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In Vitro Metabolism of ^{14}C-Dieldrin and Some of its Metabolites in Isolated Nerve Cords of *Blaberus discoidalis*

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In some electrophysiological studies on the mode of action of dieldrin, it has been found that two of its metabolites, namely cis- and trans-aldrindiol were neuroactive (WANG & MATSUMURA 1970, WANG et al. 1971, AKKERMANS et al. 1973, 1975, BERKEN & NARAHASHI 1974). Dieldrin, when applied to preparations of the nervous system, was found to possess a latent period of nearly one hour, whereas the action of trans-aldrindiol at the same concentration occurred within 5-15 min after its application (WANG et al. 1971). This led to the belief that dieldrin was metabolised in insect nervous system to a more active form(s). It was therefore considered important to study the possible metabolic pathway of dieldrin in the insect nervous system (NARAHASHI 1976, BROOKS 1974, 1977). Recently SCHROEDER et al. (1977) reported that dieldrin was hydrated to cis- and trans-aldrindiol in nerve cords of intact Periplaneta americana. The metabolites of dieldrin detected in the nerve cords in vivo may in part be due to the activity of other tissues like the gut, haemolymph, fat body etc. Therefore, to study the metabolism of dieldrin exclusively in the nervous system, it is important to study its degradation in the nerve cords in vitro. The present work was therefore undertaken to study the metabolism of dieldrin and some of its metabolites in the isolated nerve cords of Blaberus discoidalis.

MATERIALS AND METHODS

Authentic samples of dieldrin and its metabolites (non-radioactive) were supplied by C.T.Bedford of Shell Bioscience Laboratory, Sittingbourne, Kent. These were found to be pure by thin layer chromatography (tlc). ^{14}C-Dieldrin (85 mCi/mmol) was purchased from Radiochemical Centre, Amersham, and was used after purification by tlc. The radioactive metabolites of dieldrin were produced by the method given below. The reduced form of nicotinamide adenine dinucleotide phosphate (NADPH) was obtained from Sigma Chemical Corp, London. Precoated glass silica Gel G plates (0.25 mm layer

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thickness) were supplied by Whatman Lab Sales, Kent. X-ray plates (KODIREX-EP) were obtained from KODAK LTD., U.K. All other chemicals used were purchased from FISON LTD., and were of A.R. grade. Insects used were adult males of B.discoidalis. These were out of the stock culture kept in this department.

Production of radioactive metabolites of ^{14}C-dieldrin. Radioactive metabolites were produced by incubation of ^{14}C-dieldrin with microsomes prepared from the fat body of B.discoidalis. The fat body from five male cockroaches was homogenised in 10 mL of an ice-cold mixture containing 2.5 x 10^{-2}M KCl, 1 x 10^{-2}M $MgCl_2$, 3.5 x 10^{-1}M sucrose, and 5 x 10^{-2}M Tris-buffer. The homogenate was strained through a single layer of cheesecloth and the filtrate was centrifuged at 10,000 g. for 30 min. The 10,000 g supernatant was then centrifuged at 100,000 g for 1.0 h. The microsomal pellet obtained was suspended in 1mL of an incubation mixture containing 5 x 10^{-1}M Tris buffer, 5.9 x 10^{-3} NADPH and 3.6 x 10^{-4}M ^{14}C-dieldrin added in 5 µL of ethanol. The incubation was carried out for 3 h at 35°C. At the end of 3 h, the reaction was stopped by adding 50 µL of acetone. Dieldrin and its metabolites were extracted with 10 mL of diethyl ether: dichloromethane (2:3 v/v) followed by two 10 mL portions of ethyl acetate. The combined extract was dried over sodium sulphate and evaporated. The residue was dissolved in acetone. Metabolites of dieldrin i.e. M1 and cis- and trans-aldrindiol were isolated by tlc as given below. Experiments for the production of radioactive metabolites were done several times and individual metabolites from all the experiments were pooled. Purity was checked by tlc as mentioned below. The purified metabolites were stored in acetone at -20°C. They appeared to be stable under these conditions.

Incubation of nerve cords with ^{14}C-dieldrin or its individual metabolites. Acetone solutions of ^{14}C-dieldrin or its metabolites M1, cis-aldrindiol or trans-aldrindiol were evaporated in separate 10-mL centrifuge tubes under a stream of nitrogen. The residue was dissolved in 0.5 mL of ethanol which was evaporated to 50 µL. To this was added 0.5 mL of Rice's insect saline (FINLAYSON & OSBORNE 1970) and the tube shaken on a whirly mixer. The amount of ^{14}C-dieldrin in the saline was adjusted to 90.5 pmoles or in the case of its metabolites, to 20 pmoles.

Groups of five cockroaches were dissected in the saline and the nerve cords isolated. The five nerve cords were rinsed in fresh saline and incubated

in the 0.5 mL saline containing dieldrin or one of its metabolites. The incubation was carried out for 48 h at room temperature. The control experiment consisted of ^{14}C-dieldrin incubated in saline.

