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9. Quantitative differences such as these are inconclusive since they suggest only that certain structures are less frequent in mother's gestures than in the child's. However, in order to argue that the child induces consistent structure from the infrequent instances of structure found in his mother's gestures, one must allow that the child is coming to the learning situation with a bias toward making those inductions.
10. Although the camera might have inhibited the mother's gesture production overall, it is unlikely that the camera affected the way in which the mother structured or failed to structure her gestures. The "camera-shy" hypothesis is further weakened by the fact that, for five mothers, the rate of single-sign production was higher for mother than for her child (although each mother's rate of production of the more complex sign sentence form was half that of her child).
11. It is possible that individuals other than parents (such as siblings or the experimenters themselves) influenced the development of the gesture system, but three of the ten deaf children had no siblings, suggesting that interaction with a sibling was not necessary to develop a structured gesture system. Experimenters made every effort not to gesture to the deaf children, and few of the children's gestures were imitations of the experimenter's gestures (2 percent, 1 of 58, overall); the experimenter produced so few gestures on videotape that analysis for structural properties was unnecessary.
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13. We thank L. Gleitman, J. Huttenlocher, M. McClintock, W. Meadow, E. Newport, M. Shatz, M. Silverstein, and T. Trabasso for comments and R. Church, E. Eichen, M. Morford, and D. Unora for videotape coding. Supported by NSF grant BNS 77-05990 and by grants from the Spencer Foundation.

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Metabolic Detoxification: Mechanism of Insect Resistance to Plant Psoralens

Abstract. *Larvae of the black swallowtail butterfly,* Papilio polyxenes *Stoll, forage successfully on plants that contain high levels of photosensitizing psoralens. These insects rapidly detoxify psoralens, particularly in the midgut tissue prior to absorption, with the result that appreciable levels of unmetabolized phototoxin do not enter the body circulation where deleterious light-induced interactions with dermal or subdermal tissues would occur.*

Psoralens (linear furocoumarins) are photoactive compounds that readily alkylate DNA when activated by longwave ultraviolet light (*1*). The biological activities of psoralens include uses in human medicine (*1, 2*) and they pose significant toxicological risks to man and other organisms (*3, 4*). Psoralens occur naturally in plant species, where they act as generally effective deterrents against feeding by insects and other herbivores (*4, 5*). Certain insects, however, particularly the larvae of some butterflies of the family Papilionidae, feed successfully and preferentially on plants that contain psoralens (*6*). The mechanism of insect resistance to the phototoxic effects of these chemicals has heretofore not been considered and is the subject of our report. We have shown that larvae of the black swallowtail butterfly, *Papilio polyxenes* Stoll (Lepidoptera: Papilionidae), rapidly degrade 8-methoxypsoralen (xanthotoxin) to nonphotosensitizing metabolites in the midgut tissue prior to absorption; thus, appreciable levels of the intact phototoxin are not available for light-induced interactions with body tissues. In contrast, identically exposed larvae of a psoralen-susceptible insect, the fall armyworm, *Spodoptera frugiperda* J. E. Smith (Lepidoptera: Noctuidae), metabolize xanthotoxin at a much slower rate, with the result that levels of the absorbed photosensitizer in *S. frugiperda* are more than 50 times greater than those seen in *P. polyxenes*.

