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MUSHROOM CHEMICAL DEFENSE: Food Aversion Learning Induced by Hallucinogenic Toxin, Muscimol

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Abstract—Wild animals eat fungi, yet mushroom poisonings in nature are unknown. The opossum *Didelphis virginiana* readily consumed the toxic mushroom *Amanita muscaria*, became ill, and then developed an aversion to the fungus. Both the illness and the aversion were due, in part at least, to the toxin muscimol. This appears to be the first demonstration of a mushroom chemical defense against fungivores and the first reported role in nature for an hallucinogen.

Key Words—Mushroom, antifeedant, food aversion, muscimol, hallucinogen, opossum, *Didelphis virginiana*, *Amanita muscaria*, plant-herbivore coevolution.

INTRODUCTION

Approximately 350 cases of mushroom poisoning occur annually in the United States, and it is estimated that since 1900, 1500 people worldwide have died from eating toxic mushrooms (Lincoff and Mitchel, 1977). Surprisingly, nothing is known about mushroom poisonings of animals in the wild. Red squirrels (*Tamiasciurus hudsonicus*) eat some species of toxic fungi with impunity (Metcalf, 1925; Klugh, 1927; Hatt, 1929; Fogel and Trappe, 1978). Numerous other mammals eat fungi (Fogel and Trappe, 1978) and undoubtedly encounter potentially toxic mushrooms. Do these animals discriminate between toxic and edible fungi and learn to avoid those species that are poisonous or unpalatable?

In this report I show that the opossum *Didelphis virginiana,* a nocturnal marsupial that eats fungi in the wild (Fogel and Trappe, 1978), eats the toxic

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mushroom *Amanita muscaria* and becomes ill. The animal then develops an aversion to the fungus due, in part at least, to the mushroom's content of muscimol. A γ-aminobutyric acid agonist in the central nervous system of several vertebrates (Chilton, 1978), muscimol is an hallucinogen to humans (Lincoff and Mitchel, 1977; Chilton, 1978). This work appears to be the first demonstration of a chemical defense against fungivores, the first reported role in nature for muscimol, and the first demonstration of a role in nature for a compound hallucinogenic to humans.

METHODS AND MATERIALS

Ten opossums that readily accepted the commercial mushroom *Agaricus bisporus* were used in these experiments. There were four male and six female animals (ages 9 months to 2 years) either born in captivity or obtained as young from the pouch of their mother. They were maintained on a diet of dog food and water. The animals were tested once daily just prior to their regular feeding time.

Experiment 1: Dose-Response Tests with Muscimol. Tests were performed with the toxin muscimol to establish whether this compound could account for the toxicity of *Amanita muscaria.* This mushroom contains two related isoxazoles, muscimol and ibotenic acid. In man both induced symptoms of hallucination, delirium, muscular spasm, stomach upset, and vomiting. Ibotenic acid, however, is labile, readily decarboxylating to form muscimol, and it has been suggested that a significant portion of the activity of administered ibotenic acid may actually be due to muscimol formed secondarily by the decarboxylation reaction (Chilton, 1978). Ibotenic acid was therefore omitted from the tests.

Nine opossums were fed muscimol added to their standard diet of dog food pellets. Six dosages varying between 0.6 and 3.0 mg/kg body weight (1–7 mg/animal) were administered by topically applying the muscimol to a moistened dog food pellet so that the toxin would adhere. There were a total of 34 trials. A dosage of no more than 7 mg was employed in a test for fear of harming the animal. This dosage was chosen as the upper limit as it represents the amount of muscimol potentially available in a single *A. muscaria* mushroom (Chilton, 1978). The oral LD_{50} of muscimol for rats is 45 mg/kg body weight, far lower than that employed in these tests.

