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MICROSOMAL MIXED-FUNCTION OXIDASES IN INSECTS—I

LOCALIZATION AND PROPERTIES OF AN ENZYME SYSTEM EFFECTING ALDRIN EPOXIDATION IN LARVAE OF THE SOUTHERN ARMYWORM (*PRODENIA ERIDANIA*)*

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Abstract—An extremely active microsomal epoxidase has been obtained from larvae of the southern armyworm (*Prodenia eridania*) and optimal assay conditions *in vitro* have been established. Maximum enzyme activity is associated primarily with the larval mid-gut. Specific activity of the enzyme changes markedly during larval development and reaches a maximum in the sixth instar.

THE VAST majority of lipophilic xenobiotics, including drugs and insecticides, are subject to primary oxidative detoxication by enzymes comprizing the mixed-function oxidase complex associated with mammalian liver microsomes.[1, 2] These enzymes, requiring NADPH and O_2, show a remarkable ability to metabolize the diversity of structural features commonly incorporated into drugs and pesticides and their reactions include the hydroxylation of aromatic[3] and alicyclic[4] rings, the *N*-dealkylation of substituted amines,[5, 6] and the *O*-dealkylation of aromatic ethers,[6] the oxidation of phosphorothionates (P=S) to phosphates (P=O)[7–10] and the epoxidation of double bonds.[11–14]

In contrast to the considerable amount of information which is presently available on the microsomal enzymes obtained from mammalian liver, current knowledge of the nature of these enzymes in insect species is sparse and incomplete, though it is clear that they play a dominant role in insecticide metabolism,[15–17] and are, therefore, of fundamental significance in determining such important phenomena as selective toxicity and insect resistance to insecticides.

Although it appears likely that the microsomal enzymes in insects are basically similar to those found in mammalian liver, their complete analogy should not be tacitly assumed and many fundamental problems regarding the insect's detoxication system remain unsolved.

Based on this premise, the following investigation involving the southern armyworm comprizes part of an extensive study directed towards obtaining basic information on the microsomal enzymes of insect species. The epoxidation of aldrin is employed throughout as a measure of microsomal-enzyme activity.

* A preliminary report of this work was presented at the National Meeting of the Entomological Society of America, New York City, November 27–30, 1967.

MATERIALS AND METHODS

Insects. Larvae of *Prodenia eridania* (Cramer) were reared continuously under greenhouse conditions with natural lighting from eggs originally supplied by Niagara Chemical Division, FMC Corp., Middleport, N. Y. Larvae were fed on bifoliate leaves of red kidney bean (*Phaseolus vulgaris*) in $2\frac{1}{2}$ ft $\times$ $2\frac{1}{2}$ ft $\times$ 2 ft cages covered with nylon organdy and provided with a 2-in. base of sand for pupation. Some larvae of each generation were reared through to adults to maintain the cultures. Adults were kept under conditions of low-light intensity and were provided with sucrose solution. Eggs were laid on wax paper and subsequently transferred to bean plants.

Chemicals. Analytical grade samples of aldrin (1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-1,4-endo-exo-5,8-dimethanonaphthalene) and its 6,7-epoxide, dieldrin, were kindly supplied by the Shell Development Co., Modesto, Calif. Smith, Kline & French, Philadelphia, Penn., furnished the 2-diethylaminoethyl-2,2-diphenylvalerate (SKF 525-A) and the 4,5,6,7-tetrachloro-1,3-benzodioxole was synthesized in this laboratory by the procedure described elsewhere.[18] Glucose 6-phosphate (G6-P), G6-P dehydrogenase, NADP, NADH, FAD, FMN and reduced glutathione were purchased from Calbiochem, Los Angeles, Calif. All other chemicals and solvents employed were analytical reagent grade.

Tissue preparations. Following excision of the head and tip of the abdomen, the entire larval gut was removed posteriorly and was placed on a paper tissue. The dark green gut contents were effectively removed by a rolling motion of the finger and the remaining gut tissue placed immediately in ice-cold 1·15% (w/v) KCl. After thorough rinsing, the gut tissues were homogenized in a hand-operated, Potter-Elvehjem tissue grinder in additional cold 1·15% KCl such that the concentration was five guts per ml with the sixth instar. Preparations from earlier instars entailed the use of more larvae to attain approximately equivalent protein concentration. The fraction, henceforth designated crude homogenate, was obtained as the supernatant of the initial homogenate following centrifugation at 1000 g_{max} in an International Equipment Company (IEC) clinical centrifuge. Gut-tissue microsomes were obtained by centrifugation for 1 hr at 105,000 g_{max} of the supernatant resulting from an earlier 15 min spin at 12,000 g_{max}. Centrifugation was accomplished with an IEC B-60 ultracentrifuge employing an A-211 angle-head rotor and all operations were effected at 0–4°. The microsomal pellet was resuspended in an appropriate volume of 1·15% KCl and, as with crude homogenate, was used without delay.

