How the cell deals with drugs like actinomycin is not known. We did find that when treated cells were washed with cold medium and then fed with warm medium containing labeled uridine immediately or at intervals up to 6 hours after being held at 8°C, RNA synthesis was related to the time at 37°C, not to the total time after withdrawal of drug. Therefore, "reversal" cannot be attributed solely to simple diffusion which is only twofold slower at the lower temperature. The data suggest that there is a previously unknown temperature-dependent cell process involved.

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10. Supported by NIH grant AI-06864. We thank Dr. Edward Reich and Dr. Lon Hodge for advice and interest.

23 November 1970

Detoxication Enzymes in the Guts of Caterpillars: An Evolutionary Answer to Plant Defenses?

Abstract. *Higher activity of midgut microsomal oxidase enzymes in polyphagous than in monophagous species indicates that the natural function of these enzymes is to detoxify natural insecticides present in the larval food plants. Differing strategies of adaptation to plant defenses may partly account for the great diversity of insect herbivores.*

Many plant species are chemically defended. Their defensive toxins or inhibitors belong mostly to the large class of secondary substances (*1, 2*), and include such compounds as alkaloids, some terpenoids and steroids, rotenoids, and organic cyanides. Herbivores and pathogens exploiting a defended plant species are presumably the relatively few species that have evolved appropriate countermeasures against the particular toxins present in their hosts (*2, 3*). Sometimes these toxins may even contribute to the behavioral clues which help such an herbivore to find its chosen food plants (*4*).

In the livers of mammals, birds, and fish there are enzymes that promote metabolic attack on a variety of drugs and pesticides (*5, 6*). The remarkable diversity of reactions catalyzed by these microsomal mixed-function oxidases includes oxidation of thioethers; aromatic, aliphatic, and alicyclic hydroxylation; *O*-, *S*-, and *N*-dealkylation; and epoxidation. These reactions affect a wide variety of substrates; they serve both to speed excretion of a foreign compound from the body and generally, also, to decrease its toxicity. Similar enzymes are known in invertebrates (*7*), and recent work in our department has revealed their presence in lepidopterous insect larvae, where their activity is concentrated in the midgut tissues (*8*).

Microsomal detoxication enzymes certainly existed long before the advent of modern drugs and pesticides, yet their natural role and substrates have received surprisingly little attention. Here we describe experimental results which support the hypothesis that these enzymes exist in lepidopterous caterpillars for metabolizing potential toxins present in the natural food. Such a function for the equivalent enzymes in vertebrates was suggested in 1922 by Sherwin (*9*), and more recently by Williams, Brodie, and others (*6, 10*). Gordon (*11*) has proposed a similar hypothesis for insects.

Different species of phytophagous Lepidoptera have widely differing ranges of larval food plants, varying from strict monophagy in some species to wide polyphagy in others. Biosynthesis and metabolic action of their microsomal detoxication enzymes must require expenditure both of energy and of nutrients. If metabolism of plant secondary substances is the chief natural function of these enzymes, and if natural selection acts to reduce energetic and nutritional waste, we would expect to find a correlation between enzyme activity and degree of feeding specialization. In our experiment, the microsomal oxidase activities in the larval midguts of 35 species of Lepidoptera were estimated in terms of the rate at which aldrin was epoxidized to dieldrin (*8*).

Midguts from last instar larvae were cleared of gut contents (which contain an inhibitor of oxidase) (*8, 12*) and homogenized in ice-cold 0.15*M* KCl with a Ten Broeck tissue grinder. Samples (0.5 ml) of this homogenate, containing 1 to 10 mg of protein, were added to a medium (total volume 5.0 ml) containing tris-HCl buffer (50 mmole/liter, *p*H 7.8), 2.4 m*M* glucose-6-phosphate, 51 μM nicotinamide-adenine dinucleotide phosphate, glucose-6-phosphate dehydrogenase (1.6 enzyme units), and 2.7 m*M* KCl. After addition of aldrin (100 μg) in ethanol (25 μl), mixtures were incubated at 30°C for 10 or 15 minutes, after which the reactions were terminated by the addition of acetone (4.0 ml). The chlorohydrocarbons were then extracted quantitatively into petroleum ether, and dieldrin production was measured by electron-capture gas chromatography (*8*). The protein content of each homogenate was estimated by the Biuret method of Fincham (*13*).

Epoxidase activities are listed in three categories (Table 1) according to the range of host plants used by the larvae (*14*): (i) species normally confined to plants of one family, or two closely related families, (ii) species known to feed on between two and ten families of plants, and (iii) species which normally feed on plants of more than ten families. These categories represent positions along the traditional scale from monophagy, through oligophagy, to polyphagy; they depict the scale of host plant range in the absence of more precise knowledge of larval feeding habits. Epoxidase activity was found to be higher in the polyphagous group than in the more oligophagous group ($P < .01$) and higher in the oligophagous group than in the mo-

nophagous species ($P < .01$) (*15*). No significant differences in epoxidase activity were found in larvae of the same species which had been reared to the last instar on different food plants (*16*).