Experiment 2: Palatability of Different Mushroom Species. A measure of the relative palatability of various wild mushroom species was obtained in a feeding protocol previously described (Camazine et al., 1983). The assay was performed as follows. Individual opossums, tested in daily feeding sessons, were offered fresh pieces (approximately $2 \times 2 \times 2$ cm) of a given test species of mushroom and comparable pieces of *Agaricus bisporus* as the edible

control. A total of 18 species of mushroom common to the northeastern United States were collected for testing. Each species was presented in a single feeding session to several (6–10) opossums. Mushroom pieces were presented one at a time in three-item sequences consisting of two pieces of *Agaricus* and one randomly interspersed piece of test mushroom. Each item was left with the animal for a maximum of 30 sec. The total number of items (test plus control) presented to each opossum per session ranged from 15 to 27. Results were scored as fate of individual mushroom pieces. If an item was totally consumed, it was scored as eaten; if it was partially eaten, rejected on close inspection (tasted, sniffed, and/or manipulated), or ignored from a distance, it was scored as rejected. If an item at the end of a session was rejected, it was not tallied since the failure to eat might have been due to satiation of the animal. For each mushroom, the scores from all the opossums were combined to calculate a palatability rating defined as the percent of the total number of test items eaten. On a given day each opossum was presented with a single species of test mushroom. There were a total of 35 tests over a period of 5 months.

Experiment 3: Tests with Calvatia gigantea *and Muscimol.* Two additional experiments were performed using muscimol, both utilizing a bioassay similar to the mushroom assay described above except that test items consisted of a mushroom to which a topical dosage of the toxin was added. The mushroom was moistened with water so that the crystalline toxin would adhere to the surface. The control mushrooms to which no toxin was added were similarly moistened.

In the first of these experiments, I attempted to create a food aversion by adding toxic doses of muscimol to the mushroom, *Calvatia gigantea*. In experiment 2, three months earlier, nine opossums had been tested with *Calvatia gigantea* to determine whether it was palatable. Experiment 3 consisted of six trials spaced over 20 days using eight of the same nine opossums. On day 1, seven pieces of the *Calvatia gigantea* each poisoned with 1 mg of muscimol and 14 pieces of the control (*Agaricus*) were presented to the same opossums using the identical protocol as in the previous mushroom tests. The same test was repeated on day 2 except that only the first four of the seven pieces were poisoned as this amount was adequate to make the animals ill. In the remaining trials (days 3, 6, 13, and 20), no muscimol was added to the *Calvatia*.

Experiment 4: Tests with Panellus serotinus *and Muscimol.* The second experiment with muscimol assessed the significance of food novelty. In this case muscimol was applied to the familiar *Agaricus* mushroom on days 1 and 2, rather than to the test mushroom, and presented to the same eight opossums in conjunction with the nonpoisonous mushroom, *Panellus serotinus*, that the animals had never previously eaten. The protocol was identical to that in the *Calvatia*-muscimol experiment.

RESULTS AND DISCUSSION

Experiment 1: Dose-Response Tests with Muscimol. Dose-response tests with muscimol at dosages of 1–3 mg/kg body weight applied to the dog food pellet resulted in vomiting in 79% of the trials. Smaller dosages did not appear to induce any illness.

Experiment 2: Palatability of Different Mushroom Species. Of the 18 mushrooms tested for their palatability, only *Amanita muscaria* and *Calvatia gigantea* were completely eaten by all the opossums. The other fungi ranged in palatability from 2 to 96% (Camazine et al., 1983). At first this result was surprising due to the known toxicity of muscimol. However, within half an hour after ingestion of the first piece of *Amanita muscaria*, six of the nine opossums vomited. The initial acceptance of the *Amanita* mushrooms and the delayed illness induced by the toxin suggested the possibility that the opossums might learn to avoid the poisonous fungus in subsequent tests. The identical test was repeated one day later. The results (Figure 1) show that the acceptability of the *Amanita* decreased markedly from 100% to 17%. Four of the nine opossums refused the *Amanita* completely. The palatability of the