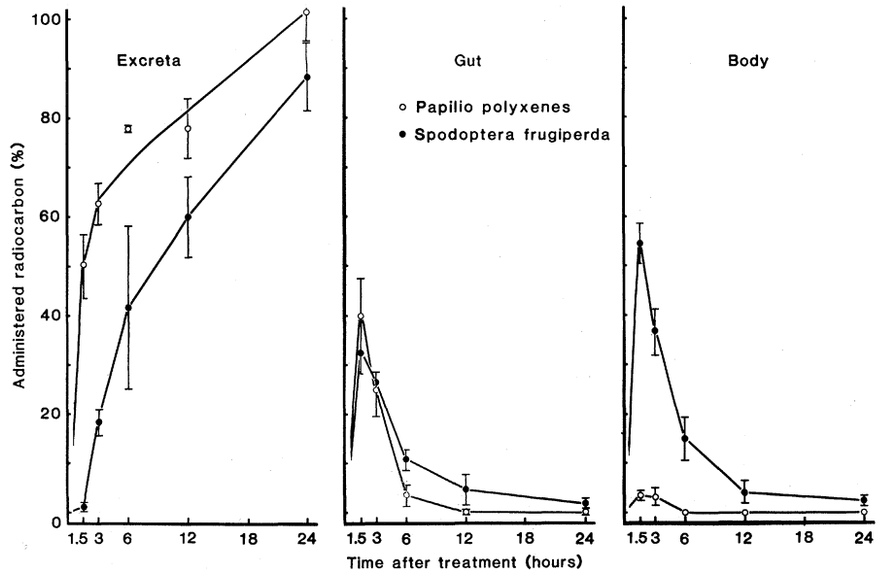

Fig. 1. Patterns of ^{14}C excretion and distribution in last instar larvae of *Papilio polyxenes* and *Spodoptera frugiperda* after oral treatment with [^{14}C]xanthotoxin at 5 μg/g. Data points are means (N = 3 or more) with standard deviations indicated by the bars.

While collecting plants for studies of the toxicology and psoralen chemistry of the livestock photosensitizing weed *Thamnosma texana* (Gray) (Rutaceae) (*7*), we observed larvae of *P. polyxenes* feeding on populations of *T. texana* in direct sunlight in Medina County, Texas. The larvae were collected and reared to pupae in the laboratory on harvested, moistened *T. texana*. Emerging adults were fed a mixture of honey and water, and 2- to 3-day-old females were subsequently mated with 1- to 2-day-old males. Sprigs of fresh parsley from a local supermarket were provided for oviposition, and the hatched larvae were continuously fed a diet of fresh parsley. Newly hatched larvae of a laboratory colony of *S. frugiperda*, a highly polyphagous insect that feeds on many plant hosts other than psoralen-rich species (*8*), were transferred to and reared on fresh parsley.

A ^{14}C-labeled preparation of xanthotoxin, a common plant psoralen (*9*), was applied in acetone solution to small twigs of parsley, the solvent was allowed to evaporate, and the treated twigs were fed to last instar larvae of either insect species that had been starved for 2 hours (*10*). The dosages were equivalent to 5 μg/g, tailored to the individual prestarvation weight of each larva. Typically, larvae of both *P. polyxenes* and *S. frugiperda* consumed the entire dosage within 5 to 15 minutes. At 1.5, 3, 6, 12, or 24 hours after treatment, the gut was carefully dissected from the body, and the gut and contents, body, and any excreta eliminated during the period after treatment were analyzed separately for [^{14}C]xanthotoxin and its metabolites (*11*).

Elimination of ^{14}C after oral treatment with [^{14}C]xanthotoxin is much more rapid in *P. polyxenes* than in *S. frugiperda* (Fig. 1). Within 1.5 hours, 50 percent of all the administered ^{14}C is recovered in

excreta of the treated butterfly larvae, but only about 1 percent is eliminated by the armyworms. Given the relatively large midgut capacity of *P. polyxenes* and the small amounts of excreta deposited at the early sampling intervals, it is apparent that ^{14}C in the excreta of early samples arose as a result of absorption of ^{14}C through the gut wall and subsequent excretion (presumably by the Malpighian tubules) into the hindgut.

Xanthotoxin is rapidly absorbed through the gut wall by larvae of both insect species, as indicated by similar disappearance rates from the gut; however, about 15 times as much ^{14}C accumulates in the body tissue of *S. frugiperda* than in *P. polyxenes* (Fig. 1). In armyworms, 55 percent of the administered ^{14}C is recovered in the body tissues 1.5 hours after treatment, but body burdens of ^{14}C in *P. polyxenes* are always < 4 percent of the total dose. Differences in the amounts of xanthotoxin per se in the body tissues of the treated worms are even more dramatic (Table 1). In armyworm bodies, about 42 and 20 percent of the administered ^{14}C is recovered as unmetabolized xanthotoxin 1.5 and 3 hours after dosing, respectively; comparable values for unmetabolized xanthotoxin in body tissues of butterfly larvae are 0.7 and 0.1 percent. Therefore *S. frugiperda* contains absorbed phototoxin at all intervals after treatment in amounts that are at least 50 times greater than those of *P. polyxenes*.

