much higher levels of chemotactic activity in the supernatants (Table 1 and Fig. 1).

Our investigation may provide an explanation for some negative findings (*6*). Chemotactic activity varied widely among cell lines, with negative activity observed in leukemias or lymphomas and some solid tumors (Table 1). Moreover, a prozone phenomenon was frequently encountered (Table 1 and Fig. 1). Defective in vitro chemotaxis and in vivo inflammatory responses, possibly related to soluble inhibitors, have been reported in tumor-bearing animals and humans (*9, 10*). Our results are not necessarily in conflict with these findings. For instance in mice with advanced M4 and M9 sarcomas the accumulation of macrophages in the peritoneal cavity was reduced after intraperitoneal inoculation of phytohemagglutinin. Systemic defective chemotaxis does not mean that, at the tumor site, neoplastic cells could not be attracting mononuclear phagocytes into the tissue to stimulate directly or indirectly tumor growth (*1, 11*).

Macrophage infiltration in tumors is likely to be a complex process and depend on more than one factor. Local proliferation could contribute to TAM's, and host cells (for example, fibroblasts and macrophages) could also be a source of chemotactic materials. Tumor-derived chemotactic factors may play a role in determining the entry of blood monocytes into neoplastic tissues, a suggestion supported by the statistically significant, although not strict, correlation found between chemotactic activity and TAM content of mouse tumors.

Macrophages from two sarcomas (mFS6 and MN/MCA1) enhanced tumor cell proliferation in vitro, at least at low macrophage to target cell ratios (*11, 12*). The observation of chemoattractants from these tumors supports the proposal that TAM's may stimulate tumor growth in vivo, at least at the primary tumor site (*1, 11–13*).

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Taste-Aversion Conditioning of Crows to Control Predation on Eggs

Abstract. *Free-ranging crows (*Corvus brachyrhynchos*) that ate chicken eggs that were painted green and contained a nonlethal toxin subsequently avoided green eggs at various locations, whether or not they contained toxin. The crows also continued to eat unpainted and nontoxic chicken eggs. Illness-induced aversions among predators in nature may be a powerful determiner of the evolution of Batesian mimicry and, in human hands, serve as a practical tool for wildlife ecologists.*

Some predators avoid prey that are harmless to them but resemble noxious prey. Such mimics of the noxious models represent a phenomenon known as Batesian mimicry (*1*). Some mimics appear to be avoided even when the predators have not been exposed to the noxious model (*2*). The majority of mimics, however, are apparently avoided only when predators have been exposed to the models, often becoming ill after consuming one or more model prey (*3*).

Many animal species develop an aversion to the flavor of a food if it makes them sick (*4*); this process is known as taste-aversion conditioning. In agriculture, it has been used to control predation by olfactory predators when they associate the taste and smell of live prey with those of toxic meat baits that they have eaten. The success of the process depends on hiding the toxin in the bait so that it is the taste of the prey that is avoided rather than that of the toxin (*5*). As long as taste is paired with illness, nongustatory senses may also play a role in the avoidance behavior. Thus, olfactory predators avoid by smell (*6*), raptors and pigeons by vision (*7*), and bats by hearing (*8*).

Taste-aversion conditioning has not been generally included in evolutionary models of Batesian mimicry because the research has focused on nonillness-producing noxious events and visual mimics. Research on taste-aversion conditioning has focused on learning theory and, until recently, on olfactory predators. We report two experiments designed to (i) evaluate the usefulness of the taste-aversion agent UC 27867 (*9*) in producing visual avoidance of colored eggs by free-ranging crows (*Corvus brachyrhynchos*) and (ii) measure the degree to which crows would generalize their conditioning from the toxic baits.

In the first experiment (23 June to 13 July 1981) we established five treatment and five control sites near Fargo, North Dakota (*10*), each with a line of 20 straw nests at 15-m intervals on the ground. All nests were visible from the air. Half the nests at each site contained one chicken egg painted green (*11*); the others had one white, unpainted chicken egg. The color sequence was randomized and eggs were rearranged every 3 days. Only green eggs in treatment sites were injected with UC 27867 (30 mg) (*12*). We replaced eggs every morning, noting the location and activities of crows as well as evidence of predation. Each site appeared to have a separate group of crows (*13*).

For three-way analysis of variance (*14*), averages per site were used because measurements at the different sites were considered to be independent. Only days that followed rearrangement of the color sequence were used in this analysis ($N = 7$ and $N = 8$ days for the two experiments, respectively). The statistical significance level for all comparisons was set at $P < .05$.

