sent a particularly interesting example of translational control in view of the apparent homogeneity of δ-crystallin messenger RNA (*9*). Whatever the precise mechanism, our data show that embryonic chick lenses cultured under the described conditions without their vitreous body develop cortical cataracts and undergo a specific, reversible change in δ-crystallin synthesis. Although we do not know whether there is a causal relation between this particular transient change in δ-crystallin synthesis and cortical cataracts in the cultured lens, our study demonstrates that a reversible biochemical event can occur during the development of a cataract and, therefore, it may be insufficient to limit etiological studies of cataracts to lenses that already have pronounced opacities.

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Stink of Stinkpot Turtle Identified: ω-Phenylalkanoic Acids

Abstract. *The exocrine secretion of the "stinkpot turtle,"* Sternotherus odoratus, *discharged by the animals in response to disturbance, contains four ω-phenylalkanoic acids (phenylacetic, 3-phenylpropionic, 5-phenylpentanoic, and 7-phenylheptanoic). The last two of these are new natural products. The first two are powerfully malodorous and responsible for the stench of the fluid. Lesser components, including several aliphatic acids, are also present. Only a few milligrams of secretion are discharged by a turtle at any one time. Although bioassays with fish suggest that the secretion has the potential to serve as a feeding deterrent to predators, it is argued that* Sternotherus *does not ordinarily discharge enough fluid to effect this action and may employ its secretion only as an aposematic signal that warns predators of its more generalized undesirability.*

The turtles of the family Kinosternidae include a number of species known as musk turtles, which eject an odorous secretion when disturbed (*1*). No chemical work had hitherto been done on these fluids, which in some species are strongly scented. We here report the isolation and characterization of the odorous constituents of the secretion of *Sternotherus odoratus*, a species from the eastern United States, inelegantly but appropriately called the "stinkpot." *Sternotherus* has four glands, morphologically identical, opening lateroventrally near the edge of the carapace, in front and behind the plastral bridges (Fig. 1A). They are small structures, comprising only about 0.1 percent of body weight (*2*), and capable of discharging no more than a small droplet at any one time (Fig. 1, B and C). The amount suffices, however, to impart a potent stench to a manipulated turtle. The glands are present in both sexes and are functional in juveniles as well as adults.

Two medium-sized *Sternotherus*, from Tampa, Florida, were "milked" of secretion by tapping their shells and prodding their bodies, and taking up the exuded fluid in glass capillary tubing. Analyses of the pooled milkings (in ether or amyl acetate) by gas chromatography–mass spectrometry (GCMS) revealed no highly volatile components (*3*), and exposure of the secretion to dampened lead acetate paper indicated that hydrogen sulfide was absent. Treatment of the secretion with ethereal diazomethane yielded a series of methyl esters; these were identified on the basis of GCMS data (*4*) as methyl esters of phenylacetic, 3-phenylpropionic, and 5-phenylpentanoic acids. The methyl ester of a fourth acid had a mass spectrum, *m/e* 220 (3), 188 (11), 129 (5), 117 (5), 105 (11), 104 (11), 97 (12), 92 (30), 91 (100), 87 (28), 84 (12), 74 (33), 69 (11), 65 (14), 59 (8), 55 (9), suggestive of methyl 7-phenylheptanoate. An authentic sample of this ester was prepared by treatment of 7-phenylheptanoic acid (*5*) with diazomethane, and was indistinguishable (by GCMS comparison) from the natural product. In addition, small amounts of 3-methylbutanoic, hexanoic, hexadecanoic, and heptadecanoic acids were also identified by the GCMS data obtained from their methyl esters (*4*). Upward of ten additional minor components were present, in amounts too small to permit identification.