When the gut contents of untreated *P. polyxenes* larvae were incubated with [^{14}C]xanthotoxin no metabolism of xanthotoxin was detected after 12 hours, an indication that neither the gut flora nor enzymes secreted into the gut lumen were the source of metabolic activity (*12*). Whole midguts were then removed from the last instar larvae of both *P. polyxenes* and *S. frugiperda* and slit open lengthwise; the contents were washed out in cold phosphate buffer (0.2*M*, *p*H 7.8). Samples of the unhomogenized midgut tissue (about 90 mg, wet weight) were then incubated at 32°C with 25 μg of [^{14}C]xanthotoxin in 2.0 ml of buffer. Extraction and analysis after 4 hours showed that 73 ± 12 percent of the xanthotoxin was metabolized in samples that contained *P. polyxenes* midgut tissue, whereas only 9 ± 4 percent was metabolized in samples that contained *S. frugiperda* midgut tissue. The xanthotoxin degradation in these samples was clearly metabolic in origin because no degradation of xanthotoxin was detected in samples in which the enzymatic activity of the butterfly or armyworm midguts had been destroyed by heat treatment (100°C in buffer, 30 minutes) prior to incubation.

Table 1. Unmetabolized xanthotoxin in body tissues of last instar larvae of the black swallowtail butterfly (*Papilio polyxenes*) and the fall armyworm (*Spodoptera frugiperda*) after the animals were treated with [^{14}C]xanthotoxin at 5 μg/g. The values are means ($N = 4$) ± standard deviation (S.D.).

Time after treatment (hours)	^{14}C absorbed (percent ± S.D.)*	
	Papilio polyxenes	*Spodoptera frugiperda*
1.5	0.7 ± 0.3	41.6 ± 10.0
3	0.1 ± 0.0	20.4 ± 6.2
6	0	4.0 ± 3.5
12	0	0.4 ± 0.4

*Percent of the administered xanthotoxin.

Thin-layer chromatographic (TLC) analysis of extracts from excreta, body tissues, and gut and contents from the in vivo studies and from the in vitro midgut tissue incubations indicated that the metabolic pathways for xanthotoxin are qualitatively the same in both butterfly larvae and armyworms. In all samples, two major xanthotoxin metabolites are seen, both considerably more polar than xanthotoxin on the basis of TLC behavior. Two additional minor metabolites occurred, but these collectively account for less than 10 percent of the metabolic products generated (*13*).

Larvae of *P. polyxenes* were fed large quantities of [^{14}C]xanthotoxin-treated parsley, and the two major metabolites were subsequently isolated in milligram amounts from the excreta by preparative TLC. The least polar of the two, designated metabolite **1**, was successfully crystallized from a mixture of ethyl acetate and hexane. The more polar metabolite **2** exhibited instability during workup but was ultimately isolated in pure form after methylation with diazomethane. Mass spectral and nuclear magnetic resonance studies conclusively established that both metabolites arise as a result of cleavage of the furan ring (Fig. 2) (*14*), reactions that may involve a transient 2′,3′-epoxide intermediate (*15*). The generation of metabolite **2** apparently does not involve **1** as a metabolic intermediate because *P. polyxenes* larvae fed purified ^{14}C-labeled **1** eliminated the compound unchanged in the excreta. Both **1** and **2** have been established as xanthotoxin metabolites in some mammalian systems (*15, 16*) but ours is the first report of their occurrence in invertebrates. Because of the extensive modifications in the parent psoralen nucleus, neither metabolites **1** nor **2** would be expected to have photosensitizing activity (*15*).

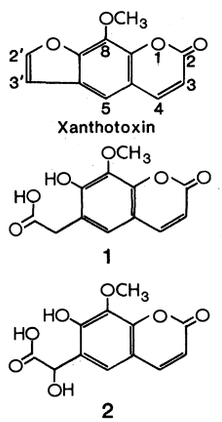

Fig. 2. Structures of xanthotoxin and its major metabolic products in larvae of *Papilio polyxenes* and *Spodoptera frugiperda*.