Preparations from tissues other than the gut were obtained in a manner analogous to that described for the crude homogenate.

The protein concentration of each preparation was determined by a modified Biuret method employing bovine serum albumin as a standard.[19]

Incubation, extraction and assay procedure. Incubations were carried out aerobically in 25-ml flasks shaken in a water bath at 30°. The procedures were similar to those previously reported.[14] Incubation time was 15 min. A standard 5·1-ml incubation mixture contained: Tris-HCl buffer, pH 7·8 ($5{\cdot}0 \times 10^{-2}$ M); G6-P ($2{\cdot}4 \times 10^{-3}$ M); G6-P dehydrogenase (1·6 units); NADP ($5{\cdot}1 \times 10^{-5}$ M); KCl ($2{\cdot}7 \times 10^{-3}$ M) and 0·5 ml of either crude homogenate or microsomal suspension. Following temperature equilibration, the reaction was initiated by addition of 100 μg aldrin in 100 μl ethanol. The reaction was terminated by addition of 4 ml acetone, the flask contents transferred to 45-ml glass-stoppered tubes and the reaction flasks rinsed with an additional 3 ml

acetone. Following addition of a small amount of sodium sulfate, the contents of each tube were extracted twice with 4-ml aliquots of petroleum ether, shaking being accomplished with a vortex mixer. The combined extract was adjusted to 10 ml and after appropriate dilution was assayed for dieldrin.

Dieldrin assay was carried out with a Research Specialities gas chromatograph provided with a ^{90}Sr ionization detector connected for electron capture. All-glass columns (4′) packed with 5% SE 30 on Gas Chrom Q were employed and column temperature was 180°. Nitrogen was the carrier gas. Dieldrin was determined by the peak-height method from standard curves obtained under identical gas chromatographic conditions on the same day and under the conditions of analysis employed, no metabolite other than dieldrin was observed.

Throughout this paper a unit of enzyme activity is defined as 1 millimicromole of dieldrin produced per minute per milligram of protein.

RESULTS

Optimal assay conditions

The following data, except for those of the tissue distribution study, were obtained using microsomes or crude homogenates of gut tissue from sixth instar *Prodenia* larvae, after having previously established that epoxidation activity was chiefly associated with that organ (see later section). All the results reported comprize the mean values of several incubations.

pH Optimum for microsomal epoxidation

The pH optimum was established with two buffer systems, Tris-HCl and Tris-phosphate (both 5×10^{-2} M), over the pH range 6·1–8·8 (Fig. 1). In both cases a

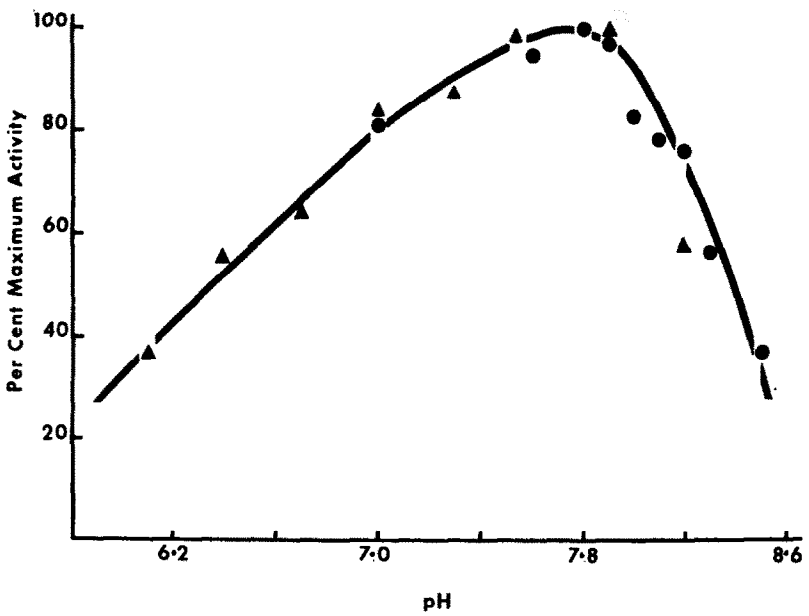

FIG. 1. Effect of pH on aldrin epoxidation. Incubation mixture and conditions are as described in Table 1 except 0·5 ml crude homogenate employed. ▲ = Tris-phosphate and ● = Tris-HCl buffer.

steady increase in epoxidation was observed as the pH was increased above 6·0 and optimum activity was found at pH 7·8, thereafter declining with further increase in alkalinity.