Table 1. Activities of gut epoxidase of last-instar larvae of 35 species of Lepidoptera in relation to range of larval food plants. Activities are expressed as nanomoles of dieldrin per milligram of protein per minute times 10^3. For each determination, gut homogenates were prepared from a minimum of five larvae in the actively feeding stage of the last instar. Standard incubation mixtures contained aldrin (100 μg) and were incubated for 10 or 15 minutes. Values represent means of at least two determinations. Larvae for the experiments were kindly supplied by several members of the Department of Entomology, Cornell University. Most insects were collected from wild populations in central New York State. PF, plant family containing larval food plants.

Insect family and species	Epoxidase activity in larvae feeding on		
	1 PF	2 to 10 PF	11 or more PF
Saturniidae			
Telea polyphemus			39
Callosamia promethea		13	
Hyalophora cecropia			82
Philosamia cynthia		29	
Antheraea pernyi		147	
Saturnia pyri		90	
Lasiocampidae			
Malacosoma americanum	80		
Malacosoma disstria			108
Geometridae			
Erannis tiliaria		34	
Sphingidae			
Ceratomia catalpae	3		
Notodontidae			
Datana integerrima	32		
Lymantriidae			
Porthetria dispar			175
Noctuidae			
Polia purpurissata			732
Polia lutra		113	
Lacinipolia vicina			408
Orthodes cynica			334
Morrisonia evicta		50	
Xylomiges dolosa	3		
Orthosia rubescens			159
Orthosia revicta		205	
Pseudaletia unipuncta		26	
Leucania inermis	13		
Leucania juncicola	13		
Euplexia benesimilis		26	
Phlogophora iris			208
Prodenia eridania			659
Panthea furcilla		513	
Charadra deridens		5	
Trichoplusia ni		42	
Arctiidae			
Holomelina fragilis		14	
Pyrrharctia isabella			418
Spilosoma virginica			211
Hyphantria textor		53	
Nymphalidae			
Vanessa atalanta	11		
Danaidae			
Danaus plexippus	8		
Mean epoxidase activity	20.4	90.7	294.4

Enzyme activities listed in Table 1 thus probably reveal genetic differences between the species rather than differences due to enzyme induction.

To the extent that the rate of epoxidation of aldrin to dieldrin typifies the activity of the enzymes toward a wider range of substrates, these results support the hypothesis that detoxication of secondary substances in food plants is the chief function of the mixed-function microsomal oxidases in the midgut tissue of lepidopterous larvae. Enzymes which specifically metabolize certain plant toxins, such as cyanide, are already known in insects (*17*); experiments with synthetic chemicals suggest that the activity of the gut microsomal oxidases may be restricted to lipophilic compounds (*18*). Variations in activity between different species within each of the three feeding groups may reflect (largely unknown) qualitative and quantitative differences in the content of secondary substances between different plants. The unusually high activity found in *Panthea furcilla*, for example, may reflect the high content of terpenoids found in its coniferous food plants (*19*). Pyrethrins are among the toxins known to be metabolized by the mixed-function oxidases in certain insects. Sesamin, a lignan which inhibits these enzymes, occurs together with pyrethrins in *Chrysanthemum cinerariaefolium* flowers; it is thus a natural synergist, possibly evolved by the plant to neutralize the ability of some of its enemies to detoxify pyrethrins (*20*).

Our results indicate, also, that the enzyme activity is adjusted by natural selection to the content of secondary substances present in the larval food plants. This agrees with a suggestion by Gordon (*11*) that "the extraordinarily high and generalized tolerance of the larval feeding stages of relatively polyphagous holometabolous insects to contact insecticides is probably the result of selection for endurance of prolonged and varied biochemical stresses associated with the diversity of their natural food plants." During the elaborate coevolution between plants and phytophagous insects (*3*) it seems that plants have evolved an enormous variety of natural pesticides, only some of which are metabolized by any given insect species. Monophagous insects save expenditure of nutrients and energy by restricting their countermeasures to one or a few potentially toxic substances, which they may even reuse for their own defense against predators (*21*). Polyphagous species, by contrast, must be prepared to counter a wider range of potential toxins and perhaps, also, the higher concentrations of some of these compounds likely to be found in several of their food plants. Differing strategies of counteradaptation to plant defenses may help to explain one of evolution's most striking products, the great diversity of phytophagous insects.