Five additional turtles were milked, including a third unsexed medium-sized specimen from Tampa, and four sexed adults from Philadelphia, Pennsylvania, (three males and one female). The three males were milked twice, with 3 weeks between milkings. As is seen from Fig. 2, which incorporates the data from all seven turtles, the secretion consistently contained the four ω-phenylalkanoic acids, in relative percentages that varied somewhat between individuals and milkings (*6*). The aliphatic acids (not tabulated) were present at concentrations not exceeding 1 percent relative to the ω-phenylalkanoic acids. As is evident from the four cases where both secretory output was weighed and the total ω-phenylalkanoic acid content of the secretion was calculated (*7*), the turtles discharge the acids in microgram quantities, with only milligrams of fluid. Our own olfactory sensitivity to the acids is evidently high. Subjective sniff testing of the secretion and its components showed that the stench of the secretion is attributable

chiefly to phenylacetic and 3-phenylpropionic acid.

Nothing can be said with certainty about the function of the *Sternotherus* secretion. A defensive role is most generally assumed, since the turtles appear to eject the fluid only in response to provocation, but supportive evidence is lacking. In our view, it seems unlikely that the small quantity of secretion discharged by *Sternotherus* could in itself suffice for direct chemical deterrence of a predator. It could, however, serve as a readily detectable chemical warning that alerts an attacking predator to other undesirable qualities of the turtle. *Sternotherus* flesh is distasteful to humans (*1*), and it might be similarly distasteful to some predators. Moreover, as anyone knows who has handled them, *Sternotherus* are unusually pugnacious, being able to bite and scratch vigorously (*1*). And, of course, they can make themselves relatively inaccessible by drawing back into their shells. Given the possibility that predators could, either through learning or because of innate predisposition, make the association between odorous warning and undesirability of prey, the secretion might have evolved primarily as an aposematic signal, comparable in every way to a conventional visual aposematic signal (*8*), but chemical in character. Such chemical aposematism could operate in water as on land, the two habitats frequented by *Sternotherus*, and unlike visual aposematism could operate also at night, when the turtles are most active.

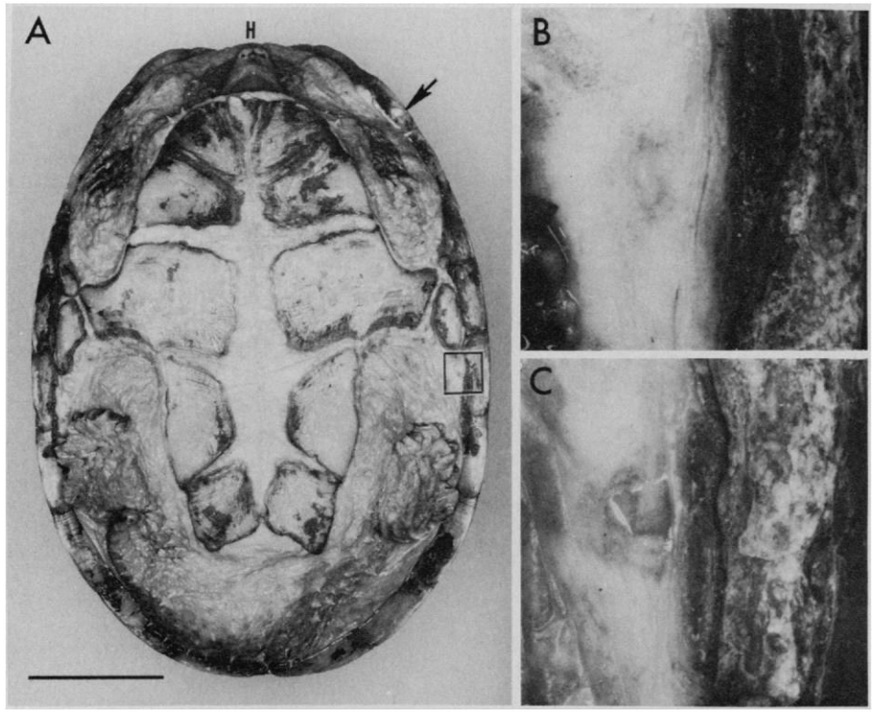

Fig. 1. (A) Ventral view of an alarmed male *Sternotherus*, with head (*H*) and legs withdrawn, showing the location (arrow, square) of the two left "stink glands"; (B) enlarged view of a gland [same region as denoted by square in (A)]; (C) comparable to preceding, showing discharged secretion. Reference bar in (A) is 2 cm; the three pictures are from three different turtles.