A total of 400 eggs were available at each treatment and control site during

the 20 days of experiment 1. Of the 140 eggs at each site included in the analysis (ten green and ten white from the 7 days following rearrangements), the number eaten by crows residing at treatment sites (46.8 eggs per site) was equivalent to those eaten by crows residing at control sites (58.2 eggs per site). Treatment site crows ate fewer green eggs (4.8 eggs per site) than white eggs (42 eggs per site) and fewer green eggs than did control site crows (23.2 eggs per site). Treatment site crows ate white eggs in numbers similar to their control counterparts (35 eggs per site). The basis for food selection appeared to be visual; 87.3 percent of green eggs observed to be passed over at treatment sites were avoided by birds flying at heights of more than 5 m.

Consumption of green eggs at treatment sites indicated successful taste-aversion conditioning (*15*): at all but one site high initial consumption rates dropped to virtually no consumption by about day 9 (Fig. 1). By day 10, the presence of toxin was functionally irrelevant: green eggs could as well have been nontoxic mimics, and survived intact. Consumption of white eggs at treatment sites and consumption of both green and white eggs by crows at control sites (Fig. 2) did not show similar patterns (*16*). The mean numbers of crow sightings at each treatment (8.8) and each control site (6.5) were not significantly different, nor were the mean numbers of crows observed per sighting, which stabilized during this experiment at 3.0 ± 0.36 (± standard error of the mean) and 3.6 ± 0.56, respectively.

In the first experiment, the cost to the crows of avoiding some eggs was low, since others were readily available nearby. In the second experiment, the strength of the taste aversion already established among treatment site crows was tested by denying them safe white eggs at what had become dependable feeding sites. For this experiment (14 July to 3 August 1981), a line, 4 km long, of 15 nests was established at the original five treatment and five control sites. Each site had five central nests, spaced at 15-m intervals, and ten more nests at 400-m intervals, five at each end of the central nests. Treatment site central nests had one toxic green egg and those at control sites had one nontoxic green egg. At each site half the dispersed nests had a single white egg and half had a nontoxic green one. Egg replacement and observations were conducted as described for the first experiment.

A total of 300 eggs were available at each treatment and control site during the 20 days of the experiment. Of the 120 eggs at each site included in the analysis (five central green, five dispersed green, and five dispersed white eggs from the 8 days following rearrangements), the number eaten by treatment site crows (17.4 eggs per site) was significantly less than that eaten by control site crows (37.2 eggs per site). Treatment site crows did not distinguish significantly between the toxic green eggs in the central nests (2.6 total eggs eaten per site) and the nontoxic green eggs in the dispersed nests (4.8 total eggs eaten per site), but they ate significantly more white eggs (10 eggs per site) than green ones. Control site crows ate green eggs at the rate of 15.2 per site in central nests and 12.2 per site in dispersed nests, both significantly more than at treatment sites but not significantly different from each other. Consumption of dispersed white eggs was the same in both treatment and control sites.

Treatment site crows, denied access to white eggs at the central nests, apparently shifted their feeding to wheat fields and avoided the green eggs of both the central and the dispersed nests. We saw significantly fewer crows at central treatment nests (2.2 per site) than at central control nests [9.8 per site; $t(18) = 2.4$] and significantly more crows in wheat fields within 1 km of treatment sites (20.0 per site) than in control sites [1.8 per site; $t(18) = 2.74$].