Linearity of epoxidation with time and protein concentration

As indicated in Fig. 2, the rate of epoxidation remained linear up to about 20 min, after which it rapidly leveled off. All incubations were, therefore, effected over a 15-min period.

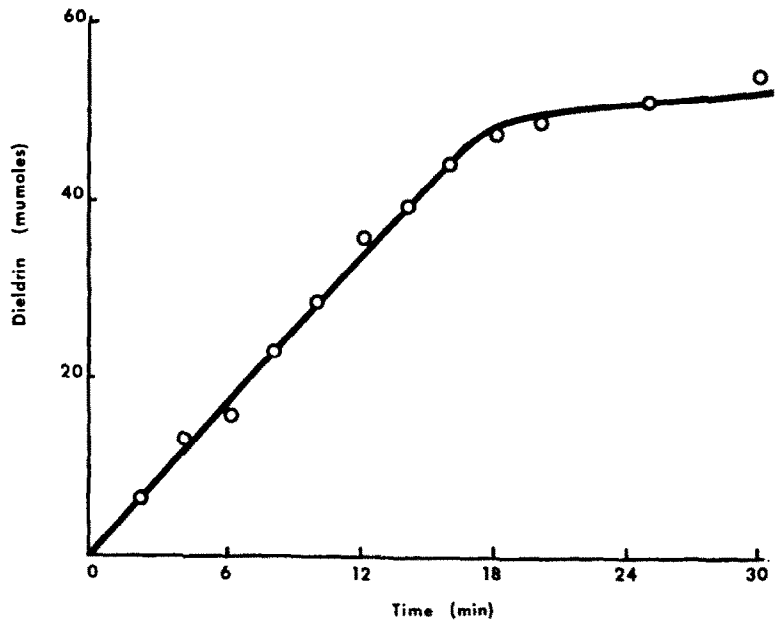

FIG. 2. Rate of aldrin epoxidation. Incubation in 250-ml Erlenmeyer flask with final concentrations of reagents as indicated in Table 1. Five-ml aliquots withdrawn at appropriate times.

The formation of dieldrin was found to be essentially linear with protein concentration over the range (2 ± 1 mg protein/incubation) normally employed. However, plots of epoxidation vs. protein concentration (Fig. 3) consistently exhibited an exponential trend, the reason for which is not immediately obvious, although this may be associated with substrate insolubility as discussed later.

Effect of cofactors and inhibitors on epoxidation

Dieldrin formation was dependent on the presence of both NADPH and oxygen (Table 1) and the former requirement was not satisfied by NADH. Omission of G6-P dehydrogenase from the NADPH generating system resulted in only a slight decrease in dieldrin formation, thus indicating the presence of this enzyme in the microsomal preparation. Apart from FMN which showed slight inhibition, the other cofactors evaluated exhibited no marked effect at 10^{-3} M.

In agreement with previous reports[12, 14] both SKF 525-A and derivatives of 1,3-benzodioxole were found to be effective inhibitors of epoxidation (Table 2).

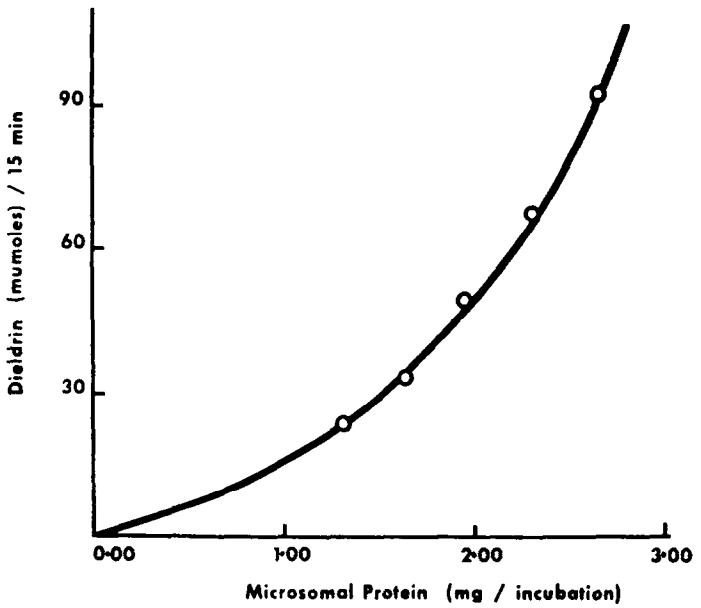

FIG. 3. Effect of microsomal protein on aldrin epoxidation. Incubation mixture and conditions as described in Table 1.