Our data show that the resistance of *P. polyxenes* larvae to the phototoxic effects of dietary psoralens is due largely to a highly efficient capacity to detoxify these chemicals, with much and perhaps almost all of the detoxification occurring in the midgut tissue prior to absorption. Such a resistance mechanism is appropriate and possibly obligatory for insects that feed on psoralen-rich plants under conditions of high light intensity because psoralens are potent nonspecific phototoxins that would be expected to undergo deleterious interactions with dermal or subdermal tissues once absorbed into the general body circulation (*17*). We do not imply that psoralen detoxification in either *P. polyxenes* or *S. frugiperda* occurs only in midgut tissues. In both butterfly and armyworm larvae, the administered [^{14}C]xanthotoxin is ultimately eliminated almost totally in the excreta as xanthotoxin metabolites; thus, the data in Table 1 for armyworms are consistent only with the conclusion that tissues outside the gut are primarily responsible for xanthotoxin degradation in this insect. In *P. polyxenes* larvae, xanthotoxin-detoxifying enzymes are active in body as well as gut tissues (confirmed by incubation with [^{14}C]xanthotoxin); thus, any psoralen that is absorbed unmetabolized by *P. polyxenes* is subject to further and probably rapid detoxification.

Because *P. polyxenes* and *S. frugiperda* larvae degrade xanthotoxin (and presumably other psoralens) by essentially identical metabolic pathways, it may be that during coevolution with its psoralen-rich host plants, the black swallowtail butterfly has simply maximized metabolic detoxification processes that

are widespread in Lepidoptera and perhaps other insect orders as well. The development of such an effective means of circumventing a rather formidable host plant resistance mechanism no doubt provides the black swallowtail with a distinct competitive advantage over other psoralen-susceptible insect herbivores.

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7. E. Oertli, L. Rowe, S. Lovering, G. W. Ivie, E. Bailey, *Am. J. Vet. Res.*, in press; E. Oertli, R. Beier, G. W. Ivie, in preparation; *T. texana* is a potent photosensitizer for livestock and contains at least eight 5- or 8-substituted psoralens (or both), including xanthotoxin and bergapten (5-methoxypsoralen).
8. M. Berenbaum [*Science* **201**, 532 (1978)] has shown that the closely related southern armyworm (*S. eridania*) is highly susceptible to the phototoxic effects of linear furocoumarins and that this species does not thrive (for one reason or another) on plants that contain appreciable amounts of psoralens.
9. [^{14}C]Xanthotoxin was prepared by demethylation of xanthotoxin to xanthotoxol, and subsequent remethylation with [^{14}C]methyl iodide. The resulting preparation (9.86 mCi/mmole) was purified on TLC to > 99 percent radiochemical purity.
10. Most *S. frugiperda* larvae were reluctant to consume xanthotoxin-treated parsley, but this refusal was overcome when the insects were starved for 2 hours before treatment.
11. Samples were diluted with water, acidified with HCl, and extracted at least three times with ethyl acetate. The ^{14}C in all phases (including extracted tissue and excreta slurries) was quantified by liquid scintillation counting (LSC). The organic extracts were concentrated and applied to silica TLC plates that were developed in a solvent system of ethyl acetate, methanol, and glacial acetic acid (150:50:2). Radioactive components were visualized by autoradiography (x-ray film) and quantified by LSC.
12. Gut contents from individual, freshly dissected *P. polyxenes* larvae were incubated with 5 μg of [^{14}C]xanthotoxin in 2.0 ml of phosphate buffer (*p*H 7.8) at 32°C under either nitrogen or carbon dioxide gas.
13. Thin-layer chromatography R_f values in the ethyl acetate, methanol, and glacial acetic acid solvent system were as follows: xanthotoxin (0.67), metabolite **1** (0.46), metabolite **2** (0.12), minor metabolites (0.51, 0.26).
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17. Some insect species may use behavioral responses to circumvent the toxic effects of plant photosensitizers. Certain leaf rolling microlepidopteran larvae that feed on photosensitizer-containing hosts are thought to utilize leaf rolling behavior primarily to avoid light.
18. We thank Jean Person for technical assistance.

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Magnesium Deficiency–Induced Spasms of Umbilical Vessels: Relation to Preeclampsia, Hypertension, Growth Retardation

Abstract. *Isolated umbilical arteries and veins, obtained from normal women at the end of pregnancy, were incubated in Krebs-Ringer bicarbonate solution and exposed to magnesium at concentrations ranging from 0 to 9.6 millimoles per liter. The basal tension of the vessels increased when magnesium was withdrawn and decreased when the concentration of magnesium was raised. Absence of magnesium in the medium significantly potentiated the contractile response of the vessels to bradykinin, angiotensin II, serotonin, and prostaglandin $F_{2\alpha}$. It appears that magnesium deficiency may be responsible for spasms of umbilical and placental vasculature. Our findings may provide a rationale for why magnesium sulfate is an effective therapy in preeclamptic syndromes in pregnant women.*