In the first experiment, crows that ate toxic green eggs subsequently avoided

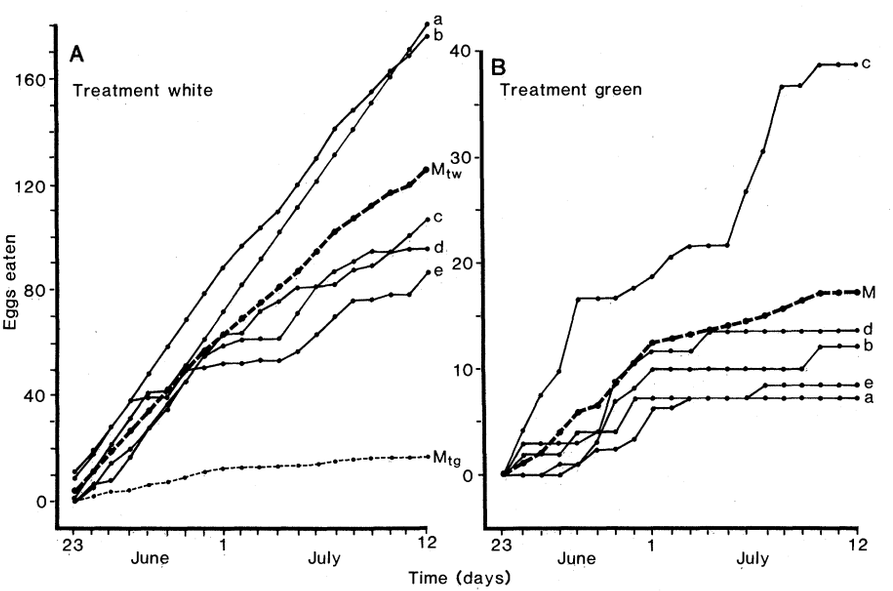

Fig. 1. Cumulative consumption of (A) nontoxic white eggs and (B) toxic green eggs at five treatment sites (a through e). Mean consumption is represented by the heavier dashed lines. The mean for treatment green (M_{tg}) appears on (A) to show scale. Only the mean rate of consumption of treated green eggs shows a significant nonlinear trend (Spearman's rho = −.55, 19 d.f., $P < .05$).

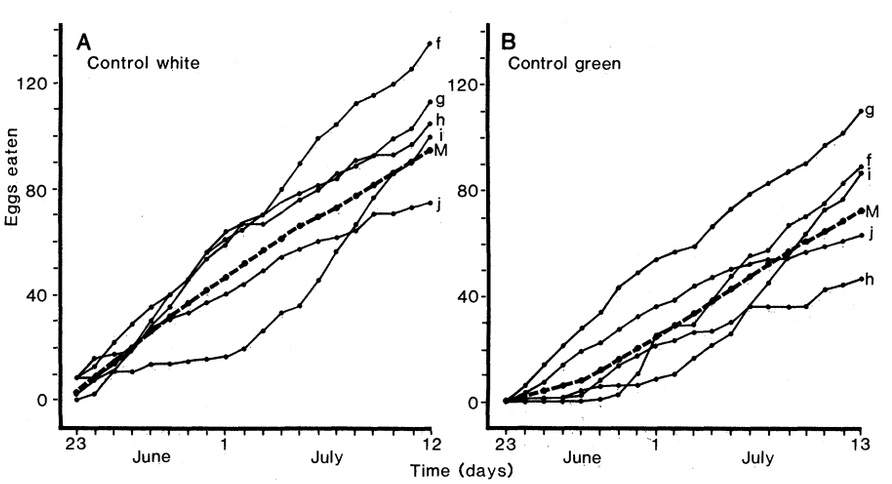

Fig. 2. Cumulative consumption of (A) nontoxic white eggs and (B) nontoxic green eggs at five control sites (f through j). Neither mean rate of consumption (*M*) shows a nonlinear trend.

green eggs but not white eggs; crows at control sites showed no such discrimination. The responses of treatment site crows to the conditions of experiment 2 were markedly different from those of crows at control sites. Treatment site crows avoided nontoxic green eggs as well as toxic ones but continued to eat white eggs. The green eggs were from 400 m to 2 km from the sites where the crows first acquired the aversion to green eggs and frequently were replaced in new random patterns. These birds moved elsewhere when central sites no longer contained alternative food.

Taste-aversion conditioning appears to be an adaptation to the presence of toxic foods in the environment since aversions develop after one or two illnesses and are associated with a taste even with a delay of hours between feeding and illness (*17*). External conditions not readily associated with food by a consumer do not effect the acquisition of a conditioned taste aversion nor its subsequent expression as avoidance (*18*). This conditioning routinely reverses otherwise robust food preferences and has been shown to influence food selection for at least 8 months under field conditions of varying deprivation and, presumably, physiological state (*19*).

Our findings indicate that a Batesian mimic relying on a toxic model capable of producing taste aversions in its predators may have a powerful adaptive advantage. In addition, a properly applied taste-aversion process may provide wildlife ecologists with a nonlethal means of controlling egg predation.

