Vertebrate Insecticide Resistance: The in vitro Endrin Effect on Succinic Dehydrogenase Activity on Endrin-Resistant and Susceptible Mosquitofish*

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Organochlorine compounds represent a major group of pesticides. As such they have received wide attention as to application, metabolism, distribution and environmental effects. The effects of these compounds on cellular respiration have been extensively studied in insects and to a lesser degree in vertebrates. Sacktor (1) reported increases of cytochrome oxidase in DDT-resistant houseflies, while Morrison and Brown (2) showed cytochrome oxidase inhibition in vitro at 10^{-3}M DDT in the American cockroach. Johnson (3) studied the effects of DDT, DDA and BHC on the succinoxidase system and cytochrome oxidase at treatment concentrations of 10^{-4}M and 10^{-5}M with no inhibition of succinic dehydrogenase. Colvin & Phillips (4) showed endrin inhibition of both cytochrome oxidase and succinic dehydrogenase in catfish liver homogenates. No studies on the respiratory enzyme activity of vertebrates resistant to organochlorine compounds have been reported. This paper presents preliminary data on the effect of endrin on succinic dehydrogenase activity of liver and brain homogenates from endrin-resistant and susceptible mosquitofish (Gambusia affinis).

Endrin-resistant mosquitofish (5) from drainage ditches at Belzoni, Humphreys County, Mississippi, and susceptible mosquitofish from ponds in Oktibbeha County, Mississippi, were used in this study. All fish were held in the laboratory for at least one week prior to use.

Liver or brain samples were pooled from 10-15 fish, homogenized in cold 0.3 M Tris buffer, pH 7.6, to make a 0.3% solution (w/v), and centrifuged at 2,000 RPM for 10 min at 0°C. In some instances this represented the tissue preparation that

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was assayed for enzyme activity. Mitochondria were prepared by the method of Hogeboom (6), and used either as intact preparations or subjected to repeated freezing and thawing before enzyme assay.

Succinic dehydrogenase was assayed manometrically by the phenazine methosulfate method (7). The main compartment of each vessel contained 2.1 ml of the homogenate or mitochondria preparation, 0.2 ml of 0.01 M KCN and 0.1 M $CaCl_2$ in 0.3 M Tris buffer. The side arms of the control vessels contained 0.2 ml of a solvent mixture of 5% ethyl alcohol, 5% acetone and 0.5% Triton X-100. Experimental vessels contained re-crystallized technical grade endrin in solvent. Endrin concentrations ranged from 1.0 mM to 0.1 uM with the concentration of each dilution checked by gas chromatography. Following an 8 min temperature equilibration period, 0.3 ml of 0.2 M sodium succinate and 0.2 ml of 1% (w/v) phenazine methosulfate were introduced from the side arm into the main compartment of the reaction vessel. The assay consisted of two 30-min periods, the first a control and the second period the treatment. Oxygen uptake was recorded at 10 min intervals during the first period of 30 min. At the end of this period either endrin or the solvent was introduced from the side arm into the main compartment and oxygen uptake was recorded at 10 min intervals for the second 30-min period. The values obtained from vessels containing solvent were used to correct for any change in oxygen uptake caused by the solvent during the 60-min experimental period. Total flask volume was 3.2 ml, with the temperatures for all runs being 37°C and the gas phase was air. Protein determinations were by the method of Lowry et. al., (8), in which a standard calibration curve using Tris buffer was prepared to correct for buffer interference.

Succinic dehydrogenase activity from intact and freeze-thawed preparations of susceptible (s) and resistant (r) fish is shown in Table 1. In the intact preparations there is no difference in enzymic activity between susceptible and resistant brains. However, between liver homogenates there is a higher enzymic activity in the susceptible preparations. In all preparations that were freeze-thawed, with the exception of s-brain, enzymic activity increased. Increases in overall enzymic activity with repeated freeze-thawing are either due to dye penetration or the protein conversion factor and not to changes in homogenate concentration. Differences in enzymic activity after freeze-thawing of susceptible and resistant preparations may be due to different degrees of membrane disruption. A series of standard dye concentrations was run with a constant homogenate concentration for each preparation to ensure that the dye was not limiting in any experimental run.

TABLE 1

Succinic dehydrogenase activity of intact and disrupted preparations. Sample size is represented by N

	Intact Preparation		Disrupted Preparation	
	N	ul O_2/30 min/mg Protein	N	ul O_2/30 min/mg Protein
r-brain	40	28.3±0.35	36	32.5±0.14
r-liver	39	16.7±0.16	36	32.5±0.20
s-brain	48	29.8±0.08	30	29.6±0.08
s-liver	48	28.5±0.27	36	37.5±0.12

There is a consistently higher fat content in the resistant liver homogenates over susceptibles. This explains in part the lower values for the resistant liver preparations. Under our experimental conditions, diet may have some effect on endrin inhibition at least in the s-liver homogenates. Material from fish fed three times a day for one week showed a decrease in endrin inhibition at lower endrin concentrations. Endrin is lipid soluble and could be tied up by an increase in tissue lipid, thereby reducing the amount of available "free" endrin in the treatment vessels. However, repeated washing of homogenates failed to change this endrin effect in either the resistant or susceptible preparations.

The endrin effect on succinic dehydrogenase activity of r-brain, r-liver, s-brain, and s-liver is shown in Table 2. At every level of endrin tested using whole homogenates, the s-brain homogenates showed endrin inhibition of succinic dehydrogenase, whereas in r-brain homogenates there were varying degrees of stimulation. The same is true of liver mitochondria from resistant and susceptible fish (i.e., inhibition at all levels tested of the susceptible and stimulation at all levels of the resistant).

