Induction of Microsomal Enzymes in the Southern Armyworm
(Prodenia eridania)  

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Levels of microsomal epoxidation, N-demethylation, and cytochrome P-450 in the gut tissues of sixth instar southern armyworm larvae were considerably enhanced following oral in vivo treatment with a series of methylbenzenes. Induction increased with increasing methyl substitution and was maximal with pentamethylbenzene. The increase in microsomal activity occurred rapidly after initiation of treatment and the final levels of induction achieved were dependent on the concentration of the inducer in the diet and the time of exposure. Microsomal enzyme activity returned to control levels following termination of exposure and induction was blocked by puromycin and cycloheximide but not by actinomycin D. The in vivo tolerance of induced worms to orally administered carbaryl was increased in a manner reflecting the enhanced microsomal enzyme activity.  

INTRODUCTION  

Induction of the hepatic microsomal drug-metabolizing enzymes following the in vivo treatment of mammals with a variety of drugs, insecticides, and other foreign compounds is a well established phenomenon which has been extensively studied in recent years (1, 2).  

Although microsomal enzyme induction has been clearly demonstrated in several insect species (3-8) most of the available data are incomplete and the investigations have often been hampered by the many problems associated with studies involving insect microsomes (9). Whether enzyme induction is reflected in an increased tolerance of insects to insecticides and whether the phenomenon can have any practical consequences under field conditions remain to be established. In view of the general need for additional data on microsomal enzyme induction in insects it was of considerable interest to establish a model system in which the effects of various inducing chemicals could be studied in detail.  

The southern armyworm (Prodenia eridania) was selected as the insect to be employed in this investigation since the microsomal enzymes of this species have already been extensively studied in this laboratory in recent years (10, 11). Furthermore the southern armyworm can be conveniently reared throughout the year and its developmental biology is such that large numbers of insects in a given physiological state are available at any given time. After preliminary studies with a variety of inducing agents, a series of alkylbenzenes were finally selected for use in this investigation.  

MATERIALS  

Insects. A colony of the southern armyworm was maintained under greenhouse conditions as described earlier (10) and was supplemented with eggs kindly pro-

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vided by the Niagara Chemical Division, FMC Corp., Middleport, NY.

Chemicals. Chromatoquality α-, m-, and p-xylene were purchased from Matheson Coleman & Bell, Norwood, Ohio. Analytical reagent grade toluene was from Mallinckrodt Chemical Works, St. Louis, MO, and 1,2,4-trimethylbenzene, durene (1,2,4,5-tetramethylbenzene), pentamethylbenzene, hexamethylbenzene, and p-dimethylaminobenzaldehyde were purchased from Eastman Kodak Co., Rochester, NY.

Vitamin free casein, Salt mixture W, cholesterol (U.S.P.), alphacel, linolenic acid (55%), and Vanderzant’s vitamin mixture were from Nutritional Biochemicals Corp., Cleveland, OH. Bacto-agar was from Difco Laboratories, Detroit, MI, and wheat germ from Kretschmer Wheat Germ Products, Minneapolis, MN.

Analytical grade aldrin (1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-1,4-endo-endo-5,8-dimethanonaphthalene) and dieldrin (the 6,7-epoxide of aldrin) were supplied by Shell Development Co., Modesto, CA. Glucose-6-phosphate (G-6-P), G-6-P dehydrogenase, NADP, NADPH, p-chloro N-methylaminiline, p-chloroaniline, bovine serum albumin, actinomycin D, and cycloheximide (B grade) were purchased from Calbiochem, La Jolla, CA. Horse heart cytochrome c (type III), and puromycin dihydrochloride were purchased from Sigma Chemical Co., St. Louis, MO. Carbaryl (1-naphthyl N-methylcarbamate), mp 142°C, was recrystallized from an analytical grade sample obtained from Union Carboide, New York, NY.

**METHODS**

Administration of chemicals. Armyworms were reared on bean plants until the late fifth instar when they were transferred to 12-in. diam plastic containers holding approximately ½ in. of autoclaved soil. Newly molted sixth instar worms were transferred to other plastic containers at intervals depending on the degree of age homogeneity which was required; for some experiments the age variation within each group was restricted to 3 hr or less if the numbers proved adequate. The worms were provided ad libitum with an agar-based semidefined artificial diet modified from Feeny (12) in which the test compounds were incorporated.