TABLE 1. REQUIREMENTS OF THE GUT-MICROSOMAL EPOXIDASE*

Incubation medium	Per cent activity
Complete	100
Minus NADP	1
Minus G6-P dehydrogenase	79
Anaerobic (N_2)	4
Minus NADP plus NADH (10^{-3} M)	4
Plus FMN (10^{-3} M)	88
Plus FAD (10^{-3} M)	107
Plus EDTA (10^{-3} M)	112
Plus nicotinamide (10^{-3} M)	100
Plus glutathione (10^{-3} M)	100

* Incubations at 30° for 15 min. Complete incubation medium (final concentration): Tris-HCl, pH 7·8 (5×10^{-5} M); G6-P ($2{\cdot}4 \times 10^{-3}$ M); KCl ($2{\cdot}6 \times 10^{-2}$ M); NADP ($5{\cdot}1 \times 10^{-5}$ M); G6-P dehydrogenase (1·6 units); 0·5 ml microsomal suspension (approximately 1·5 mg protein); aldrin (100 μg) in ethanol (100 μl); total volume, 5 ml.

TABLE 2. EFFECT OF INHIBITORS ON MICROSOMAL EPOXIDATION*

Inhibitor	I_{50} (molar)
SKF 525-A	$1{\cdot}3 \times 10^{-5}$
4,5,6,7-tetrachloro-1,3-benzodioxole	$7{\cdot}7 \times 10^{-7}$

* Incubations at 30° for 10 min. Complete incubation medium as in Table 1. Reaction initiated with addition of microsomal suspension.

Effect of metal ions on epoxidation

The effect of various metal ions on the epoxidation *in vitro* of aldrin by gut microsomes is shown in Table 3. Of those evaluated the most effective inhibitor was cupric ion which resulted in 9 per cent inhibition at a concentration as low as 10^{-7} M. Some inhibition was observed with cobalt and nickel at 10^{-5} M but even at concentrations of 10^{-3} M no marked effects were noted with the remainder.

Effect of substrate concentration

A typical Lineweaver–Burk reciprocal plot for epoxidation by *Prodenia* gut tissue is shown in Fig. 4. This indicates a K_m value of $5{\cdot}9 \times 10^{-5}$ M.

TABLE 3. EFFECT OF METAL IONS ON EPOXIDATION*

Addition to complete incubation medium		Per cent activity
None		100
Cu^{++}	10^{-3} M	0
	10^{-5} M	29
	10^{-7} M	91
Co^{++}	10^{-3} M	55
	10^{-4} M	60
	10^{-5} M	87
Ni^{++}	10^{-3} M	57
	10^{-4} M	62
	10^{-5} M	78
Fe^{+++}	10^{-3} M	79
Mn^{++}	10^{-3} M	78
Mg^{++}	10^{-3} M	94

* Incubation at 30° for 15 min. Complete incubation medium as detailed in Table 1.

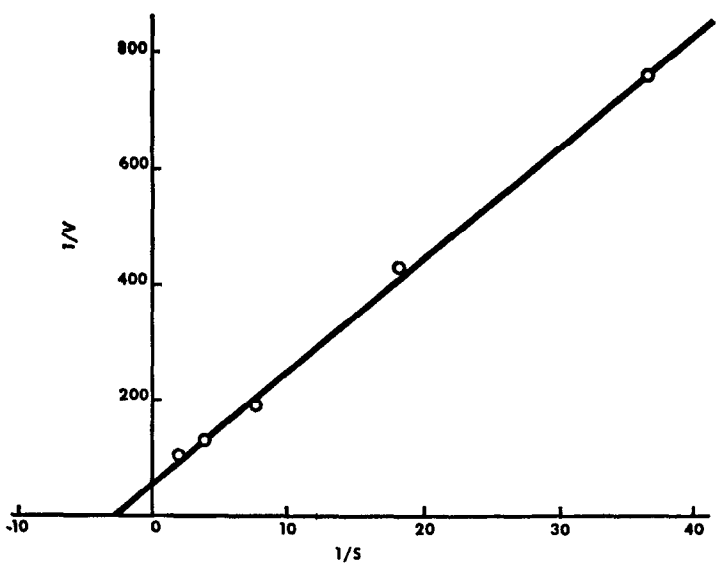

FIG. 4. Lineweaver–Burk plot for dieldrin formation. Incubation mixture and conditions as indicated in Table 1. S = aldrin added (μmoles) V = dieldrin produced (μmoles/min).

