Comparison of the Metabolism of Parathion by Lobsters and Rats

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It is well known that the phosphorothionate insecticides require activation to their oxygen analogs by enzymatic mechanisms in order to be lethal to the species involved. At the same time there are competing reactions to detoxify the compounds. Activation of parathion to paraoxon has been studied by POTTER and O'BRIEN (1964) in both aquatic and terrestrial vertebrates including turtles, trout, mud puppies, frogs, pigeons, rabbits, toads, guinea pigs, mice, rats and pigs. The pathways have been studied in rats, mice and guinea pigs in detail by NEAL (1967 a,b).

With regard to marine species, little is known concerning the effect of the organophosphate insecticides on these animals or the metabolism of these compounds by the species. It was of interest, therefore, to attempt to correlate the toxicity of parathion with its metabolism by lobsters. This species has already been shown by BRODIE and MAICKEL (1962) to be able to metabolize several drugs and that the activity is primarily in the hepatopancreas. MELLETT et al. (1969) reported that lobsters could also metabolize the drug cyclophosphamide. Since a search of the literature failed to reveal data on the susceptibility of lobsters to organophosphate poisoning, a study was also done on the acute toxicity of parathion to this species.

MATERIALS AND METHODS

Adult male rats were obtained from Charles River Breeding Laboratories (Wilmington, Massachusetts). They were housed in air-conditioned rooms and supplied food and water ad libitum. Male lobsters were obtained locally (Pt. Judith Fishermen's Cooperative, Galilee, Rhode Island) and maintained in artificial seawater at 10°C.

Parathion for the lethality study was supplied by the Monsanto Company (99.7%). The parathion (99.1%) and paraoxon for the metabolism studies were gifts of the American Cyanamid Corporation. The parathion was dissolved in 20% ethanol - 80% propylene glycol for the lethality study and in ethanol alone for the metabolism experiments. Appropriate controls for the solvents

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were employed. Acetylcholinesterase from bovine red blood cells was purchased from Sigma Chemical Corporation.

To test for the acute lethality of parathion, it was injected at various doses into the cheliped sinus of the lobster. Individual lobsters were then placed in large glass jars containing approximately three liters of aerated artificial seawater at 13°C. The experiment was terminated at the end of 48 hours.

Livers from rats and hepatopancreases from lobsters were removed, blotted gently, weighed and homogenized in 1.15% KCl. Amount homogenized and percent homogenate varied depending on the activity of the tissue. A 9,000 x g supernatant was prepared by centrifuging for 20 minutes in a Servall refrigerated centrifuge. To obtain a microsomal fraction, the 9,000 x g fraction was centrifuged for 1 hour at 105,000 x g in an IEC ultracentrifuge.

The metabolism of parathion to paraoxon was studied by incubating varying amounts of microsomes with 1 ml phosphate buffer (0.1M at pH 8.0 or as otherwise indicated), 0.1 ml NADPH (10 mg/ml), 0.1 ml glucose-6-phosphate (20 mg/ml), 0.025 ml parathion (4.0 mg/ml ethanol), and sufficient water to make a total of 2.0 ml. Incubation was carried out at 20° or 37°C for 20 minutes in 20 ml beakers. At the end of that period, the paraoxon formed was assayed for by incubating 0.10 to 0.40 ml of this with 1.0 ml of acetylcholinesterase (0.32 units), 0.6 ml acetylcholine chloride (18.2 mg/ml), and sufficient calcium free Ringer's bicarbonate buffer to make three ml. Incubations were carried out in a Warburg apparatus according to the method of DUBOIS and MANGUN (1947). A standard curve for paraoxon inhibition was used.

The metabolism of parathion to p-nitrophenol was measured using the method of VILLENEUVE et al. (1970). A 25% whole homogenate was used for rats and a 50% whole homogenate for lobsters.

Protein content was determined using the method of LOWRY et al.