	turtle No.	turtle weight (g)	total secretion (mg)	ω-phenylalkanoic acids: μg in secretion	relative %*: I	II	III	IV
Tampa	1+2				21	48	25	6
	3	35			2	18	39	41
Philadelphia	4♂	134			62	18	13	7
			1.0	0.65	43	32	22	3
	5♂	171			70	2	7	21
			3.4	1.53	60	10	22	8
	6♂	196			38	11	29	22
			5.8	8.92	49	22	16	13
	7♀	148	5.0	6.12	65	28	6	1

* I = phenylacetic acid; II = 3-phenylpropionic acid; III = 5-phenylpentanoic acid; IV = 7-phenylheptanoic acid

▨ = no data

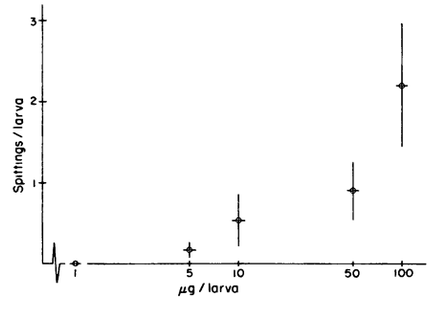

Fig. 2 (left). Secretory output, ω-phenylalkanoic acid content of secretion, and relative percentage of ω-phenylalkanoic acids, in secretion of the seven *Sternotherus* studied. Fig. 3 (right). Feeding deterrence of ω-phenylalkanoic acids to fish (*Xiphophorus helleri*). Deterrence is expressed as the frequency with which insect larvae (*Tribolium confusum*) coated with a mixture of the acids are spat out by the fish and is plotted as a function of the dosage of mixture applied to the larvae. Transverse bars give means; vertical bars give one standard error on each side of means; $N = 30$.

Evidence was obtained suggesting that ω-phenylalkanoic acids are feeding deterrents to fish and potentially, at least, employable as direct defensive agents. Beetle larvae (*9*) that were topically coated with a given quantity of a mixture of ω-phenylalkanoic acids (*10*) were presented to swordtail fish (*Xiphophorus helleri*) that were confined in groups of 12 in individual aquaria. Unlike untreated larvae, which were invariably eaten outright, treated larvae were usually first spat out by one fish or a sequence of fish, before finally being taken by an individual. The frequency of rejection, scored as the number of individual spittings per larva, provided a measure of the distastefulness of an item. In a given feeding session, ten treated and ten untreated larvae were offered in randomized alternation to the fish in one aquarium. Five dosages of the mixture were tested (1, 5, 10, 50, and 100 μg), each in three feeding sessions, with each session being conducted in a different aquarium. As is indicated by the results (Fig. 3), the two dosages (5 and 10 μg) that spanned the upper limit of secretory output of the turtles were only moderately deterrent. Against real piscine predators of *Sternotherus*, which would inevitably be considerably larger than the 5- to 6-cm-long *Xiphophorus* used in these tests, the secretion could be even less deterrent. Interestingly, juvenile *Sternotherus* are said to be taken by largemouth bass (*1*). Other predators are also reported to take the turtles on occasion, including bullfrogs, cottonmouths, skunks, raccoons, herons, and crows (*1*), but predation data are generally scant for this turtle. Nothing can be said about the possible effect of the secretion on ectoparasites such as leeches.

ω-Phenylalkanoic acids are not widely distributed in nature. Neither 5-phenylpentanoic acid nor 7-phenylheptanoic acid has previously been reported as a natural product. Phenylacetic acid has been identified as a scent-marking pheromone in a male gerbil (*11*), and both phenylacetic and 3-phenylpropionic acid have been reported, without functional assignment, from sheep urine (*12*) and some microorganisms and plants (*13*). ω-

Phenylalkanoic acids also have antimicrobial properties (*14*), suggesting yet another, albeit remote, functional possibility for the turtle secretion.