Localization of microsomal epoxidase activity

Initial experiments showed that crude homogenates or microsomes prepared from whole sixth instar larvae contained no epoxidation activity. Active preparations were obtained, however, after fractionation of larvae into gut, fat body, Malpighian tubules, head and skeletal tissue, the latter consisting mainly of muscle and exoskeleton. The results are shown in Fig. 5, from which it is clear that maximum activity is localized in the gut tissues. The Malpighian tubules and, rather surprizingly, the

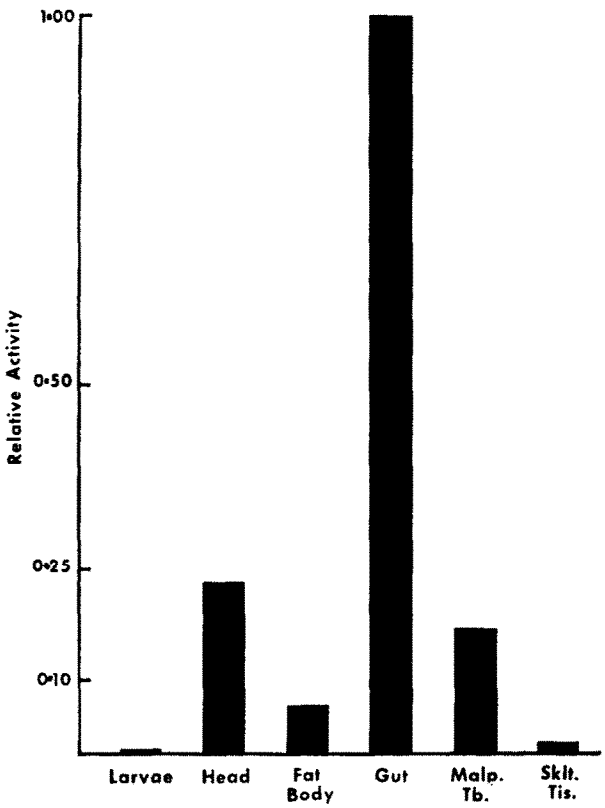

FIG. 5. Localization of epoxidase activity. Crude homogenates of the several tissues incubated under conditions described in Table 1. Specific activities indicated relative to the gut preparation.

head capsule show activities of 0·15 and 0·22 relative to that of the gut. Only marginal activity was obtained from homogenates of fat body and skeletal tissue. Subsequent fractionation studies established that activity was almost exclusively associated with the mid-gut (Fig. 6), relatively slight activity (about 0·1) being found with homogenates of fore-gut or hind-gut.

Intracellular localization of enzyme activity

Table 4 shows the epoxidation activity observed in several sub-cellular fractions obtained by differential centrifugation. The microsomal fraction clearly exhibits the greatest activity of 2·145 units. The 0·065 units of activity in the 12,000 g_{max} sediment,

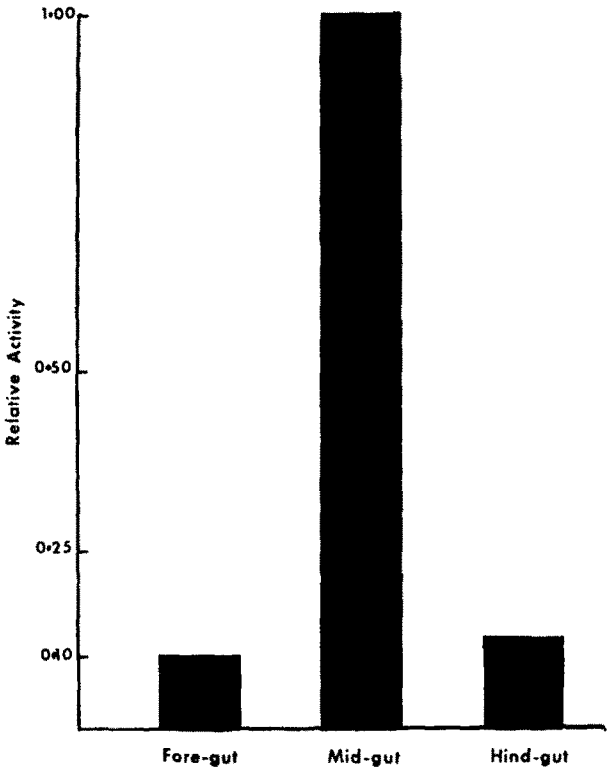

FIG. 6. Relative specific activities of three sections of the larval gut. Crude homogenates incubated under conditions described in Table 1.