The diet was prepared by mixing 7.0 g bean leaf powder, 1.5 g alphacel, 1.5 g vitamin-free casein, 1.5 g sucrose, 0.75 g Vanderzant’s vitamin mixture, 0.50 g wheat germ, 0.15 g cholesterol, 0.15 g salt mixture W, 0.15 g linolenic acid, 2.0 g agar, and 250 ml water. The agar was dissolved in 100 ml boiling water and after cooling to 40–50°C it was mixed with the dry ingredients and additional cold water in a Waring Blender for 1 min. Appropriate amounts of inducers or protein synthesis inhibitors were incorporated into the diet at the time of mixing in the Waring Blender. The diet was transferred to 8-oz ice-cream cups and was stored in the refrigerator up to 3 days. The armyworms were provided with fresh diet every 3–4 hr during the daytime and once over night. The bean leaf powder was prepared from red kidney or lima bean leaves which were freeze-dried and finely powdered in a ball mill, U.S. Stoneware, Akron, OH.

Actinomycin D was administered to the caterpillars either in the diet or on pieces of bean leaf in a manner similar to that described for the bioassay technique.

**Enzyme preparations.** Enzyme preparations were made from two separate samples, each consisting of 12 worms, taken from groups of 30 subjected to each feeding regimen. This was repeated at least twice for any particular treatment. Whole guts were removed from groups of 12 sixth instar armyworm larvae and the gut contents removed as previously described (10). The cleaned guts were further rinsed and homogenized for 15–20 sec in ice-cold 1.15% KCl in a ground glass tube with a motor-driven Teflon pestle. The homoge-
nate was adjusted to 3.0 ml with 1.15% KCl and was used without delay for measurement of epoxidase and N-demethylase activities; in certain cases, as specified, the 10,000g, 10-min supernatant of a homogenate of isolated midguts was used for these assays. For assay of NADPH-cytochrome c reductase activity, 0.05 M potassium phosphate buffer, pH 7.8, mM with respect to cyanide, was used for cleaning and homogenization of isolated midguts opened by longitudinal section and for cytochrome P-450 determinations, 0.1 M potassium phosphate buffer, pH 7.8, was employed for this purpose. These assays were carried out with microsomes obtained by centrifugation of the 10,000g, 10-min supernatant for 1 hr at 100,000g at 2-4°C in an International Equipment Co. (IEC) B-60 preparative ultracentrifuge with a fixed angle rotor (A-211) and resuspension of the pellet in the appropriate buffer.

Malpighian tubules and fat bodies from sixth instar armyworm larvae were rinsed and homogenized in 1.15% KCl and the 10,000g, 10-min supernatant was employed for epoxidase and N-demethylase assays.

Enzyme assays. The epoxidation of aldrin and the N-demethylation of p-chloro N-methylaniline were measured in 5-ml incubation mixtures containing 0.25 mmole Tris-HCl buffer, pH 7.8, 0.06 mmole KC1, 0.20 μmole NADP, 0.012 mmole G-6-P, 1.6 units of G-6-P dehydrogenase, and 0.26 μmole aldrin or 3.0 μmole p-chloro N-methylaniline. The reactions were initiated by addition of 0.5 ml enzyme suspension containing 1.0 ± 0.2 (epoxidation) or 2.0 ± 0.4 (N-demethylation) μg protein and incubations were carried out at 30°C for 10 and 20 min, respectively (10); the respective reactions were terminated by addition of 5 ml pesticide-quality acetone and 2 ml 0% p-dimethylaminobenzaldehyde in 3 N sulfuric acid. Dieldrin formation was measured by gas-liquid chromatography and p-chloroaniline determined spectrophotometrically as previously described (10, 11). All assays were carried out in duplicates with each enzyme preparation.