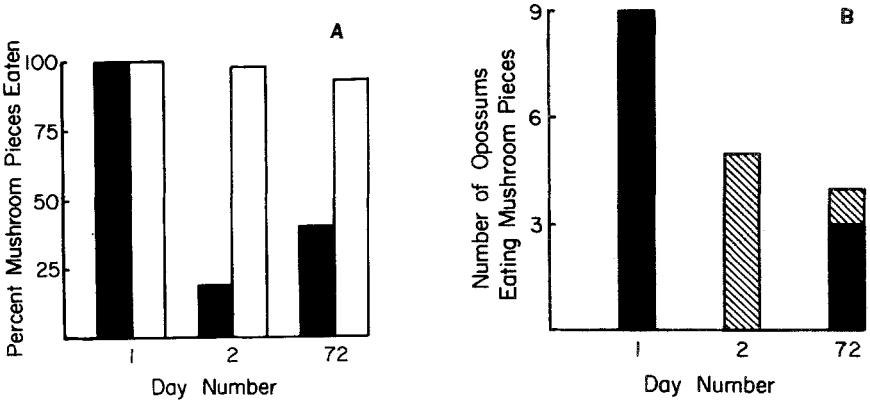

FIG. 1. (A) Palatability of the test mushroom, *Amanita muscaria* (solid bars), and the control mushroom, *Agaricus bisporus* (open bars) during three trials spanning 72 days. Data are expressed as percent total mushroom pieces eaten by all animals. Nine opossums were tested each day and each was fed nine test and 18 control pieces of mushroom on days 1 and 2. On day 72, five test and 10 control pieces were offered. There is significant difference (paired t test statistics) between the number of test pieces eaten on day 1 and day 2 ($P < 0.01$). (B) The same data for the palatability of the test mushroom, *A. muscaria*, expressed as the response of the individual opossums. Solid bars show the number of animals eating all the pieces of the test mushroom, and the cross-hatched bars show the number of animals eating some, but not all, of the pieces presented in the feeding session.

Agaricus control was essentially unchanged (98% consumed). The opossums had evidently learned to avoid the toxic *Amanita* mushroom yet continued to eat the nontoxic control mushrooms.

The animals were retested 70 days later to determine whether the food aversion still prevailed (Figure 1). Only 40% of the mushroom pieces were eaten. Five of the nine opossums again refused the *Amanita* mushrooms entirely (Figure 1B). Other animals also retain lasting food aversions, which may span weeks in the case of the slug *Limax maximus* or even decades for man (Gelperin, 1975; Garb and Stunkard, 1974).

Experiment 3: Tests with Calvatia gigantea *and Muscimol.* In experiment 2, three months earlier, each animal consumed all the pieces of *Calvatia gigantea*, confirming that the mushroom was palatable. On day 1 of experiment 3, all the mushrooms pieces were eaten, and within 75 min of eating the first mushroom piece, seven of the eight opossums vomited. On day 2, in which the identical protocol was repeated except that only the first four of the seven pieces of *Calvatia* were poisoned, 75% of the *Calvatia* pieces were consumed including all the poisoned pieces (Figure 2A). Four of the six animals that ate the *Calvatia* vomited with this dosage. The palatablity of the control mushroom was essentially unchanged (98% consumed). Only 16% of the *Calvatia* pieces were eaten on day 3; five of the animals refused any test mushroom and the remaining three ate some but not all of the pieces (Figure 2B). The palatability of the control mushroom was 75%. In further testing, there was a gradual extinction of the learned aversion; the palatability of the *Calvatia* increased nearly to baseline levels by day 20. This experiment demonstrates that, after two trials, a food aversion to a mushroom can be established using the toxin, muscimol.

In the *Amanita muscaria* and the *Calvatia*–muscimol tests, the mushrooms were initially palatable but were rejected after they had caused an illness in the animals. The opossums may not have associated the illness with the muscimol itself (which may lack a distinctive taste or odor) but instead may have learned to avoid the carrier mushroom which is remembered as the novel food item consumed just before the onset of the illness. The significance of food novelty was confirmed in experiment 4 in which muscimol was applied to the familiar *Agaricus* mushroom, rather than to the test mushroom, and presented to the opossums in conjunction with the novel mushroom, *Panellus serotinus*.

Experiment 4: Tests with Panellus serotinus *and Muscimol.* The results of this experiment (Figure 3) and the *Calvatia*–muscimol experiment are similar. This result would be expected if food novelty is an important factor in the evaluation of potential foods. Both the *Panellus* and the *Agaricus* were initially palatable (*Panellus*, 88%; *Agaricus*, 96%), but after two sessions in which the toxin was applied to the *Agaricus*, the novel *Panellus* mushroom was almost totally rejected (palatability = 2%), while the palatability of the