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8 February 1971

Mycorrhizal Enhancement of Water Transport in Soybean

Abstract. *Mycorrhizae produced by* Endogone mosseae *decrease the resistance to water transport in soybean* (Glycine max *L.). The decrease was associated with an increase in the growth of shoots but not of roots.*

Vesicular-arbuscular (VA) endomycorrhizae increase the rate of growth of many plants (*1*), but the mechanism of the enhancement is only partially understood. There is evidence that increased growth could result from the increased uptake of various nutrients (*2–4*). Mycorrhizal plants are known to utilize less available forms of phosphorus more efficiently than nonmycorrhizal plants (*3*), and studies with ^{32}P show that segments of mycorrhizal roots have higher phosphorus contents after a period of uptake than comparable nonmycorrhizal segments from either mycorrhizal or nonmycorrhizal plants (*4*). On the other hand, the growth stimulation by mycorrhizae could be associated with the production of growth regulators. An ectomycorrhizal fungus has been shown to produce three growth substances identified as cytokinins (*5*).

The hyphae of VA mycorrhizae are associated with roots in such a way that they ought to increase the absorptive surface. If so, the presence of the hyphae could enhance water uptake which, in turn, might affect growth. In this report we show for the first time that mycorrhizae reduce the resistance to water uptake by plants.

Methods for growing VA mycorrhizal fungi in axenic culture are unknown, and cultures are maintained on potted plants (*6*). An Illinois isolate of *Endogone mosseae* (*7*), known to produce VA mycorrhizae on the roots of soybean and other plants (*8*), was grown on maize in an autoclaved sand-soil mixture. About 110 g of this mixture, which contained maize mycorrhizae and spores of *E. mosseae*, were used as inoculum for soybean (variety Harosoy 63) and added to 5-inch (12.7-cm) pots containing an autoclaved sand-soil mixture. A similar portion of autoclaved inoculum was added to the control pots, along with a water filtrate from unsterilized inoculum, which had been passed four times through a sieve with 44-μm openings (smaller than the spores of *E. mosseae*). This procedure ensured that contaminating microorganisms, which can affect nutrient availability and plant growth (*9*), would also be present in control pots. Soybean seeds were soaked in a soybean rhizobium culture for 3 minutes prior to planting. Plants were grown in a controlled environment [14-hour photoperiod; light intensity, 0.15 cal cm^{-2} min^{-1} (fluorescent and incandescent); day temperature, 30° ± 1°C; night temperature, 24° ±1°C)]. After planting, 50 ml of nutrient solution (*10*) was added to each pot. Plants were watered with distilled water daily.

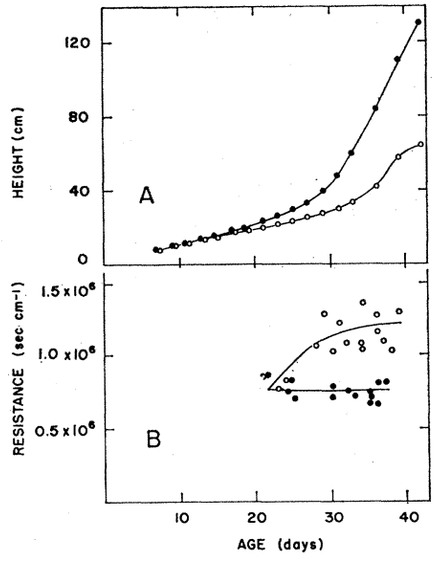

Fig. 1. (A) Height of mycorrhizal (solid circles) and nonmycorrhizal (open circles) soybean plants at various times after planting. Data represent the averages for six plants. (B) Whole plant resistances to water transport in mycorrhizal (solid circles) and nonmycorrhizal (open circles) soybeans calculated from the half-time for recovery from a moderate water deficit. Data represent resistances of single plants.

Resistances to water transport in whole plants which were intact in the soil were measured by a recently described method (*11*), in which the time required for a test leaf on the plant to recover from water deficiency was determined. The delicacy of the mycorrhizae made this method, which does not disturb the soil-plant system, particularly suitable.

Determinations were made with a thermocouple psychrometer that measures the water potentials of intact leaves (*12*). The recovery conforms to a well-known equation describing diffusive transfer in a plane sheet as a function of time (*13*). Since the resistance of the transfer system determines the time required for recovery, the resistance of the intact plant can be calculated.

The blades of intact, moderately water-deficient soybean leaves were sealed in the chamber of the thermocouple psychrometer, the soil-root or soil-mycorrhizal systems were then submerged briefly in degassed water, and the recovery in the water potential of the intact leaf was recorded. After we had determined that the data conformed to the transfer equation for a plane sheet, resistances (in seconds per centimeter) were calculated according to (*11, 13*):

$$r = \frac{t_{1/2}}{0.195\, l}$$

where $t_{1/2}$ is the half-time for recovery (in seconds) and l is half the average leaf thickness during recovery (in centimeters). Measurements of l were made with a light microscope. Since