NADPH-cytochrome c reductase activity was measured directly in 1-cm cuvettes in a Norelco Unicam SP-800 double beam spectrophotometer. The 1.0-ml incubation mixture contained 0.1-0.15 mg microsomal protein in 0.05 M phosphate buffer, pH 7.8, 1 mM with respect to cyanide, and 0.5 mg cytochrome c. The linear increase in optical density at 550 nm was determined following addition of 0.1 μmole NADPH to the sample cuvette (13). A unit of reductase activity is defined as the amount of enzyme which causes an increase in optical density of 1.0 min in a 1-cm light path (14).

Cytochrome P-450 was measured by the method of Omura and Sato (15) in 1.0 ml of a suspension containing 0.4-0.8 mg microsomal protein and is expressed as ΔOD_{550-400} mg protein.

Protein concentrations were determined by a modified Biuret method (16) in suspensions used for epoxidase, N-demethylase, and cytochrome P-450 measurements, and by the Lowry method (17) in those employed for NADPH-cytochrome c reductase assays. In both cases bovine serum albumin was used as a standard.

Bioassays. Microliter aliquots of appropriate acetone solutions of carbaryl were applied to approximately 2 × 2 mm² pieces of fresh bean leaf with an all-glass Agla syringe placed in a Burkard hand operated micro-applicator. After the solvent had evaporated the pieces of leaf were fed individually to 72 ± 10-hr-old sixth instar armyworm larvae held in separate compartments in Teflon ice cube trays. The caterpillars were provided with fresh bean leaves 3 hr after ingestion of the carbaryl and were maintained at room temperature; mortality was recorded 24 hr after treatment. Groups of 10-12 worms were used for each of several dosage levels (based on fresh weight) replicated 3-4 times. LD₅₀ values were estimated from the linear probit-mortality log-dosage lines.
The increases in gut epoxidase, N-demethylase, and NADPH-cytochrome c reductase activities, and in the concentration of cytochrome P-450 resulting from 3-day ad libitum feeding to southern armyworm larvae of diets containing 13.5 μmole/g of diet. Under these conditions toluene and m-xylene were inactive while some evidence of activity began to occur with p-xylene and o-xylene approximately doubled oxidase activity. Increasing methyl substitution was associated with an increase in the level of induction observed which attained a maximum of approximately 3-fold with pentamethylenzene; the level of induction caused by hexamethylenzene was substantially lower, however, and was comparable with that produced by o-xylene. Cytochrome P-450 levels increased in a manner which closely paralleled the increases in epoxidase and N-demethylase activities and careful wavelength calibration using a standard Holm-
ium filter established that the spectral properties of the reduced P-450–carbon monoxide complex in microsomal preparations from induced worms were identical to those in microsomes from control insects. Although NADPH-cytochrome c reductase activity followed a similar pattern, the increase in activity of this enzyme was considerably less.

In view of recent reports that some compounds can enhance enzyme activity when added directly to microsomal preparations in vitro (18), the possibility that the observed increases in enzyme activity might result from residues of alkylbenzenes present in the tissues at the time of homogenization was investigated. The addition of low concentrations (μM) of pentamethylbenzene to the preparations had no effect on epoxidation but caused a slight increase in N-demethylation. A millimolar concentration caused about 50% inhibition of both reactions suggesting that pentamethylbenzene is probably acting as an alternative substrate under these conditions. This was supported by the fact that pentamethylbenzene (5 × 10⁻³ M) produced a Type I difference spectrum with oxidized cytochrome P-450 in preparations from control armyworms.

**Fig. 2.** Effect of inducer concentration on epoxidase activity in whole gut homogenates of the southern armyworm. Solid bars, pentamethylbenzene; striped bars, hexamethylbenzene. Inducers administered in diet for 3 days.

**Fig. 1.** Effect of inducer concentration on N-demethylase activity in whole gut homogenates of the southern armyworm. Solid bars, pentamethylbenzene; striped bars, hexamethylbenzene. Inducers administered in diet for 3 days.