Extraction of ^{14}C-dieldrin and its metabolites. At the end of the 48-h incubation period, the nerve cords were taken out and rinsed in 1 mL of fresh saline which was later mixed with the saline used for incubation of nerve cords. The washed nerve cords were homogenised in an all-glass homogeniser in 5 mL of acetone. The homogenate was centrifuged at 1500 g for 10 min. The residue was rehomogenised in 10 mL of the acetone:water mixture (1:1 v/v). The homogenate was centrifuged for 10 min at 1500 g. The supernatants from the first and second spin were combined. The combined acetone:water extract so obtained was evaporated using rotary evaporation until the acetone had evaporated. The remaining aqueous phase was partitioned three times with 10 mL portions of diethyl ether. The combined ether-phase was evaporated and the residue dissolved in 20 mL of a mixture of acetonitrile:hexane (1:1 v/v). The acetonitrile phase was transferred to a beaker and the remaining hexane phase was washed three times with 10 mL portions of acetonitrile (JONES & RIDDICK 1952). The combined acetonitrile phase was dried over sodium sulphate. The aqueous phase remaining after ether extraction was fortified with suitable volumes of conc. HCl to make the strength of the solution equal to 1N HCl (DOROUGH et al. 1974). It was heated at 90°C for 1 h then cooled and neutralised using 1N NaOH solution.

The neutralised aqueous phase was partitioned once with 10 mL of dichloromethane followed by two 10 mL portions of ethyl acetate. The dichloromethane-ethyl acetate extract was dried over sodium sulphate and mixed with the acetonitrile extract obtained above. The organic extract thus obtained was evaporated and the residue dissolved in 10 mL of acetone. This was further evaporated to 0.5 mL under a stream of nitrogen.

Dieldrin and its metabolites present in the saline were extracted with 10 mL of diethyl ether: dichloromethane (2:3 v/v) followed by four 10 mL portions of ethyl acetate. The combined extract was dried over sodium sulphate and finally evaporated to 0.5 mL as mentioned above. Suitable aliquots of the final extracts were analysed by tlc.

Samples were spotted on precoated silica gel-G tlc plates. The plates were developed in a solvent system consisting of acetone:hexane (1:3 v/v) to a distance of 15 cm and exposed to the X-ray film for 15 days. After developing the film the areas of silica gel on tlc

plates, corresponding to the spots on the X-ray film, were scraped into 1 mL of acetone in a scintillation vial. Scintillation fluid (5 mL) prepared by dissolving 4 g of PPO and 0.2 g POPOP in 1 L of toluene was added to each vial. The samples were counted at an efficiency varying from 75 to 90%. Quench correction was by the channels ratio method and the counts were automatically corrected for background. To confirm the identity of the metabolites produced, their R_f values were critically matched with those of authentic samples supplied by the Shell Company in different solvent systems. Non-radioactive organochlorines on tlc plates were detected by the method given by MATTHEWS & MATSUMURA (1969). The R_f values of dieldrin, metabolite M1, cis- and trans-aldrindiol were 0.68, 0.46, 0.24 and 0.16, respectively, in the solvent system consisting of acetone: hexane (1:3 v/v). When the plates were developed in the solvent system consisting of ether:hexane (1:1 v/v) the corresponding R_f values were found to be 0.76, 0.54, 0.16 and 0.07, respectively.

RESULTS

Dieldrin was metabolised in the isolated nerve cord and the saline yielding three metabolites. Two of these have been identified as cis- and trans-aldrindiol. The structure of the third metabolite is tentatively proposed (Fig. 1).

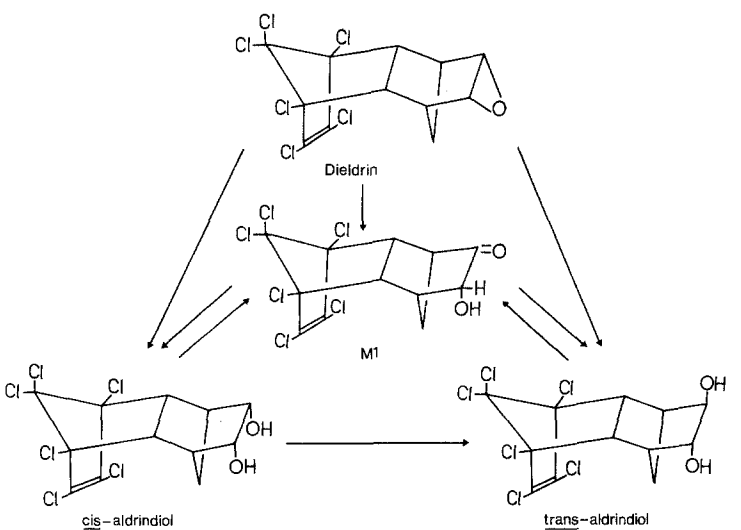

Fig. 1 Metabolic pathway of dieldrin in isolated nerve cords of B.discoidalis.