Comparisons of liver and brain material in which the mitochondria had been disrupted (Table 3) show that at every level tested in both resistant and susceptible homogenates there was endrin inhibition. Unlike the intact preparations the inhibition was directly related to endrin concentration indicating that the membranes were disrupted by freezing and thawing. There is no difference between the endrin effect either on resistant or susceptible mitochondrial preparations although the degree of inhibition by endrin on ruptured resistant mitochondria is greater than any other preparation.

TABLE 2

Succinic dehydrogenase activity of intact preparations. For each endrin concentration tested, column 1 is the first 30 min period (control) and column 2 is the second 30 min period (treatment). All values are corrected for the solvent effect. Sample size is three replicates each in triplicate

	1.25×10^{-7} M ul O_2/30 min/mg Protein		% Endrin Effect	1.25×10^{-6} M ul O_2/30 min/mg Protein		% Endrin Effect
	1	2		1	2	
r-brain	27.6±0.53	32.0±0.35	+16	29.2±0.29	33.6±0.65	+15
r-liver				14.1±0.45	16.2±0.45	+15
s-brain	31.7±0.98	21.1±0.98	-34	29.2±0.40	25.8±0.29	-12
s-liver	28.6±0.29	28.0±0.18	-2	31.7±0.15	27.2±0.24	-14

	1.25×10^{-5} M ul O_2/30 min/mg Protein		% Endrin Effect	1.25×10^{-4} M ul O_2/30 min/mg Protein		% Endrin Effect
	1	2		1	2	
r-brain	28.8±0.41	28.8±0.69	0	28.6±0.38	28.9±0.53	+1
r-liver	15.9±0.32	20.0±0.18	+26	18.1±0.41	19.0±0.56	+5
s-brain				29.3±0.30	23.1±0.18	-12
s-liver	28.1±0.19	23.8±0.27	-15			

TABLE 3

Succinic dehydrogenase activity of disrupted mitochondrial preparations. For each endrin concentration tested, column 1 is the first 30 min period (control) and column 2 is the second 30 min period (treatment). All values are corrected for the solvent effect. Sample size is three replicates each in triplicate

	1.25×10^{-7} M			1.25×10^{-6} M			1.25×10^{-5} M		
	ul O_2/30 min/mg Protein		% Endrin Effect	ul O_2/30 min/mg Protein		% Endrin Effect	ul O_2/30 min/mg Protein		% Endrin Effect
	1	2		1	2		1	2	
r-brain	32.6±0.43	28.4±0.68	-13	34.9±0.76	26.2±0.96	-25	31.8±0.56	20.4±0.92	-36
r-liver	31.4±0.43	29.2±0.61	-7	31.3±0.83	26.3±0.87	-16	35.8±1.01	27.9±0.60	-22
s-brain	24.8±0.31	24.1±0.48	-3	26.9±0.96	22.6±0.46	-16	27.9±0.95	20.4±0.29	-27
s-liver	37.2±0.45	36.2±0.33	-2	39.7±0.40	35.7±0.31	-10	37.1±0.33	30.4±0.37	-18

There is a 500 fold difference in toxicity levels of endrin between the resistant and susceptible fish based on T_m50 (9). There is no indication that there is any specific organ which accumulates endrin, although any organ or tissue with fat stores would show increased levels.

From this study, there is evidence that vertebrate resistance involves a cellular membrane barrier. Endrin inhibition of succinic dehydrogenase activity in resistant tissue only after the mitochondrial membrane is disrupted clearly demonstrates a membrane barrier that is effective in the resistance mechanism. Succinic dehydrogenase is an inseparable part of the inner membrane complex and is protected by a peripheral membrane. This membrane would serve as the principal barrier, and only after its disruption could endrin penetrate the inner membrane. This indicates, as has been suggested (4), that there is binding of endrin to the lipid-rich components of the mitochondria. This would change the integrity of the structural components necessary for catalytic activity and lead to reduced enzyme activity.

Although insect resistance to organochlorine compounds is well documented and rather widespread, vertebrate resistance is a restricted phenomenon. Using our succinic dehydrogenase-mitochondrial membrane as a model system, it is possible to suggest that vertebrate resistance is a membrane phenomenon involving either a physical alteration of the membrane, a functional modification, or both. Further, it might indicate that the action of organochlorine compounds is a general one involving disruption of cellular function in all organs and tissues of an animal and not a direct and unique involvement of the central nervous system.

References

1. Sacktor, B.,J. Econ. Entomol. 43, 832 (1950).
2. Morrison, P.E. and Brown, A.W.A., J. Econ. Entomol. 47, 723 (1954).
3. Johnson, C.D., Arch. Biochem. 30, 375 (1951).
4. Colvin, H.J. and Phillips, A.T., Bull. Environ. Contam. Toxicol. 3, 106 (1968).
5. Boyd, C.E. and Ferguson, D.E., J. Econ. Entomol. 57,430 (1964).
6. Hogeboom, G.H., Methods in Enzymology, Vol. 1, S.P. Colowick and N.O. Kaplan, Eds.,(Academic Press, New York, 1955) p. 16.
7. Bernath, P. and Singer, T.P., Methods in Enzymology, Vol. 5 S.P. Colowick and N.O. Kaplan, Eds., (Academic Press, New York, 1962) pp. 597-602.
8. Lowery, O.H., Rosebrough, N.J., Farr, A.L.,and Randall, R.J., J. Biol. Chem. 193, 265 (1951).
9. Ferguson, D.E., Ludke, J.L., and Murphy, G.G., Trans. Amer. Fish. Soc. 95, 335 (1966).