Effect of inducer concentration. Figures 1 and 2 show the induction of gut N-demethylase and epoxidase activities respectively resulting from a 3-day exposure to different concentrations of penta- and hexamethylbenzene in the diet; they also clearly illustrate the relative inducing capacity of these two compounds. The level of induction of both enzymes was observed to increase in an almost linear manner up to an inducer concentration of approximately 2000 ppm and N-demethylation activity continued to increase up to 4000 ppm. In contrast, the level of induction of epoxidase activity in the presence of 4000 ppm of pentamethylbenzene was somewhat less than that observed at 2000 ppm. The concentration of cytochrome P-450 50 ppm pentamethylbenzene was 3.53-fold greater than in the controls.

Effect of time of exposure to inducer. As illustrated in Fig. 3, the level of induction of gut microsomal epoxidation and N-demethylation activity is dependent on the time for which the insects are exposed to the inducing agent. Enzyme activity increased most during the first 24 hr of feeding on
diets containing 2000 ppm penta- or hexamethylbenzene although an additional increase was observed when the exposure was extended to 2 or 3 days.

A more detailed examination of the time course of induction revealed a remarkably rapid enzymatic response to the presence of the inducing agent (Fig. 4). Thus, 8 hr after starting to feed newly molted sixth instar armyworms on a diet containing 2000 ppm pentamethylbenzene, the gut N-demethylase activity was 5-fold greater than that in the control insects. Activity continued to increase during the first 24 hr of feeding and when, at this point, the inducing diet was replaced by a control diet, activity showed an immediate and rapid decline returning to the level in control insects within about 36 hr. A very similar pattern was observed when the inducing agent was administered during a 24-hr period starting 24 hr after the molt (Fig. 4). The maximum specific activity attained in the induced worms was approximately the same (18–19 nmoles produced/min/mg protein) whether the inducer was administered to newly molted or 24-hr-old sixth instar larvae. In contrast to the sharp decline in N-demethylase activity which normally occurs immediately prior to pupation, enzyme activity in induced worms remains high at this time. More detailed studies on the changes in enzyme activity occurring during the larval–pupal transformation in induced worms are presently under investigation.

Subcellular distribution of enzyme activity. The subcellular distribution patterns of epoxidase and N-demethylase activities in fractions of armyworm midgut homogenates are shown in Table 2. In preparations from control larvae both enzymes were clearly associated with the microsomal fraction (P₁₀₀₀₀₀), and little or no activity was detected in the microsomal supernatant (S₁₀₀₀₀₀). Although some activity was lost in the 10,000g 10-min pellet (P₃₀₀₀₀), which was usually discarded, the specific activity of the microsomal fraction was approximately 2-fold greater than that of the 10,000g, 10-min supernatant. In similar fractions from the midguts of armyworms induced for 3 days on a diet containing 2000 ppm pentamethylbenzene the subcellular distribution patterns remained virtually identical to those observed in control caterpillars.

![Figure 3](image-url)  
**Fig. 3.** Effect on induction of duration of exposure to 2000 ppm hexamethylbenzene in whole gut homogenates of southern armyworm. Solid bars, epoxidase activity; striped bars N-demethylase activity.

![Figure 4](image-url)  
**Fig. 4.** Time course of induction of N-demethylase activity in 10,000g, 10-min supernatant of midgut homogenates of southern armyworm. A, activity in worms fed control diet; B, worms fed 2000 ppm pentamethylbenzene during first 24 hr after molt; C, worms fed 2000 ppm pentamethylbenzene from 24 to 48 hr after molt. Age variation was 3 hr or less.
Induction of microsomal enzymes in the Southern armyworm

Electron microscopy. An examination of electron micrographs of cross sections through the midgut tissues of control armyworms (Fig. 5a) and those fed a diet containing 4000 ppm pentamethylbenzene for 3 days (Fig. 5b) revealed that the induction process was associated with structural changes in the endoplasmic reticulum (ER). Although small areas of smooth ER can be seen in the micrographs from control worms (Fig. 5a), the major structural feature in these is an extensive network of rough ER. In the midgut tissues of the induced worms the smooth ER is much more obvious and in some portions of the cell the rough ER appears to have been pushed aside to accommodate areas which are comprised predominately of smooth membranes.

