **1** is equal to the bound Zn, the total amount of **1** ($\mathbf{1}_t$) in the *cis* form becomes

$$\mathbf{1}_t = \mathbf{1} + B \quad (3)$$

Therefore

$$K = B/(\mathbf{1}_t - B)\,F \quad (4)$$

This equation can be rearranged to provide a form that is more convenient for plotting and extracting the value of K

$$B/F = K\,(\mathbf{1}_t - B) \quad (5)$$

From Fig. 3, a plot based on Eq. 5, we see that the binding of Zn to **1** follows Eq. 1 and we calculate that $K = 1.3 \times 10^5 M^{-1}$. A calculation of K from each of the individual points of the curve, with $\mathbf{1}_t = 0.6 \times 10^{-5} M$ as determined spectrophotometrically, yielded $K = 0.9 \times 10^5 \pm 0.1 \times 10^5 M^{-1}$. Therefore, our best estimate is $K = 1.1 \times 10^5 \pm 0.2 \times 10^5 M^{-1}$.

An examination of molecular models of the *cis* isomer reveals the possibility of intramolecular cooperativity between the two iminodiacetic acid groups, a phenomenon not possible in the *trans* isomer (Fig. 4). The planar *trans* form would require two molecules of **1**, and the binding of each molecule would be much weaker. These two factors probably account for the absence of Zn^{2+} binding by the *trans* isomer under the same conditions. Preliminary studies indicate that the ionization constants of *cis*-**1** and *trans*-**1** differ, which suggests that the binding of protons might also be amenable to photoregulation.

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Trauma-Induced Protein in Rat Tissues: A Physiological Role for a "Heat Shock" Protein?

Abstract. *Hyperthermic shock induces the synthesis of a novel protein (P_{71}) in many rat tissues in vivo. In incubated rat tissue slices P_{71} is the major protein synthesized even though it is undetectable in the tissues of a normal, unstressed rat. P_{71} is a "heat shock" protein, and it may be induced in vivo by stimuli other than hyperthermia. These results indicate that caution must be used in studies of protein synthesis in tissue explants, since the pattern of proteins synthesized by rat tissue slices is characteristic of stressed tissue.*

Freshly sliced rat tissues, including brain, thymus, heart, lung, spleen, liver, and kidney, rapidly synthesize a novel protein (P_{71}) which has a molecular weight of 71,000 and an acidic isoelectric point (*1*). This response was first observed in brain slices where the synthesis of P_{71} was traced to cells associated with the microvasculature (*2, 3*). P_{71} was not synthesized in unstressed brain in vivo, nor in brain slices during the first 30 minutes of incubation (*2*). In addition, it was shown that a new species of RNA was required for the synthesis of P_{71} (*2*). Although rat embryo cells in culture synthesize little or no P_{71}, these cells can be stimulated by canavanine, heat shock, or heavy metal ions to produce a protein similar, if not identical, to P_{71} (*1*). Similarly, one of the proteins synthesized in response to heat shock by *Drosophila* (*4*) and chicken embryo cultures (*5*) appears to be closely related to P_{71} (*6*). These studies showed that the induction of the heat shock proteins was accompanied by increased amounts of messenger RNA encoding these proteins.

Since P_{71} or proteins similar to P_{71} are induced by heat shock, canavanine, and heavy metal ions in various systems (in vitro), it has been suggested that this protein might be important for cell survival under conditions of environmental or physiological stress (*1, 7*). To examine the synthesis of P_{71} in vivo we used male Sprague-Dawley rats (*8*). First, we determined if P_{71} was synthesized or present in any tissues of either anesthetized or untreated rats. Second, we examined various tissues of rats stressed with hyperthermic shock for the synthesis of P_{71}. Proteins were separated and compared by a combination of isoelectric focusing (IEF) and sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE).

Newly synthesized proteins were labeled in vivo by an intraperitoneal injection of 0.3 mCi (0.3 ml) of L-[^{35}S]methionine (*9*). At the end of a 90-minute labeling period, the rats were given 0.3 ml of Somnotol (*10*) and briefly perfused with saline via the left ventricle to remove as much blood as possible from the tissues (*11*). Various tissues (0.2 to 0.3 g) were removed and placed in 1.0 ml of lysis buffer for subsequent two-dimensional gel electrophoresis according to the method of O'Farrell (*12*). Samples (130 μl) were loaded on cylindrical columns (2 by 100 mm) for IEF in the first dimension. Focusing was done at 400 V overnight (15 to 18 hours) followed by 1 hour at 800 V. The IEF gels were equilibrated for 1 hour in SDS-sample buffer and the proteins were further separated by SDS-PAGE on 7.5 percent acrylamide slab gels (*13*). The gels were stained for proteins with Coomassie brilliant blue R and destained (*14*). Labeled proteins were visualized by autofluorography (*15*).

Figure 1A shows that P_{71} was the major protein synthesized by heart slices in vitro. The slices were prepared and incubated for 30 minutes in the absence of the labeled amino acid and then for 90 minutes in the presence of the label as previously reported (*1*). The two-dimensional gels were run as described above. The P_{71} was also synthesized at high rates in tissue slices of lung, brain, thy-

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