Vertebrate Insecticide Resistance: *in vivo* and *in vitro* Endrin Binding to Cellular Fractions from Brain and Liver Tissues of *Gambusia*

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The *in vivo* and *in vitro* binding patterns of endrin-^{14}C in susceptible and resistant mosquitofish brain and liver cellular fractions were compared. The cell membrane fractions of resistant fish bind more endrin than susceptible fish, while the resistant mitochondria binds less endrin than susceptible fish mitochondria. Differences between endrin uptake in susceptible and resistant fish, retention of endrin by brain cell membranes, a blood–brain barrier, and a structural difference in myelin could account for endrin resistance in mosquitofish.

Organochlorine compound resistance in populations of *Gambusia affinis* (mosquitofish) was first demonstrated in fish collected from drainage ditches in the Mississippi Delta (Boyd and Ferguson, 1964). Culley and Ferguson (1969) reported an approximate 500-fold difference in LC_{50} values between endrin-resistant and susceptible fish. The resistant population also showed higher LC_{50} values for most other organochlorine compounds. Of the organochlorine compounds assayed, endrin was the most toxic to susceptible fish. Endrin use has been restricted for several years, and there are no residue levels of any consequence in tissues assayed by gas chromatography.

Little, if any, information is available on *in vivo* binding of insecticides in vertebrates and no binding studies have been reported on insecticide resistant vertebrates. Matsumura and Hayashi (1966), in *in vitro* studies on rat brain and axonic portions of nerve cords from the American cockroach, showed considerable membrane organelle binding of endrin and dieldrin. Similarly Telford and Matsumura (1970) treated resistant and susceptible cockroach nerves with dieldrin and noted that susceptible axonic membrane fractions bound a larger percentage. This study is intended to provide data on both *in vivo* and *in vitro* binding patterns in endrin-resistant and susceptible mosquitofish.

MATERIALS AND METHODS

Each experimental group consisted of 16 susceptible and/or resistant mosquitofish varying in size from 3.5 to 5.5 cm. Of the fish assayed, 95% were sexually mature females. All fish were retained in the laboratory for 1 week prior to assay, under the same conditions of diet, light, and temperature. *In vivo* treatments were carried out for 6 hr in 8-l. glass aquaria using 2 ppb endrin-^{14}C. During treatment susceptible fish showed increased activity, as evidenced by short darting movements, which were eventually followed by a loss of equilibrium. Only live susceptible fish were assayed. Resistant fish showed no effect of poisoning. *In vitro* treatments of whole liver, brain, relatively pure mitochondria, and myelin fractions were carried out in 2 ml of 0.3 *M* Tris–HCl buffer (pH 7.6) containing 500 ppb endrin-^{14}C incubated for 30 min at 37° C with agitation. Livers and brains were homogenized in a Ten-Brock glass homogenizer containing either 0.25 *M* sucrose or 0.32 *M* sucrose containing 1 m*M* EDTA. Differential centrifugation methods followed Hogeboom (1955), Matsumura and Hayashi (1969), and Cuzner *et al.* (1965) in a Beckman Ultracentrifuge Model L2-50, with Type 50 and SW 50L rotors. All fractions were examined for purity and composition with an electron microscope. Counting was either by a Nuclear Chicago Mark I or a Packard Model 3320 Tri-Carb Scintillation Spectrometer. The protein content of all samples was determined by the method of Lowry *et al.* (1951).

RESULTS AND DISCUSSION

Uptake of endrin-^{14}C after *in vivo* treatment with 2 ppb of endrin was significantly greater in all susceptible brain fractions than resistant brain fractions (Table I). Except for the light microsomal fraction, all liver fractions showed the same pattern. Brain fractions washed repeatedly after *in vitro* treatments with endrin showed that susceptible fish bound more endrin than resistant fish, except in the cell membrane fraction. About 70% of the total activity was removed by washing of susceptible brain fractions while 64% was removed from resistant brain fractions. In both groups the heavy microsome showed the highest percent removal, 78% for susceptible and 73% for resistant, while the cell membrane–nuclear fractions were the lowest, 69% for susceptible and 60% for resistant. The pattern of removal with washing was essentially the same in both treatments. The resistant cell membrane binds twice as much endrin as the susceptible cell membrane and apparently reduces the amount of endrin entering the cell. All other fractions contained considerably less insecticide. An opposite pattern appears in susceptible fish. The susceptible brain cell membrane binds considerably less than other fractions. The nuclear fraction contained about two times more endrin than the cell membrane.