Induction in tissues other than gut. In addition to the induction observed in the gut tissues, epoxidation and N-demethylation activities in the fat body and Malpighian tubules were also enhanced in armyworms fed on diets containing 2000 ppm pentamethylbenzene for 3 days (Table 3). The percent increases in these tissues relative to the controls were considerably lower than those occurring in the gut and the net increase was quite small in each case, particularly in the fat body.

Effect of protein synthesis inhibitors. The effects of enzyme activity of three protein synthesis inhibitors (actinomycin D, cycloheximide and puromycin) were investigated by incorporating them in appropriate diets in both the presence and absence of the inducer, pentamethylbenzene (2000 ppm); the results are shown in Table 4. Cycloheximide (50 ppm) and puromycin (2 and 10 ppm) caused a significant decrease in the levels of induction of both epoxidation and N-demethylation caused by pentamethylbenzene. At these concentrations the materials also caused a substantial decrease in enzyme activity in control larvae although the worms appeared to be healthy and exhibited normal size, weight, and feeding characteristics. Figure 6 shows the time course of the effect of puromycin (10 ppm) on N-demethylase activity in both induced and control worms and clearly shows that induction is rapidly and effectively counteracted by this compound. The effect of puromycin on epoxidase activity showed a similar pattern.

### Table 2

Distribution of Epoxidase and N-Demethylase Activities in Subcellular Fractions of Malpigh from Sixth Instar Armyworms Fed Control Diet or a Diet Containing 2000 ppm Pentamethylbenzene for 3 Days

<table>
<thead>
<tr>
<th>mg Protein</th>
<th>Epoxidation</th>
<th>N-demethylation</th>
</tr>
</thead>
<tbody>
<tr>
<td>(total)</td>
<td>Specific activity</td>
<td>Relative activity</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crude</td>
<td>29.1</td>
<td>2.066</td>
</tr>
<tr>
<td>S10,000</td>
<td>11.9</td>
<td>2.585</td>
</tr>
<tr>
<td>P10,000</td>
<td>16.9</td>
<td>1.155</td>
</tr>
<tr>
<td>S100,000</td>
<td>4.5</td>
<td>ND</td>
</tr>
<tr>
<td>P100,000</td>
<td>1.1</td>
<td>5.503</td>
</tr>
<tr>
<td>Induced</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crude</td>
<td>24.2</td>
<td>5.928</td>
</tr>
<tr>
<td>S10,000</td>
<td>9.2</td>
<td>9.021</td>
</tr>
<tr>
<td>P10,000</td>
<td>14.0</td>
<td>4.141</td>
</tr>
<tr>
<td>S100,000</td>
<td>3.6</td>
<td>ND</td>
</tr>
<tr>
<td>P100,000</td>
<td>4.6</td>
<td>18.715</td>
</tr>
</tbody>
</table>

* S = supernatant, P = pellet, ND = not detected.
FIG. 5. Electron micrographs of sections of (a) control and (b) induced armyworm midgut tissues, $\times 50,000$. Induction with 4000 ppm pentamethylbenzene for 3 days.
Actinomycin D had little or no effect on induction by pentamethylbenzene. At low concentrations it appeared to have a stimulatory effect on both reactions and although at the highest concentration used (2 μg g⁻¹ 12 hr) some inhibition of epoxidase induction occurred there was no similar effect on N-demethylation (Table 4).

*In vivo significance of induction.* The effect of induction on the in vivo tolerance of sixth instar armyworms to orally administered carbaryl is shown in Fig. 7.

**TABLE 3**

<table>
<thead>
<tr>
<th></th>
<th>Epoxidation</th>
<th>N-demethylation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>umole/mg protein/min</td>
<td>Net increase</td>
</tr>
<tr>
<td>Midgut</td>
<td></td>
<td></td>
</tr>
<tr>
<td>control</td>
<td>2.189</td>
<td>6.348</td>
</tr>
<tr>
<td>induced</td>
<td>8.537</td>
<td></td>
</tr>
<tr>
<td>control</td>
<td>0.320</td>
<td>0.732</td>
</tr>
<tr>
<td>Malpighian tubules</td>
<td></td>
<td></td>
</tr>
<tr>
<td>induced</td>
<td>1.052</td>
<td>0.595</td>
</tr>
<tr>
<td>control</td>
<td>0.410</td>
<td></td>
</tr>
<tr>
<td>Fat body</td>
<td></td>
<td></td>
</tr>
<tr>
<td>induced</td>
<td>1.005</td>
<td></td>
</tr>
</tbody>
</table>