TABLE 4. INTRACELLULAR LOCALIZATION OF GUT EPOXIDASE*

Fraction	Total protein (mg)	Total activity (mμmoles dieldrin/min)	Relative specific activity
Tissue homogenate	96·5	43·5	0·37
Mitochondria, nuclei, cell debris	64·5	14·2	0·17
Microsomal fraction	17·8	22·2	1·00
Soluble fraction	7·5	3·3	0·09

* Fractions obtained by centrifugation as outlined under Materials and Methods. Incubation at 30° for 15 min. Complete incubation mixture as described in Table 1.

comprizing the mitochondria, nuclei and larger cell debris, and also the slight activity (0·011 units) in the microsomal supernatant may be due to microsomal contamination.

Development of epoxidation through larval instars

Hepatic microsomal enzyme activity is known to vary considerably with age in mammalian species[20, 21] and it was, therefore, of interest to examine whether similar changes occur through the several developmental stages of the southern armyworm. For this purpose larval instars were differentiated by head capsule measurement as described by Redfern.[22] A profile of the epoxidation activity of gut homogenates of

the several instars (Fig. 7) shows that quite remarkable changes in specific activity take place as the larvae mature. As a result of the small size of first and second instar it was not possible to prepare gut homogenates and, as with the later instars, preparations from whole larvae were devoid of measurable activity. Epoxidation activity of gut homogenates of third and fourth instar larvae was observed to be low, but during the fifth instar this was found to rise dramatically and eventually reach a

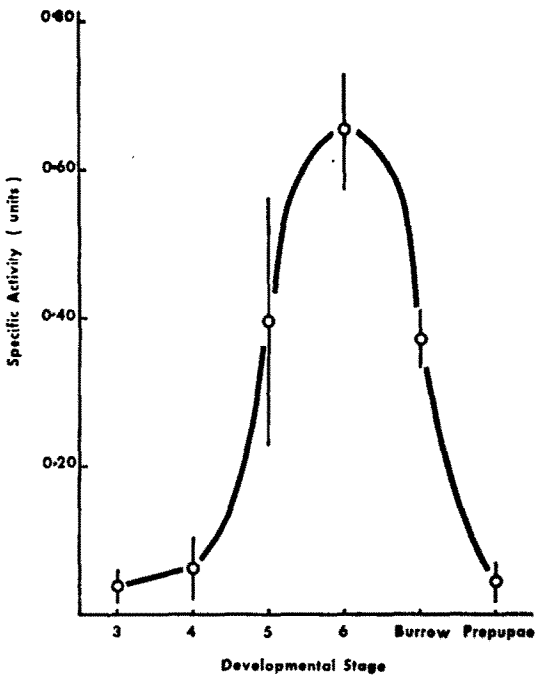

FIG. 7. Development of epoxidase through several instars. Incubation mixture and conditions as described in Table 1, but employing 0·5 ml crude homogenates. Standard deviation from mean indicated by vertical bars. Replication of each instar as follows: third (8), fourth (7), fifth (9) and sixth (13) burrowing larvae (5) and prepupae (6).

maximum in the sixth instar. The net change from third to sixth instars represents a 30-fold increase in specific activity of the epoxidase enzyme. After achieving maximum size in the sixth instar, the larvae clear their guts of food material, move from the plant and burrow into the sand prior to pupation. Epoxidation activity of gut homogenates of the "burrowing larvae" was found to be considerably lower than that in actively feeding sixth instar larvae, and by the time the pre-pupal stage was reached the activity had decreased to a level approximately equal to that in the third instar. No epoxidation was observed in egg homogenates and the morphological nature of the threadlike adult gut prohibited any determination of enzymatic activity in this tissue.

DISCUSSION

In mammalian species, the mixed-function oxidases responsible for drug and insecticide metabolism are conveniently associated with the endoplasmic reticulum of one organ, the liver, the latter thus providing a relatively homogenous source of

material from which to prepare microsomes. In contrast to this, investigators working with insect species have relied chiefly on microsomal preparations from whole insects.

