Epoxidation of Aldrin to Dieldrin by Lobsters

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The metabolism of insecticides is important in regard to the toxicity and storage as either the parent compound or as metabolites. Of particular interest to many investigators has been the conversion of aldrin to dieldrin. This epoxidation has been shown to occur in several species including mammals and poultry (BANN et al., 1956), houseflies (BROOKS, 1960), locusts (COHEN, and SMITH, 1961), soil microorganisms (LICHTENSTEIN and SCHULZ, 1960), a large number of Lepidoptera species (KRIEGER et al., 1971), freshwater fish (LUDKE et al., 1971), and a number of freshwater invertebrates including protozoa, coelenterates, worms, arthropods and mulluscs (KHAN et al., 1972b).

KHAN et al. (1972b) point out the importance of the systems responsible for such pesticide conversions as regards the susceptibility of a particular species and also other members in a food chain. Of particular interest to this laboratory is pesticide metabolism in lobsters (Homarus americanus), an important economic species utilized in great numbers by man for food. Little is known, however, about the ability of this species to metabolize foreign organic compounds with which it comes into contact in its environment. BRODIE and MAICKEL (1962) found that the hepatopancreas was capable of metabolizing several drugs. MELLETT et al. (1969) found that lobsters could metabolize the drug cyclophosphamide. Previous studies (CARLSON, 1973) have also demonstrated the in vitro metabolism of parathion to p-nitrophenol by lobster hepatopancreas.

MATERIALS AND METHODS

Male lobsters were obtained locally (Pt. Judith Fishermen's Cooperative, Galilee, Rhode Island) and were maintained in artificial seawater at 10°C. Adult male rats were obtained from Charles River Breeding Laboratories (Wilmington, Massachusetts) and were housed in air-conditioned rooms with free access to food and water. Both groups of animals had lighting maintained on 12 hr

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light-dark cycles. The aldrin used as substrate and dieldrin as authentic product were 99+% pure (Supelco, Inc., Bellefonte, Pa.).

The incubation medium was similar to that of KHAN et al. (1972a). Hepatopancreases from lobsters were removed, blotted gently, weighed and homogenized in 0.1 M phosphate buffer (pH 8.0) to make a 20 per cent homogenate. The homogenate was centrifuged at 9000 g in a Servall refrigerated centrifuge for 20 minutes. The reaction was initiated by the addition of 0.40 ml of the 9000 g fraction to the incubation medium which contained 0.1 ml NADPH (48 mg/ml), 0.1 ml glucose-6-phosphate (48 mg/ml), 0.01 ml aldrin (18.25 mg/5 ml isopropyl alcohol), and 1.39 ml phosphate buffer (0.10 M, pH 8.0). The incubations were carried out in a Dubnoff metabolic shaker and were found to be linear for 30 min at the end of which time the reaction was terminated by the addition of 2 ml of hexane. After thorough mixing and separation of the layers, 10 µl of the upper hexane layer were analyzed for dieldrin using a Packard gas chromatograph equipped with an electron capture detector. The temperatures were: inlet 230, column 195 and detector 200°C. The column was 1.5% SP-2250 and 1.95% SP-2401 on Supelcon AW DMCS. Peak areas were compared with dieldrin standards.

When subfraction experiments were conducted, the 9000 g fraction of lobster hepatopancreas was further centrifuged at 105,000 g for 1 hr in an IEC Ultracentrifuge. The microsomes were then resuspended in phosphate buffer.

The measurement of cytochrome P-450 was carried out according to the method of DALLNER (1963). Microsomes from lobster hepatopancreas were prepared as above except that it was necessary to add 1 mM dithiothreotol in order to prevent breakdown to cytochrome P-420. Microsomes were also prepared from the livers of rats which were perfused with isotonic KCl. Microsomal protein content was determined using the method of LOWRY et al. (1951).

RESULTS

The pH profile of the epoxidation reaction was determined by incubating at 37°C using phosphate buffers of pH 6.0, 7.0, 8.0 and 9.0. The data in Table 1 indicate that the pH optimum was 8.0. There were severe decreases one pH unit either above or below 8.0. This is similar to the sharp peak found for aldrin to dieldrin conversion in crayfish hepatopancreas and gut (KHAN et al., 1972a, b).