**TABLE 4**

<table>
<thead>
<tr>
<th></th>
<th>Percent of control activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Epoxidation</td>
</tr>
<tr>
<td></td>
<td>Control + agent</td>
</tr>
<tr>
<td>Actinomycin D</td>
<td></td>
</tr>
<tr>
<td>0.8 ppm in diet</td>
<td>100</td>
</tr>
<tr>
<td>4.0 ppm in diet</td>
<td>107</td>
</tr>
<tr>
<td>1 μg g⁻¹ 24 hr</td>
<td>97</td>
</tr>
<tr>
<td>2 μg g⁻¹ 12 hr</td>
<td>92</td>
</tr>
<tr>
<td>Cycloheximide</td>
<td></td>
</tr>
<tr>
<td>30 ppm in diet</td>
<td>41</td>
</tr>
<tr>
<td>Puromycin</td>
<td></td>
</tr>
<tr>
<td>2 ppm in diet</td>
<td>69</td>
</tr>
<tr>
<td>10 ppm in diet</td>
<td>27</td>
</tr>
</tbody>
</table>

*Inducer (2000 ppm) and/or inhibitors administered in diet for 2 days prior to assay.

*Administered on pieces of leaf to individual worms for two days prior to assay.*
The $LD_{50}$ of carbaryl to control worms (72 ± 10 hr of age) was 30 μg/g under the conditions employed. Worms of the same age which had been maintained on a diet containing 2000 ppm hexamethylbenzene, a moderate inducer, had a slightly increased tolerance to carbaryl ($LD_{50}$ value of 67 μg/g) whereas those fed a diet containing 2000 ppm pentamethylbenzene exhibited a dramatic 11-fold increase in tolerance to this insecticide ($LD_{50}$ of 350 μg/g).

DISCUSSION

Most previous studies on microsomal enzyme induction in insects have been conducted with adult resistant houseflies (Musca domestica) which appear to respond quite readily to inducing agents such as DDT, dieldrin, and phenobarbital (3, 4, 19). In some cases, however, a high concentration of the inducing agent is required to produce an effect. Extremely high dietary levels of chloroecyclizine (5000 ppm) and phenobarbital (10,000 ppm) were required to enhance microsomal activity in the gut tissues of wax moth larvae (Galleria mellonella) (5) and in this investigation substantial levels of induction in southern armyworm larvae were obtained only at dietary concentrations of alkylbenzenes in excess of 500 ppm. This apparent resistance to induction in insects, particularly in immature stages, might reflect more rigid metabolic control mechanisms which serve to decrease their sensitivity to external chemical stimuli. It might also explain earlier unsuccessful attempts in this laboratory to induce armyworm larvae with materials such as DDE and dieldrin (11) since the levels of these agents required for induction are probably greater than those causing toxic effects.

The series of methylated benzenes used in this investigation constitute ideal model inducing agents since they are nontoxic and have no obvious effects on larval feeding, development, subsequent pupation, adult emergence, or reproduction. They can therefore be administered in high concentrations without any detrimental effects to the larvae. The inducing efficiency of these compounds increases markedly with increasing methyl substitution up to pentamethylbenzene which was clearly the most potent member of the series (Table 1).

Although it is possible that the levels of
induction obtained with the lower, more volatile homologs are modified somewhat by evaporation from the diets, the decreased activity of hexamethylbenzene suggests that inducing efficiency may be related to specific structural or physico-chemical properties.