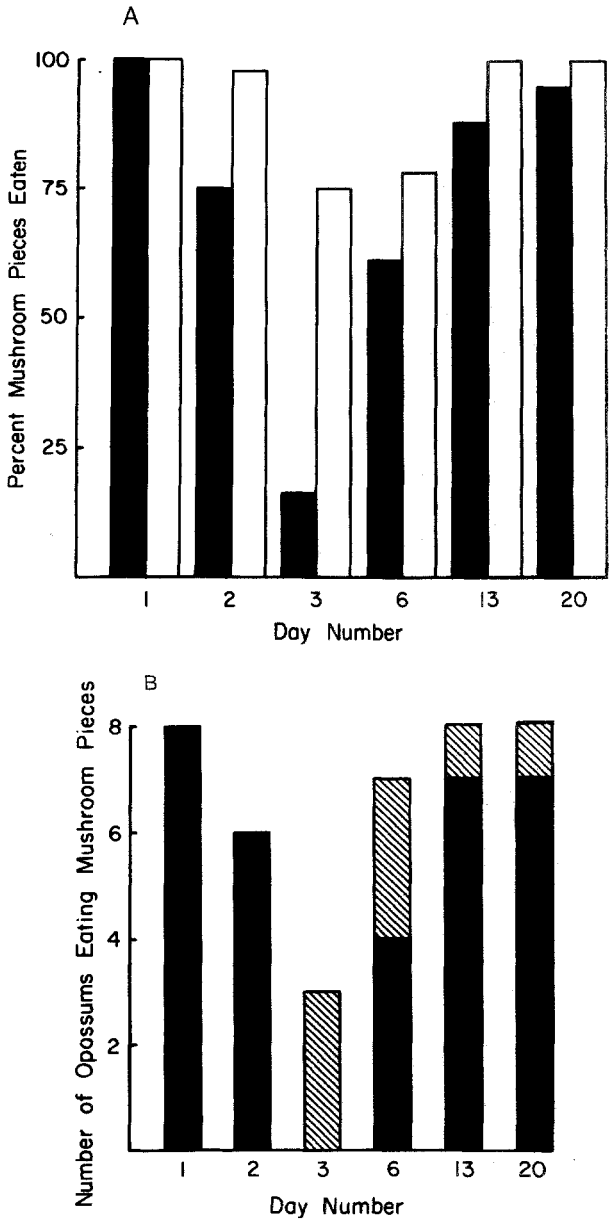

FIG. 2. (A) The palatability of the test mushroom, *Calvatia gigantea* (solid bars), and the control mushroom, *Agaricus bisporus* (open bars) over a period of 20 days. The test mushroom was poisoned on days 1 and 2 by topically applying muscimol. Data are expressed as percent total mushroom pieces eaten by all animals. During each trial eight opossums were offered seven pieces of test mushroom and 14 pieces of control mushroom. There is a significant difference between the number of test pieces eaten on day 1 and day 3 ($P < 0.01$). (B) The same data of the palatability of *C. gigantea* expressed as the response of the individual opossums, drawn as in Figure 1 (B).