Although the emphasis of this study is on binding in brain tissue, of interest is binding in an organ other than the target organ such as the liver. All susceptible liver fractions showed a significantly higher endrin binding. Repeated washing increased the S/R ratios greatly, with the mitochondria

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Table I. *In vivo* **and** *in vitro* **Endrin Binding in Susceptible (S) and Resistant (R) Brain and Liver Particulate Fractions. Samples Represent the Mean of Three Individual Treatments of 16 Fish. Mean Values Expressed as counts/min/mg Protein**

	Cell membrane	Nucleus	Mitochondria	Myelin	H microsome	L microsome	Total binding
Brain							
in vivo							
S	228		495		614	147	407
R	139		145		169	76	139
S/R[b]	1.65[a]**		3.41**		3.63**	1.91**	2.92**
in vitro							
S	515		731		793	281	685
R	231		413		324	205	397
S/R[b]	2.23[a]**		1.77**		2.45**	1.37	1.73**
in vitro (washed)							
S	293	712	329	770	409	123	487
R	501	203	167	386	207	132	259
S/R[b]	0.59**	3.51**	1.97**	1.99**	1.99**	0.94	1.89**
Liver							
in vivo							
S	1644		1367		1381	201	1293
R	1478		1038		763	406	1003
S/R[b]	1.11[a]**		1.32**		1.80**	0.49**	1.16**
in vitro							
S	3.98		265		343	182	312
R	2.96		199		183	100	208
S/R[b]	1.34[a]**		1.33**		1.87**	1.82**	1.53**
in vitro (washed)							
S	779	447	417		584	324	465
R	170	54	60		134	193	108
S/R[b]	4.58[a]**	8.28**	6.85**		4.43**	1.68**	4.30**

[a] Treatments include nuclear fractions. [b] Significant difference between S and R at 0.05 (*) or 0.01 (**) level of confidence as determined by t test.

showing the most significant increase. This is the same pattern seen in *in vitro* brain mitochondrial treatments and indicates again that resistant membranes prevent endrin entrance without binding.

Resistant fish livers are considerably larger and possess a much greater amount of fat (Fabacher and Chambers, 1971). The high lipid solubility of organochlorine insecticides should promote retention in fatty areas. Washing of *in vitro* fractions removed the endrin solubilized in fat, leaving only the tightly bound endrin. The washed resistant fractions contained 85–93% of the total endrin taken up, and the liver fat of susceptible fish contained 73–90% of the total endrin taken up. Therefore, compartmentalization of endrin by fat would not seem to be a factor in mosquitofish resistance.

Comparisons of binding patterns based on percent distribution between susceptible and resistant tissue show the relative buildup of endrin within a cell and may be indicative of a resistant mechanism. The resistant brain cell membrane fractions in both *in vivo* and *in vitro* treatments bind more endrin than the susceptible fractions (Figure 1). Except for the light microsome fractions, this pattern is reversed in the other membrane organelles.

The total percent distribution of *in vitro* washed fractions (Figure 2) indicates a greater binding in resistant brain cell membranes. Endrin binding in susceptible fish nuclei (51.2%) was significantly greater than in resistant fish (22.1%). Ferguson *et al.* (1966) treated susceptible and resistant mosquitofish to lethal doses of endrin and reported uptake to be the same in both populations. Our data do not support this observation.