It is of interest that all four microsomal parameters measured in this investigation respond in a parallel fashion to the various inducers employed (Table 1). In contrast to the results of some investigations (8, 20) the increase in cytochrome P-450 was quantitatively similar to that of both epoxidation and N-demethylation and the spectrum of the induced P-450 was identical to that in preparations from control larvae. Since the relative distribution of epoxidation and N-demethylation activities in subcellular fractions from control and induced worms are virtually identical (Table 2), it appears certain that the enhanced activity is associated with the microsomal fraction of the cell. This is supported by the proliferation of smooth ER seen in electron micrographs of the midgut tissues of induced worms (Fig. 5b). The proliferation does not occur in a uniform manner throughout the cell, however, but appears to be localized in specific areas so that some portions of the cell consist almost entirely of agranular membranes or membrane vesicles. A similar increase in the smooth endoplasmic reticulum of the liver is often associated with increased hepatic drug metabolizing activity in induced mammals (1) and recent studies with insects have shown numerous changes in cell ultrastructure following treatment with a variety of inducing agents (21, 22).

That the observed induction in the armyworm is due in part to an increase in protein synthesis is illustrated by the data in Table 4. Actinomycin D, which reportedly inhibits the transcription process (23) but which is also considered to interfere with the cytoplasmic synthesis of a soluble repressor (24), caused a considerable stimulation of induction at low concentrations, a phenomenon commonly reported in mammalian studies (24). At the highest concentration it appeared to cause a slight reversal of the epoxidase induction although the significance of this is questionable since no similar effect was observed with the N-demethylase induction. Cycloheximide, an inhibitor of translation (25), which also blocks transcription through inhibition of DNA-dependent RNA polymerase (26), caused an essentially complete reversal of enzyme induction (Table 4) at dietary concentrations of 50 ppm and puromycin, another inhibitor of protein synthesis (27), had a similar effect at much lower concentrations. In contrast to actinomycin D which caused little effect when administered alone to larvae, both cycloheximide and puromycin drastically reduced microsomal enzyme activity in control larvae and the gut tissues of the treated worms were noticeably thinner and more fragile. Both these compounds are reported to similarly reduce hepatic microsomal enzyme activity in rats (24). In studies with adult houseflies (3, 6) an almost complete block of induction by actinomycin D and cycloheximide was observed whereas puromycin had little or no effect. Although the mechanism with which induction occurs remains unclear the data in Table 4 would indicate that de novo protein synthesis is involved in the process.

The level of induction attained is directly related to the degree of exposure of the worms to the inducing agent in the diet. This is true with respect to both concentration (Figs. 1 and 2) and time (Figs. 3 and 4) as has been observed by several other investigators (5, 18). In contrast to N-demethylase activity which increases in an essentially linear manner up to dietary concentrations of pentamethylbenzene of 4000 ppm, epoxidase activity appears to be maximally induced at a much lower concentration and shows little additional response to concentrations of the inducer in excess of 2000 ppm; indeed there appears
to be a slight decrease in activity at high inducer concentrations. The reasons for this are not clear although Yu and Terriere (28) reported a similar effect while studying epoxidase activity in the housefly in response to increasing dosages of several cyclodiene inducers.

In agreement with the results of previous studies with insects (5, 19), the level of induction in the armyworm increases with increasing duration of exposure to the inducing agent, the largest effect being observed during the first 24-hr period and smaller though significant increments occurring during the next two days (Fig. 3). When the time course of the induction process is studied in more detail (Fig. 4) it becomes obvious that the microsomal enzymes in the armyworm gut tissues respond with extraordinary speed to the presence of the inducer. Because of the natural age-dependent changes in microsomal activity which occur during the course of the sixth instar, the levels of induction relative to the controls vary with age and are highest in newly molted larvae where activity is normally low. This clearly emphasizes that in order to obtain reproducible data it is essential to have control and induced insects exactly synchronized with respect to age (13). The maximum specific N-demethylase activity achieved with diets containing 2000 ppm pentamethylbenzene appears to be the same (18-19 nmoles product/min mg protein) whether the inducer is administered during the first or second 24 hr-period of the sixth instar. This suggests that under given conditions of inducer exposure, enzyme activity approaches a constant upper limit which is independent of the baseline activity in control insects. The remarkable flexibility of the microsomal control mechanism is further illustrated by the rapidity with which enzyme activity returns to control levels following termination of exposure to the inducer (Fig. 4). It probably reflects the rapid disappearance of the inducer from the tissues and is consistent with the view that induction constitutes a temporary adaptation which enables the organism to survive short-term exposure to environmental chemical stress (13).