Among the reactions shown to be catalysed by suitably fortified microsomes from whole houseflies are the hydroxylation of naphthalene,[3] the epoxidation of aldrin[13, 14] and heptachlor,[12] and the hydroxylation of other cyclodienes[4] and DDT.[23] Aldrin epoxidation* and DDT hydroxylation[23] have also been demonstrated in microsomes prepared from whole homogenates of the German cockroach (*Blattela germanica*) and parathion activation has been shown to occur in similar preparations from the rice stem borer (*Chilo suppressalis*).[24] Primary oxidative metabolism of the carbamates,[5,6,25] pyrethrins,[26] rotenone[27] and the 1,3-benzodioxole synergists[28] is effected by microsomes prepared from housefly abdomens. These investigations have succeeded in establishing that, qualitatively at least, the pathways of microsomal metabolism in insects are very similar to those found in mammalian liver. There are, however, a number of serious objections to using microsomes prepared from such heterogenous material as whole insects and it is questionable whether the results accurately reflect the real situation.

Whether, as in mammals, insects possess some specific organ or tissue in which drug-metabolizing enzymes are localized has been a question open to some discussion. Figures 5 and 6 clearly show that in the case of the southern armyworm epoxidation activity is almost exclusively confined to the tissues of the gut, more specifically the mid-gut. Additional evidence obtained in this laboratory from about twenty other species suggests that this is a pattern of localization common to lepidopterous larvae. These data are in apparent contradiction to results obtained with *Periplaneta americana* in which epoxidation[12] and desulfuration[9, 29] activity were found to be associated chiefly with the fat body, though some desulfuration was also determined in gut, caeca and Malpighian tubules. It has also been established that with the locust, homogenates of fat body show maximum activity with regard to the oxidation of *p*-nitrotoluene.[30] As data on more species are accumulated it will be of considerable interest to note whether the patterns of localization differ in different orders of insects.

It is, therefore, clear that the microsomal pellet obtained from homogenates of whole insects contains a large percentage of material arising from the endoplasmic reticulum of inactive tissues, the net result of which will be to lower the specific activity of the enzyme as measured *in vitro*. This factor alone could account for the apparently low activity *in vitro* often observed in homogenates of whole insects. As can be seen from the comparative data in Table 5, the specific activity of the epoxidase enzyme from *Prodenia* gut tissues is considerably greater than that previously reported for a number of other organisms. It surpasses the most active mammalian liver preparation by a factor of almost 10 and is 50-fold greater than the values reported for microsomal preparations from whole houseflies. Probably of more importance than the tissue dilution factor is the fact that homogenization of whole insects often results in the liberation of materials which are effective inhibitors of microsomal mixed-function oxidation.[15, 30]

A number of what appear to be different inhibitors have now been demonstrated in several species including houseflies,[31] where they appear to be concentrated in the head and thorax,[25, 32] and also in homogenates of locust guts and fat bodies.[30, 32]

* J. W. Ray, personal communication.

TABLE 5. ACTIVITY OF MICROSOMAL EPOXIDASE SYSTEMS IN VARIOUS SPECIES

Source	Specific activity (mμmoles/min/mg protein × 10^3)	Reference No.
Vertebrate (liver)		
Rabbit*	344†	12
Rat*	274†	12
Pig‡	273	14
Mouse‡ (male)	78	34
(female)	176	34
Quail (male)	108	33
(female)	9	33
Rat‡ (male)	84	33
(female)	21	33
Trout‡	6	33
Invertebrate		
Southern armyworm (gut)‡ (*Prodenia eridania*)	2145	
Housefly (whole)‡ (*Musca domestica*)	41	33
Housefly (whole)‡ (*Musca vicina*)	25	13
Blowfly (whole)‡ (*Phormia regina*)	3	33

* Microsomal protein nitrogen estimated by micro-Kjeldahl method.
† Calculated assuming 1 mg protein nitrogen equivalent to 6·25 mg microsomal protein.
‡ Biuret protein estimation using bovine serum albumin standard.

Although the natural function *in vivo* of these inhibitors remains to be elucidated, their presence undoubtedly explains the enzymatic inactivity of many preparations from whole insects. The complete absence of any epoxidation activity in whole armyworms initially suggested the presence of some endogenous inhibitory material in this species. Subsequent investigations have shown that a potent inhibitor of microsomal enzymes is indeed present in the gut contents of the larvae and work is presently in progress to characterize this material. Initial data indicate that the inhibitor is, or is closely associated with, a soluble, high molecular weight protein. In addition to inhibiting microsomal enzymes from armyworm gut tissues, the material inhibits mixed-function oxidation in microsomes obtained from mouse liver.