TABLE 1

Effect of pH on Aldrin Epoxidation

pH	N	Dieldrin Formed[a]
6.0	7	1.3 ± 0.19[b]
7.0	5	0.9 ± 0.18
8.0	9	1.9 ± 1.24
9.0	2	1.1 ± 0.08

[a]Nanograms dieldrin/mg tissue/30 min

[b]Mean ± S.E.

The effect of temperature on the conversion of aldrin to dieldrin was investigated by incubating one portion of a 9000 g fraction and another portion of the same sample at 20°C. As shown in Table 2 the reaction was not very temperature sensitive. The 20 per cent difference in dieldrin formation is not statistically significant using Student's t test at the 5% level. Subsequent experiments, however, were carried out at 20°C. If the tissue was heated for 10 min in a boiling water bath, no dieldrin was found indicating that the system was destroyed by boiling and that there was no nonenzymatic conversion of aldrin to dieldrin under the conditions employed.

TABLE 2

Effect of Temperature on Aldrin Epoxidation

Temperature (°C)	Number	Dieldrin Formed[a]
20	9	1.2 ± 0.15[b]
37	9	1.0 ± 0.16

[a]Nanograms dieldrin/mg tissue/30 min

[b]Mean ± S.E.

Further centrifugation of the 9000 g fraction at 105,000 g allowed for the separate 105,000 g soluble and microsomal fractions to be assayed. The values given in Table 3 are expressed as per equivalent to mg of whole tissue. Although much of the activity was lost by this procedure, most of the activity remaining appeared to be in the soluble portion rather than the microsomal fraction.

TABLE 3

Subcellular Distribution of Aldrin Epoxidation Activity

Fraction	Dieldrin Formed[a]
105,000 g Soluble	0.14 ± 0.05[b]
Microsomal	0.04 ± 0.02

[a]Nanograms dieldrin/equivalent of 1 mg whole tissue/30 min

[b]Mean ± S.E. for 3 replications

The findings by others of mixed function oxidase activity in lobsters and the detection in these experiments of aldrin epoxidation implied that lobster hepatopancreas contains an electron transport system of some sort. It was important, therefore, to determine if lobster hepatopancreas contained cytochrome P-450 and to compare the amount found to the much more enzymatically active rat liver. The results of the cytochrome P-450 determinations are presented in Table 4. The cytochrome P-450 content of lobster hepatopancreas was less than one-fourth that of rat liver on a per milligram of protein basis.

TABLE 4

Cytochrome P-450 Content of Lobster and Rat Microsomes

Species	Number	P-450[a]
Lobster	5	87 ± 9.3[b]
Rat	3	381 ± 62.9

[a] $\Delta OD_{450-500}$/mg microsomal protein

[b] Mean ± S.E.

DISCUSSION

These in vitro experiments indicate that the lobster does possess the capability for the metabolism of aldrin to the more stable dieldrin. Characterization of this epoxidation in the hepatopancreas revealed that the pH optimum is 8.0 and that there is slightly greater activity at 20°C than at 37°C. This higher activity at lower temperature is in agreement with data reported for the metabolism of the organophosphate insecticide parathion to p-nitrophenol (CARLSON, 1973).

The subcellular distribution studies which demonstrated that most of the activity was located in the 105,000 g soluble portion of the hepatopancreas rather than in the microsomes are surprising. KHAN et al. (1972a) reported that in crayfish, 80% of this epoxidase activity resided in the microsomal fraction. This low activity may be a reflection of the small amount of cytochrome P-450 which was found to be present in the lobster microsomes.

The findings of this investigation that lobsters have the ability to oxidize aldrin to dieldrin along with the studies of other investigators demonstrating other oxidase activities indicate that this species is capable of metabolizing xenobiotics such as pesticides and other pollutants it may encounter in its marine environment. These studies also support the idea of KHAN et al. (1972a, b), LUDKE et al. (1972) and others that methodology should be adapted to the particular species being studied in order to obtain meaningful results.

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