The symptoms of preeclampsia in pregnant women include hypertension, edema, increased vascular reactivity to pressor substances, uteroplacental changes (ischemia, infarctions), cerebral and visual disturbances, and coagulation defects (*1, 2*). In addition, almost half of all fetuses delivered from preeclamptic mothers exhibit growth retardation. It is estimated that, worldwide, the syndrome kills 5 million pregnant women and fetuses annually (*1, 3, 4*). Although preeclampsia has been known for centuries (*5*), its pathophysiology remains unresolved (*1–4, 6*). Its incidence is highest among indigent populations, perhaps because of malnutrition (*1, 5, 7, 8*). The preferred treatment for severe cases is parenteral administration of $MgSO_4$, which somehow restores normal blood pressure and vascular reactivity (*1, 2, 4, 6*).

Hypomagnesemia has been seen with preeclampsia (*9–12*). Normal pregnant women often show progressive hypomagnesemia during the last 2 months of pregnancy (*11–13*). According to recent surveys (*11, 14*), the dietary intake of Mg^{2+} among pregnant women worldwide has been steadily declining since around 1900, to the point where the Mg^{2+} balance of many is negative (*11, 12, 14*). Acute hypomagnesemia in animals and humans is often associated with increases in blood pressure and in peripheral vascular resistance (*11, 15*). Artificial lowering of the Mg^{2+} content of isolated peripheral, coronary, and cerebral vessels from rats, rabbits, dogs, and piglets induces rapid contractile responses (*15–17*). Acute hypermagnesemia reduces both spontaneous and drug-induced tone in these peripheral arteries and veins (*15–17*). Such evidence suggests that extracellular Mg^{2+} plays a role in regulating vasomotor tone.

There are reports that placentas from women with preeclampsia or eclampsia exhibit decreased Mg^{2+} and increased Ca^{2+} (*11, 18*). A higher than normal ratio of Ca^{2+} to Mg^{2+} has been shown to provoke vasospasm in coronary, cerebral, and certain peripheral blood vessels in animals (*15, 16*). It was once suggested that peripheral vasospasm might play a role in preeclampsia (*19*). To investigate the possibility that vasospasm of the umbilical arteries and veins can be produced by Mg^{2+} deficiency, we determined the influence of sudden Mg^{2+} withdrawal and hypermagnesemia (2.4 to 9.6 m*M*) on vascular tone and on vasoactive drug–induced responses in human umbilical arteries and veins.

Arteries and veins, obtained from the normal umbilical cords of 34 pregnant women at full-term spontaneous delivery, were helically cut and set up isometrically (*20*). The tissues were equilibrated in normal Krebs-Ringer bicarbonate solution (118 m*M* NaCl, 4.7 m*M* KCl, 2.5 m*M* $CaCl_2$, 1.2 m*M* KH_2PO_4, 1.2 m*M* $MgCl_2$, 10 m*M* glucose, and 25 m*M* $NaHCO_3$) and aerated with 95 percent O_2 and 5 percent CO_2 at 37°C. After 3 hours of incubation under tension, the vessels were exposed randomly to normal (1.2 m*M*), zero, and high (2.4, 4.8, and 9.6 m*M*) concentrations of Mg^{2+} and their contractile responses were measured with Grass FT-03 force-displace-

Table 1. Influence of extracellular Mg^{2+} on the basal tone of human umbilical arteries and veins. The number of donors represented in each group is given in parentheses. Tissues were first incubated in Krebs-Ringer bicarbonate solution containing 1.2 m*M* Mg^{2+}. All the values (means ± standard errors) differ significantly from those for the 1.2 m*M* Mg^{2+} solution ($P < 0.01$, paired *t*-test). Minus signs signify relaxation.

Mg^{2+} (m*M*)	Tension (mg)	
	Arteries	Veins
0	1436.8 ± 295.2 (34)	2157.6 ± 395.0 (33)
2.4	−110.2 ± 28.2 (6)	−250.0 ± 54.2 (6)
4.8	−250.0 ± 56.2 (6)	−516.7 ± 124.8 (6)
9.6	−576.3 ± 61.5 (19)	−655.2 ± 62.1 (19)