Previous studies have established that although some activity is associated with the fat body and Malpighian tubules, the microsomal enzymes in the southern armyworm and other lepidopterous larvae are located mainly in the midgut tissues (10, 11). It has been suggested that the relative in vivo significance of the enzymes in different tissues in this and other insect species might depend on the route by which various foreign compounds enter the insect (13). Thus compounds entering via the digestive tract may be metabolized by the gut enzymes whereas those effecting direct cuticular penetration may be detoxified mainly by the enzymes in the fat body or Malpighian tubules. Based on similar reasoning the relative degree of microsomal enzyme induction in different tissues might be expected to depend on the route of administration of the inducer. Although in this investigation epoxidation and N-demethylation activities in tissues of the gut, Malpighian tubules and fat body were all enhanced following the treatment of armyworms for three days on diets containing 2000 ppm pentamethylbenzene (Table 3), the level of induction in the gut was substantially greater than that in the other tissues investigated. In contrast, studies on Madagascar cockroaches (Gromphadorhina portentosa) (29) have revealed that microsomal activities in gut, fat body, and Malpighian tubules are equally induced following injection with pheno-barbital when the inducer would be expected to have a more general distribution throughout the tissues.

The route of inducer administration and consequent variations in the distribution of induction in different organs and tissues might also provide an explanation for some of the in vivo effects of induction on insecticide tolerance. In mammalian induction studies the decrease in hexobarbital
sleeping time is commonly used as an *in vivo* indicator of enzyme induction (1), but similar changes in insecticide tolerance in insects has not always been observed following induction (19, 30). In an early attempt to establish a relationship between induction and insecticide tolerance, Morello (31) observed a 12% decrease in mortality to DDT and an 11% increase in the *in vivo* production of polar metabolites from DDT following the treatment of *Triatoma infestans* with 3-methylcholanthrene. More recently Yu and Terriere (19) have demonstrated that although microsomal oxidation in houseflies was enhanced up to 25-fold following phenobarbital treatment this had only a small effect on the *in vivo* susceptibility of the flies to propoxur (o-isopropoxphenyl N-methylecarbamate). In this investigation, however, the phenobarbital was administered orally to the flies and the toxicity of the propoxur was assessed by topical application. Under these conditions it is possible that the major site of induction is in the gut tissues where enhanced enzyme activity may be of little consequence in protecting the flies from propoxur entering via the cuticle. The results of the present investigation in which the inducer was administered orally to the armyworms, and where a definite effect on microsomal activity in the gut tissues was demonstrated, reveal a dramatic effect on the *in vivo* susceptibility of the larvae to orally administered carbaryl which is known to be metabolized mainly by microsomal oxidation (32). Thus armyworms with microsomal activity in the gut tissues enhanced 3-fold as the result of a 3-day exposure to diets containing 2000 ppm pentamethylbenzene exhibited a remarkable 11-fold increase in tolerance to orally administered carbaryl (Fig. 7). A similar though less marked effect was also observed following treatment with hexamethylbenzene, a less potent inducing agent. Ahmad and Brindley (5) found that orally administered chlorcyclizine, phenobarbital, and aminopyrine were all effective in protecting sixth instar wax moth larvae from the oral toxicity of parathion. Consequently, it would appear that in insects, induction is related to *in vivo* tolerance to insecticides provided that the inducing agent is administered by a route which induces the enzymes in the tissue likely to be most important in the detoxication process.

Whether enzyme induction has any practical importance in enabling pest insects to survive insecticide treatment under field conditions is open to some question. The possibility that this could occur as a result of the presence of the insecticide _per se_ is doubtful since, at least with lepidopterous larvae which appear to be quite refractory to induction, it is almost certain that the lethal effects of the insecticide would precede enzyme induction. However, in view of the inductive potency of several of the lower aromatic hydrocarbons used in this study the possibility that induction might occur from the presence of various spray solvents and other insecticidal inert additives deserves further attention.

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