Statistical analysis was done using Student's two-tailed t test for comparison between means. A paired t-test was used for the temperature study where samples were from the same animal.

RESULTS

The results of injecting various doses of parathion into the cheliped sinus of lobsters is presented in Table I. An approximate LD_{50} value would be 0.3 mg/kg. DUBOIS et al. (1949) have reported that the intraperitoneal LD_{50} for male rats is 7 mg/kg. An LD_{50} on the strain of rats employed in this study was carried out as a class exercise and also yielded a value of approximately 7 mg/kg. Although it is difficult to compare the two routes of administration, it would appear that lobsters are more sensitive to the lethal effects of parathion than are rats. It is of interest, however, that while rats either die within a few hours or survive, in the lobsters deaths at the lower doses often occurred during the period 24 to 48 hours after injection.

TABLE I

Lethality of Parathion[a] Injected into Cheliped Sinus of Lobsters

Dose (mg/kg)	Number Injected	Deaths in 48 hr	Percent Deaths
2	2	2	100
1	2	2	100
0.5	4	4	100
0.4	3	2	67
0.3	2	1	50
0.2	3	1	33
0.1	1	0	0
0.05	1	0	0

[a]Dissolved in 20% ethanol - 80% propylene glycol.

Preliminary experiments on the conversion of parathion to paraoxon by rats using the method outlined above indicated that initial incubation of tissue containing 0.2 to 0.5 mg protein resulted in paraoxon concentrations which could be measured by acetylcholinesterase inhibition. Nine male rats in which the microsomal protein content of the first incubation ranged from 0.26 to 0.48 mg yielded an average production of 139 nanograms of paraoxon per mg protein per hr with a standard error of 14.9 when the reaction was carried out at 37°C at pH 8.0. In the case of lobsters, no paraoxon formation could be detected using this method even

though up to 12.2 mg microsomal protein were employed. The reaction was also tested at pH 6.0 and pH 7.0 and at a temperature of 20°C to see if perhaps the conditions for formation by the lobster needed to be different from that for the rat, but again no paraoxon was detected.

The data on the formation of p-nitrophenol from parathion by rats and lobsters are presented in Table II. It is readily apparent that rats metabolize parathion (either directly or after transformation into paraoxon) to p-nitrophenol several times faster in vitro than do lobsters. It is of interest that when the temperature of incubation was decreased to 20°C there was a significant decrease in activity in the rats but a significant increase in activity in the lobsters. This increase is in contrast to the decrease in activity with temperature in the clam (Mercenaria mercenaria) nitroreductase system (CARLSON, 1972) but is in agreement with the findings of BUHLER and RASMUSSON (1968) who found that the microsomal metabolizing systems from fish have lower temperature optimum than mammalian systems.

TABLE II

Metabolism of Parathion to p-Nitrophenol by Rats and Lobsters

Species	Temperature	
	20°C	37°C
Rat	275 ± 11.3[a]	463 ± 30.1[b]
Lobster	36 ± 2.0	28 ± 1.0[b]

[a]Nanograms p-nitrophenol/mg protein/hr; mean ± standard error for groups of three animals.

[b]Significantly different from 20°C group at 5% level.

DISCUSSION

Although the metabolism of parathion involves more than one pathway, it would appear from the in vitro metabolic studies alone that lobsters should be quite resistant to parathion since the hepatopancreas was able to detoxify parathion to p-nitrophenol but no conversion of parathion to its toxic metabolite paraoxon could be detected. However, this insensitivity was not supported by the actual toxicity data. The reason for this lack of correlation between

the in vitro and in vivo data is not readily apparent. One possibility is that the in vitro reaction rates do not correlate with the actual in situ reaction rates. Another possibility is that other organs in the lobster are responsible for the conversion of parathion to paraoxon and thus the lethality of the parent compound. Additional studies on in vivo metabolism and metabolism in other organs are necessary before the question can be fully answered.

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