Integumental glands occur in all four orders of reptiles, but their products have so far been identified in only few species, including a crocodilian, which produces citronellol (*15*), a leptotyphlopid snake, which discharges a mixture of glycoprotein and long-chain aliphatic acids (*16*), and a gopher tortoise, which also secretes aliphatic acids. In the tortoise, the glands are well developed in the male only, and the secretion has a proven pheromonal role (*17*). For biologists and chemists working in concert, reptilian glands could provide a fruitful subject of research.

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Herbivore-Plant Interactions: Mixed-Function Oxidases and Secondary Plant Substances

Abstract. *The mixed-function oxidases of a polyphagous insect larva (the southern armyworm,* Spodoptera eridania*) were found to be induced by a diversity of secondary plant substances. The induction proceeds rapidly and in response to a small quantity of secondary substance. Following induction, the larva is less susceptible to dietary poisoning. It is argued that mixed-function oxidases play a major role in protecting herbivores against chemical stress from secondary plant substances.*

The so-called mixed-function oxidases (MFO) are a group of enzymes widely distributed in organisms and are best known for their well-established role in the primary degradation of drugs, pesticides, and other synthetic compounds. These enzymes are attached to the endoplasmic reticulum of cells in association with an electron transport pathway that delivers reducing equivalents from NADPH (reduced nicotinamide adenine dinucleotide phosphate) to the terminal oxidase, cytochrome P-450. Here, as a consequence of binding and subsequent reaction with activated oxygen, the compounds undergo any of several types of oxidative transformations (*1*).

Of the large number of foreign compounds to which organisms are exposed, the lipophilic ones are frequently more hazardous than others since they are often difficult to excrete and tend to accumulate in body tissues. The primary general function of MFO enzymes is to convert such lipophilic compounds into more polar hydrophilic metabolites that are more readily excreted (*2*). The fact that these enzymes metabolize the many lipophilic synthetic organic chemicals represented by modern drugs and insecticides should be viewed as a consequence of this basic capacity. The decrease in toxicity (detoxification) usually associated with the MFO system is secondary to the primary effect of increasing hydrophilicity.

The MFO system is ideally suited for its role as a general clearinghouse for lipophilic compounds. It is nonspecific in accepting a large variety of compounds as substrates, and effects the lipophile-hydrophile conversion by means of numerous reactions, including aromatic and aliphatic hydroxylations and epoxidations, *N*- and *O*-dealkylations, and nitrogen and thioether oxidations (*1*). In addition, its ability to be induced by many chemicals (*3*) provides the system with the proper flexibility for responding to conditions of increasing environmental chemical stress.

An unsettled question concerns the evolutionary "what for?" of MFO enzymes. Given their proven defensive action against man-made chemicals, and the fact that they are involved in the metabolic degradation of many naturally occurring compounds, including toxicants such as nicotine, rotenone, and the pyrethrins (*4*), it was proposed that the MFO enzymes play a major role in the feeding strategies of herbivores (*5*). Demonstration of a correlation between polyphagy and high MFO activity led to the conclusion (*6*) that the evolution (or at least evolutionary refinement) of the MFO system in herbivorous animals might have been forced by exposure to the so-called secondary substances of plants—compounds such as phenolics, quinones, terpenoids, and alkaloids, which are widely distributed in plants and frequently repellent or toxic to animals, and hence in themselves defensive (*7*). The critical questions are (i) whether the MFO enzymes of an animal are induced by secondary plant substances in its food, and (ii) whether the induction proceeds with enough speed and to sufficient levels to provide the animal with increased protection against these potentially offensive dietary factors. We now provide evidence that, for a broadly polyphagous insect, the larva of the southern armyworm moth *Spodoptera eridania*, the answers to these questions are indeed in the affirmative.

Our experiments were done with sixth (last) instar larvae that had been raised through the fifth instar on kidney bean plants (*Phaseolus vulgaris*) and shifted after the last molt to a semidefined artificial diet to which various known secondary plant substances were added. Control larvae were fed the artificial diet without the compounds. All larvae used in any given test were of closely matched age, having undergone the previous molt within a 3- to 4-hour period. MFO activity was routinely assayed by *p*-chloro *N*-