RESISTANCE PATTERN OF *ANOPHELES ALBIMANUS* WIED. FOLLOWING SELECTION BY PARATHION[1]

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ABSTRACT. Laboratory selection pressure by parathion on *Anopheles albimanus* larvae from El Salvador resulted in 216X resistance to this chemical and in lower levels of cross-resistance toward variously related organophosphates. Cross-resistance was influenced markedly by changes in either the alkyl or the aromatic moieties of parathion. Parathion and propoxur resistance in this strain, and possibly cross-resistance to closely related compounds, derives primarily from reduced sensitivity of acetylcholinesterase, as already demonstrated. Resistance toward dieldrin, which was present at significant levels at the time of strain colonization, has increased during selection by parathion.

DDT-resistant strains of *Anopheles albimanus* appeared in El Salvador in 1958, and were soon followed by strains resistant also to dieldrin (Brown and Pal 1971). As a result of expansion and intensification of resistance to these insecticides, attention was directed to other chemicals, especially organophosphates (OP) and carbamates, as substitute insecticides. But resistance to malathion became evident in El Salvador in 1969 (Breeland et al. 1970), and soon afterward it was demonstrated that this resistance extended to several other OP's as well as to carbamates (Georghiou et al. 1972). Biochemical investigations in our laboratory revealed that high levels of resistance to propoxur (>1000X) and parathion (ca. 83X) in this population are due to a substantial reduction in sensitivity of acetylcholinesterase (AChE) to inhibition by these chemicals (Ayad and Georghiou 1975).

To perform the biochemical investigations in question, it was essential that we develop a homogeneously resistant strain by further selection in the laboratory. Here we report the results of such selection by parathion and discuss the outcome of cross-resistance in the light of the known defense mechanisms of the population.

MATERIALS AND METHODS

MOSQUITO STRAINS. Four strains were employed in this investigation:

1. Carb.-L., derived by propoxur pressure from a field strain originating in El Salvador, which displayed initially a low level of tolerance to OP and carbamate insecticides (Ariaratnam and Georghiou 1971).

2. OP-R, originating from F_8 of Carb.-L and developed by intensive selection with parathion for 17 additional generations. Cross-resistance to parathion in Carb.-L at F_8 was 23.8X.

3. St. Susc. (*striped* susceptible), derived from a field collection made in Haiti (Georghiou and Gidden 1965, WHO unpublished Document) and being susceptible to OP, carbamates and DDT (Nakashima 1973).

4. H.-P. (Haiti-Panama), obtained by combining strains from Haiti and Panama, both susceptible to OP and carbamates.

TESTING AND SELECTION METHODS. All insecticides were of technical or analytical grade. Solutions were prepared in acetone on a w/v basis and were tested on early 4th instar larvae as described previously by Georghiou et al. (1966). Tests were replicated on at least 4 different

days, and the data were subjected to probit analysis (Finney 1952).

When selection involved less than 1,000 larvae, these were treated in waxed paper cups. Approximately 50 larvae were placed in each cup with 100 ml of tap water, and parathion of the desired concentration was added at the rate of 1 ml per cup. When more than 1,000 larvae were available for selection, enameled pans holding up to 1,500 larvae in 1 liter of tap water were used. In the latter case 10 ml of the insecticide solution were added to each pan. Treated larvae were kept at room temperature (25–28°C) for 24 hours. Survivors were rinsed with tap water and placed in "recovery" pans. Pupae were transferred to adult colony cages.

The parathion dosage used for selection was calculated to produce approximately 50% mortality after 24 hr of exposure, thus providing a large enough number of survivors to maintain genetic variability. This moderate pressure was expected to preserve both recessive as well as dominant factors which might exist in the populations.

Up to generation F_{17}, the selecting dosage was increased gradually from an initial level of 0.075 ppm to a final level of 0.6 ppm. During this period, each generation was kept separately. By F_{17} an apparently maximal level of resistance had been reached, hence thereafter the strain was maintained under uniform pressure with 0.6 ppm parathion. Larvae used in cross-resistance tests were obtained from a part of the maximally resistant colony which was reared for one more generation without pressure to provide the needed large numbers of larvae.