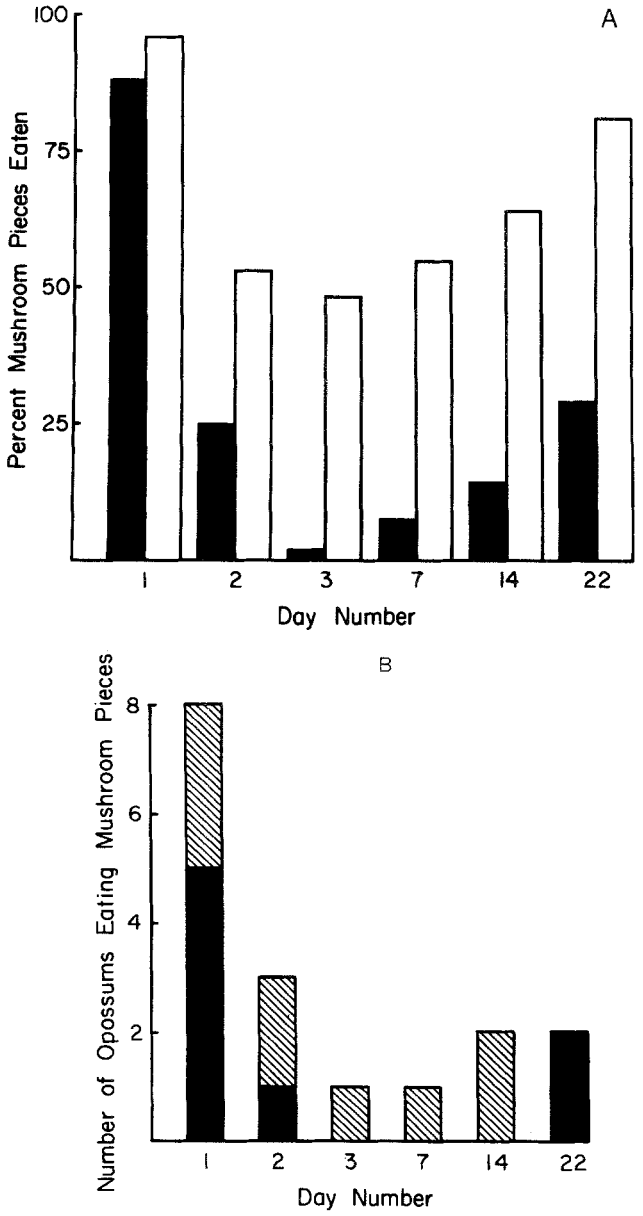

FIG. 3. (A) The palatability of the novel test mushroom, *Panellus serotinus* (solid bars) and the familiar control mushroom, *Agaricus bisporus* (open bars) over a period of 22 days. The *Agaricus* mushroom was poisoned on days 1 and 2 by topically applying muscimol. Data are expressed as percent total mushroom pieces eaten by all animals. The animals used in this experiment and the numbers of mushroom pieces presented in each session are the same as in the *Calvatia*–muscimol experiment except that one animal was not available for retesting on days 14 and 22. There is a significant difference between the number of test pieces eaten on day 1 and day 3 ($P < 0.01$). (B) The same data for the palatability of *P. serotinus* expressed as the response of the individual opossums, drawn as in Figure 1 (B).

familiar *Agaricus* mushroom decreased to 48%. This decrease in the acceptibility of the *Agaricus* control was somewhat unexpected but may be explained by a generalized association of the muscimol-induced illness with all foods presented to the opossums by the experimenters. The animals may begin to associate the entire experimental situation with their previous illnesses and become reluctant to eat under these conditions. Such behavior has been demonstrated in pigeons (Garcia and Hankins, 1977).

Learned aversions to a novel food item associated with a postingestional illness have been demonstrated in a variety of vertebrates including rats, dogs, guinea pigs, turtles, fish, and birds (Domjan, 1977). Polyphagous animals in particular tend to sample novel foods judiciously as if to assess the food for delayed ill effects before eating larger amounts that could be lethal. Deer cautiously taste new plants in small amounts (Nichol, 1938), and wild rats reluctantly sample novel substances (Wallace, 1976).

What sensory cues enable opossums to distinguish among mushroom species? Opossums are nocturnal and possess a keen sense of smell (Moulton, 1973). The distinctive odor of a particular mushroom may be the major sensory stimulus that the animal learns to associate with the toxic effect of a fungus and may serve as an olfactory warning signal (Edmunds, 1974; Eisner and Grant, 1981). Except in a few instances, pieces of mushroom are rejected after being closely approached and sniffed. Occasionally the mushroom is tasted, either being licked or placed briefly in the mouth, chewed, and then spit out. Working with rats, Garcia and his collaborators have shown that when taste and odor are combined to form a conditioning stimulus for a delayed poison, the odor alone may exert a depressive effect upon consumption even after the aversion to taste is completely extinguished (Garcia and Rusiniak, 1980). The adaptive value of this form of learning is apparent; on subsequent encounters, an animal may reject a toxic food on the basis of odor alone and thus avoid repeated, potentially lethal, taste "trials."

These experiments demonstrate that the palatable mushroom toxin, muscimol, causes a delayed illness that effectively conditions a learned food aversion in a vertebrate fungivore. This aversion is striking in that it occurs after a few trials, may be retained for many days, and can be established when the delay between food ingestion and illness is as long as an hour or more. In these respects, food-aversion learning is well suited to meet the demands placed upon a polyphagous animal, which may continually encounter in its environment a variety of novel foods with toxic effects. As an adaptation to poisonous compounds present in a variety of potential foods, the oppossum is able to learn and to retain for long periods the knowledge that a particular fungus is toxic. Some mushrooms, in turn, may have adapted to fungivory by evolving noxious compounds along with distinctive cues (such as tastes, odors, or colors) that facilitate learned food aversions.

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