Studies *in vitro* indicate that the epoxidase from *Prodenia* gut tissues is a typical mixed-function oxidase and, as with those from mammalian liver, is associated with the microsomal fraction of cell homogenates (Table 4). The optimum pH of 7·8 (Fig. 1) lies between the values reported for mammals (pH 7·2–7·4)[14] and houseflies (pH 8·2).[13]

In agreement with its classification as a mixed-function oxidase, the epoxidase system was found to require both NADPH and oxygen, anaerobic incubations in a nitrogen atmosphere reducing epoxidase activity by 96 per cent. The almost complete failure of NADH to promote epoxidation indicates a high degree of selectivity of the enzyme for NADPH. Both SKF 525-A and 4,5,6,7-tetrachloro-1,3-benzodioxole are effective inhibitors of epoxidation (Table 2). The I_{50} values of $1{\cdot}3 \times 10^{-5}$ M and $7{\cdot}7 \times 10^{-7}$ M, respectively, indicate high sensitivity of the enzyme to these compounds and substantiate the finding that the mixed-function oxidases in insects are generally more susceptible to inhibition than similar enzymes from mammalian liver.[14]

Microsomal lipid peroxidation is often found to compete with measurements *in vitro* of aldrin epoxidation in microsomal preparations from mammalian liver and this

problem can be overcome by the inclusion of EDTA, Mn^{++}, Co^{++} or antioxidants in the incubation medium.[14] Although some stimulation of epoxidase activity was observed in the presence of 10^{-3} M EDTA, the inhibition resulting from inclusion of Mn^{++} and Co^{++} in the incubation mixture suggests that, as with houseflies,[14] microsomal lipid peroxidation is of little significance in gut tissue preparations from the southern armyworm. Nicotinamide, which is often added routinely in microsomal preparations to inhibit the pyridine nucleotidase responsible for breakdown of NADP,[33] showed no simulatory effect on epoxidation and was omitted from the incubation mixture.

The apparent K_m value for the epoxidase from *Prodenia* gut is calculated as $5{\cdot}9 \times 10^{-5}$ M. However, as discussed elsewhere,[14] the real significance of K_m value is open to some question when applied to substrates such as aldrin which have extremely low aqueous solubility. In agreement with a previous report,[14] the amount of dieldrin produced by a given amount of enzyme was found to be independent of the total volume of the reaction mixture (over a 5-fold range) and was related directly to the absolute amount of aldrin added. It, therefore, appears that the aldrin is solubilized in the microsomal lipids and is thus effectively removed from the bulk of the reaction mixture, although it obviously remains available to the enzyme in this form. The exponential nature of the protein vs. activity plot (Fig. 3) could result from this phenomenon as at the low levels of enzyme employed it is possible that the correspondingly low microsomal lipid concentration becomes a limiting factor. Thus, the observed activity of the enzyme at low concentration will be smaller than expected as a result of saturation with aldrin of the microsomal lipids and a consequent lack of availability of substrate to the active site of the enzyme.

The remarkable changes in gut-epoxidase activity through the different larval instars of *Prodenia eridania* are of considerable biochemical interest as well as potential practical significance. Similar activity profiles have been observed through the larval instars of a number of other species suggesting that this may be a general phenomenon in lepidopterous larvae. The situation is not entirely dissimilar to that found in vertebrates where new-born mammals are found to lack the necessary enzymes for hepatic drug metabolizing activity.[20, 21] It is not yet clear whether the large increase in gut tissue epoxidase activity from the third through the sixth instars (Fig. 7) is controlled hormonally (internal stimulus) or whether it is in some way related to the feeding habits of the larvae (external stimulus). Certainly the activity increases in a manner which directly parallels the amount of food material consumed and begins to decrease at about the time that feeding ceases in the sixth instar and the larvae prepare to pupate. It is, therefore, of interest to speculate whether the observed increase in microsomal mixed-function oxidation represents some form of natural induction process reflecting a response of the insect to materials in its food. Work is in progress to determine if the nature of the host plant has any effect on the oxidative capacity of the larvae. Alternatively, it is entirely possible that the changes in epoxidase activity are under hormonal control and that the endogenous inhibitor, to which reference has already been made, is in some manner associated with the control mechanism *in vivo*. More information is required before any definite conclusion can be reached on this interesting finding.

It is clear from the results presented here that in all biochemical investigations involving insects, every species should initially be treated as a new and different

problem. Although it is likely that the enzymes themselves are indeed similar to those found in mammalian liver, their patterns of tissue localization and development cannot be assumed or predicted. Such information is urgently required to enable us to better understand our insect pests and to devise new methods to effect their control.

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