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9. Union Carbide 27867 is 2,3,5- and 3,4,5-trimethylphenyl methyl carbamate, an aversion agent undetectable by taste at effective doses in eggs.
10. We arranged the ten sites in a ring with a mean distance between sites of 7.5 km (ranges 6.5 to 9.0 km) enclosing 375 km^2 of farmland north of Fargo, N.D., and Moorhead, Minn. Each site was placed as near as possible to an isolated roost.
11. Krylon green spray paint (John Deere) was used. Green color appears to have no special significance: comparable results were obtained with brown spotted eggs as toxic models and green eggs as safe alternate food (L. K. Nicolaus, unpublished observations).
12. The UC 27867 was suspended in egg yolk and injected into the eggs, which were then scrambled in their shells with a rapidly rotating bent steel wire.
13. Predation was attributed to crows if we saw eggs eaten by crows, or we saw crows at the site and only evidence of crow predation was recorded anywhere at the site. Only eggs left intact for > 24 hours were listed as survivors [J. D. Rearden, *J. Wildl. Manage.* **15**, 4 (1951)]. Sightings of crows at and between sites were made as we moved along a prescribed route to replace eggs every morning. Additional counts were made in the same manner at midday and at sunset (N = 12 days) and supplemented with time-lapse photography at nest sites (each of the ten sites at least three times). We concluded that each site had a separate, apparently stable group of crows since (i) 80 percent of all crow sightings were made within 1 km of a nest site; (ii) we repeatedly saw crows approaching from the same roosts each morning and different individuals at the next site, often more than 8 km away; (iii) the number of crows sighted at each site was characteristic of the site as were movements back to roosts after feeding; and (iv) crows became increasingly brazen, approaching within a few meters to feed before we finished putting out the eggs.
14. The three-way design for the first experiment used two colors, two treatments, and five locations. That for the second experiment was the same, with the addition of an analysis of two colors, two treatments, and clumping of nests. Consumption at the central nests was also analyzed separately by two-way analysis of variance [B. J. Winer, *Statistical Principles in Experimental Design* (McGraw-Hill, New York, 1962), pp. 258–263].
15. Taste aversions appear to become established rapidly in free-ranging populations of predators when baiting procedures are adequate, and they do not appear to be extinguished quickly. Thus, the rate of bait consumption should be rapid at first and then decline to persistent low levels after aversions are established.
16. Although mammalian predators ate fewer eggs at treatment sites (7.2 per site) than at control sites (14.8 per site), they did not discriminate by color. Since neither the identity nor abundance of the predators was adequately known, no further analysis was attempted.
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Nicotinic Cholinergic Receptor Binding Sites in the Brain: Regulation in vivo

Abstract. *Tritiated acetylcholine was used to measure binding sites with characteristics of nicotinic cholinergic receptors in rat brain. Regulation of the binding sites in vivo was examined by administering two drugs that stimulate nicotinic receptors directly or indirectly. After 10 days of exposure to the cholinesterase inhibitor diisopropyl fluorophosphate, binding of tritiated acetylcholine in the cerebral cortex was decreased. However, after repeated administration of nicotine for 10 days, binding of tritiated acetylcholine in the cortex was increased. Saturation analysis of tritiated acetylcholine binding in the cortices of rats treated with diisopropyl fluorophosphate or nicotine indicated that the number of binding sites decreased and increased, respectively, while the affinity of the sites was unaltered.*

Nicotinic cholinergic receptor binding sites in the rat brain can be measured by using [^{3}H]acetylcholine as a binding ligand (*1*). In the presence of atropine to block muscarinic cholinergic receptors, [^{3}H]acetylcholine binds to a single class of sites that have the characteristics of neuronal membrane recognition sites for acetylcholine in the brain (*1*). These sites have high affinity and selectivity for nicotinic cholinergic agonists. For example, cytisine, (-)-nicotine, acetylcholine, and carbachol have apparent affinities of 1 to 13 nM for the binding site, while most nicotinic cholinergic antagonists have apparent affinities of 20 to 800 μM (*1, 2*).

We examined the in vivo regulation of this [^{3}H]acetylcholine recognition site by measuring the effects of drugs that increase stimulation of nicotinic cholinergic receptors in the brain. To increase stimulation, we repeatedly administered diisopropyl fluorophosphate (DFP), which inhibits cholinesterases (thereby prolonging cholinergic receptor stimulation by acetylcholine released at synapses), or nicotine, which stimulates nicotinic cholinergic receptors directly. Our results indicate that these two treatments produce opposite effects on [^{3}H]acetylcholine binding sites in the rat brain.

Male Sprague-Dawley rats (250 to 300 g) were housed in groups in a light- and temperature-controlled room (lights on from 0700 to 1900 hours; 23°C) and given unlimited food and water. DFP was injected subcutaneously at a dose of 1 mg/kg on day 1, 0.4 mg/kg on day 2, and 0.2 mg/kg on days 4 to 10. Control rats received injections of vehicle (sterile water) on the same schedule. Nicotine tar-