TABLE 1

In vitro metabolism of ^{14}C-dieldrin and some of its metabolites in isolated nerve cords of *B. discoidalis*

Compound incubated	Sample	Products formed (pmoles) *				Percentage of applied dose recovered **
		Dieldrin	M1	*cis*-aldrindiol	*trans*-aldrindiol	
Dieldrin	Nerve cords	69.0 ± 1.0 (76.2)	3.4 ± 0.4 (3.7)	0.83 ± 0.07 (0.9)	1.1 ± 0.2 (1.2)	95.7
	Saline	5.0 ± 0.8 (5.5)	4.9 ± 0.4 (5.4)	1.1 ± 0.3 (1.2)	1.3 ± 0.1 (1.5)	
M1	Nerve cords	N.D.	10.2 ± 2.2 (51.0)	2.1 ± 0.5 (10.7)	1.2 ± 0.2 (5.8)	93.5
	Saline	N.D.	3.7 ± 0.4 (18.6)	0.94 ± 0.06 (4.7)	0.55 ± 0.09 (2.7)	
cis-aldrindiol	Nerve cords	N.D.	2.8 ± 0.8 (13.8)	6.9 ± 1.8 (34.6)	3.3 ± 0.9 (16.2)	96.2
	Saline	N.D.	0.89 ± 0.05 (4.5)	3.7 ± 0.9 (18.4)	1.8 ± 0.1 (8.7)	
trans-aldrindiol	Nerve cords	N.D.	3.7 ± 1.0 (18.5)	N.D.	6.8 ± 1.0 (33.9)	81.6
	Saline	N.D.	1.4 ± 0.2 (6.8)	N.D.	4.5 ± 0.9 (22.5)	

* Mean ± S.D. of three replicates each consisting of five nerve cords

** Applied dose of dieldrin = 90.5 pmoles; individual metabolite = 20 pmoles
Figures given in parenthesis indicate the percentage of the applied dose.

Quantitative estimates of the results obtained are summarised in Table 1. When dieldrin was incubated with the nerve cords, the amount of M1 produced was higher than that of either of the two diols. The amount of trans-aldrindiol was higher than that of cis-aldrindiol. These metabolites were also present in the saline used for the incubation of nerve cords. The amount of an individual metabolite produced as a result of the metabolism of dieldrin was higher in the saline than their levels in nerve cords. Dieldrin in control experiments remained unmetabolised.

At the end of the incubation period, nerve cords incubated with metabolite M1 were found to contain M1, cis-aldrindiol and trans-aldrindiol. The saline also contained all three metabolites (Table 1), their levels being lower in the saline as compared to those detected in nerve cords. Cis-aldrindiol was metabolised to M1 and trans-aldrindiol. The amount of trans-aldrindiol produced was higher than M1. Nerve cords treated with the trans-aldrindiol contained M1 and trans-aldrindiol only. In this case the formation of cis-aldrindiol was not detected.

DISCUSSION

The formation of cis- and trans-aldrindiol as a result of hydration of dieldrin in nerve cords is in agreement with the results reported by SCHROEDER et al. (1977). However, the formation of the third metabolite M1 in insect nervous system has not been reported previously. If we take into consideration the results of dieldrin metabolism presented in Table 1, the higher levels of metabolite M1 make it an important metabolite. The fact that M1 is further metabolised to yield cis- and trans-aldrindiol and both cis- and trans-aldrindiols are transformed into M1 probably explains the higher level of M1 as compared to either of the diols. At this point, however, the results are different from those reported by SCHROEDER et al. (1977) who found no metabolites other than cis- and trans-aldrindiol in nerve cords of Periplaneta americana treated with dieldrin. In that study, the metabolic products in the extracts of nerve cords were silylated before further analysis. There is a possibility that silyation may derivitise the metabolite M1 to give the same retention time on GLC as either of the diols depending upon the nature of the column packing used. Therefore, it seems important that the metabolic products be analysed as extracted from the tissue without further derivitisation. Though the present study was not done on the nerve cords of P.americana, the metabolite M1 has been detected in nerve cords of three insect species treated with

^{14}C-dieldrin (G.J.P.SINGH, unpublished results). Therefore it is possible that it may be produced in P.americana as well.

The results suggest that the formation of metabolite M1 is an intermediate step in the process of hydration of dieldrin to yield the cis- and trans-aldrindiol. MATTHEWS & MCKINNEY (1974) also proposed the possibility of such an intermediate, but it was not confirmed in that study. Independent metabolism of cis and trans-aldrindiol showed that epimersation between these two diols was unidirectional and occurred in cis to trans direction. This is in agreement with the results reported previously (MATTHEWS & MCKINNEY 1974, BROOKS 1977, SCHROEDER et al. 1977).

The saline used for the incubation of nerve cords also contained all the metabolites that were present in nerve cords. The metabolism that occurred in the saline was found to be the result of enzymatic activity since dieldrin remained unmetabolised in the saline in the control experiment. When isolated nerve cords were placed in saline and removed after 30 min, then dieldrin added to that saline, the nature and number of the metabolites produced was the same as found in the saline containing nerve cords for longer period (G.J.P. SINGH, unpublished observation). Therefore the metabolism of dieldrin in the saline was probably due to the activity of enzymes lost from cut ends of the nerve cords. These results however, do not agree with those of SCHAEFER & SUN (1967), who reported that dieldrin was not metabolised in nerve cords of Musca domestica or in the saline used for incubation.

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