In an earlier study (Yarbrough and Wells, 1971), succinic dehydrogenase activity was assayed for endrin effect on intact and ruptured mitochondria. Endrin did not inhibit succinic dehydrogenase activity in resistant fish until the mitochondrial

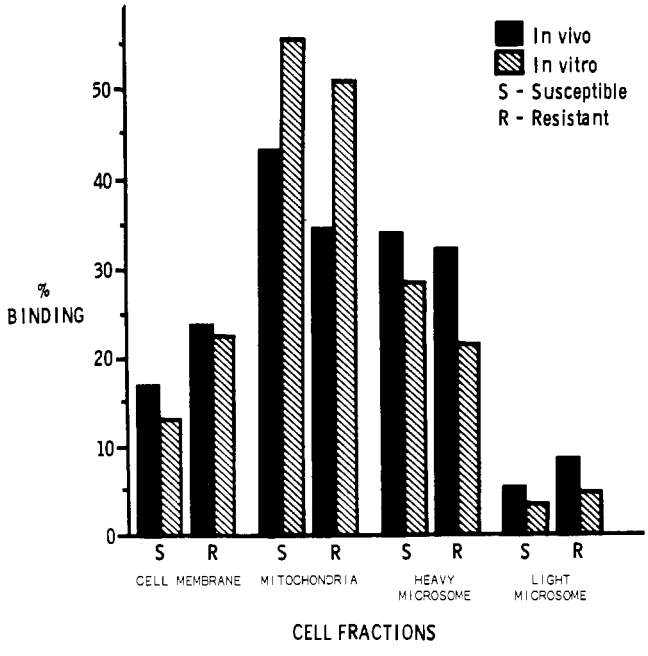

Figure 1. Distribution of bound endrin-^{14}C among cell fractions of susceptible (S) and resistant (R) brain fractions following *in vivo* **and** *in vitro* **treatment. Binding is expressed as the percent of the total endrin bound**

membrane was disrupted. However, both intact and disrupted mitochondria from susceptible fish were inhibited. In the present study, brain mitochondria and myelin fractions were prepared separately and treated to endrin. The fold differences based on S/R ratios were 1.53 for mitochondria and 1.35 for myelin. Both values were highly significant. The

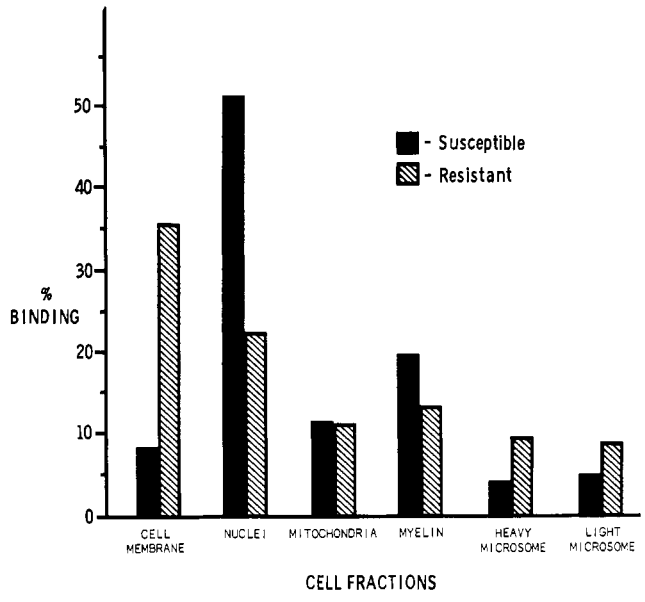

Figure 2. Distribution of bound endrin-^{14}C among cell fractions of susceptible (S) and resistant (R) brain following *in vitro* treatment. Binding is expressed as the percent of the total endrin bound following three washes

increased binding of endrin by susceptible myelin fractions may indicate a structural difference in myelin between the two populations. This increased binding by susceptible myelin could lead to interference of nerve transmission.

A blood–brain barrier is indicated by the liver to brain ratios, 3.04:1 for susceptible fish and 7.65:1 for resistant fish. This barrier appears to be more efficient in resistant mosquito-fish in which the brain contained only about one-eighth the amount of endrin found in the liver, whereas the susceptible fish brain contained one-third the amount found in the liver. *In vitro* treatment of liver and brain with lethal levels of endrin reinforces the effectiveness of the blood–brain barrier in both populations; the brains absorbed about two times as much endrin as did the livers. However, the resistant brain uptake of endrin was almost one-half that of the susceptible fish. All liver and brain fractions showed greater uptake in susceptible fish.

This study indicates that a combination of factors related to differences in membrane structure could account for endrin resistance: a membrane barrier, a blood–brain barrier, and a difference in myelin. However, it does not rule out physiological factors, and future work will include those reactions involved in active transport across cellular membranes.

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