RESULTS AND DISCUSSION

Throughout the selection process, we have monitored changes in the dosage-response line to detect any significant changes in resistance. Fig. 1 shows that only a modest increase in the LC_{50}
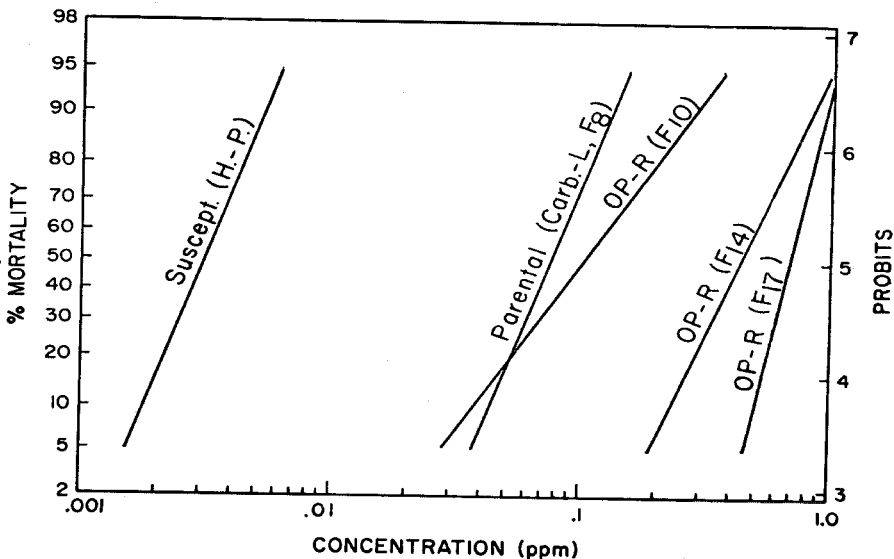

Fig. 1. Dosage-mortality relationships of larvae of susceptible, parental (Carb.-L) and parathion-selected strains of *An. albimanus*.

amounting to 1.4X had occurred during the first 10 generations of selection. However, in the next 4 generations (F_{11}-F_{14}) the increase was 4.1X, and in the subsequent 3 (F_{15}-F_{17}) 1.6X, thus attaining a total level of resistance of 216.1X by comparison with the susceptible H.-P. strain.

Fig. 1 also shows that the slope values, b, were reduced from 5.2 in F_8 to 3.0 in F_{10}, then rose to 4.4 in F_{14} and to 7.7 in F_{17}. This is the long-established pattern of changes in slope value as the population is selected toward a higher frequency of resistance genes (Hoskins and Gordon 1956).

To determine the specificity of the resistance mechanism(s), larvae were tested for susceptibility to several compounds variously related to parathion. Because the susceptible H.-P. strain referred to in Table 2 had been discontinued, these tests utilized the St. Susc. strain for comparison. The results indicate that the highest level of resistance was manifested toward the selecting agent, parathion, (Table 1).

Alterations in the alkyl moiety of parathion have caused a reduction in resistance as is clearly apparent in the case of methyl parathion (II) and isopropyl parathion (V). Likewise, desulfuration of parathion has reduced resistance from 83X (I) to 46.5X (III). The resistance was abolished almost completely when the change involved both the alkyl and the aromatic moieties, as is demonstrated by fenthion (IX) and its sulfoxide (X) and sulfone (XI) analogs.

The above data suggest that pressure by parathion has resulted in the selection of a rather specific mechanism of resistance. This was subsequently found to consist of insensitivity of AChE to inhibition by paraoxon (Ayad and Georghiou 1975). Such specificity is probably due to binding or steric fit requirements that are available only in parathion and in closely related compounds.

Table 2 indicates that the selection pressure not only increased resistance to parathion and related chemicals, but it has also imparted high levels of multiple resistance toward compounds of disparate structure such as malathion (106.5X), carbaryl (155X), and dieldrin (2926.7X). Resistance to DDT, which rose to a 20X level by propoxur selection, declined to an 8X level during parathion selection.

The apparently wide spectrum of resistance in *An. albimanus* may be attributed in part to indirect selection pressure by insecticides of various chemical groups which are applied widely on agricultural crops in Central America (Georghiou 1972, Hobbs 1973). It is suggested that the subsequent application of selection pressure by parathion in the laboratory has served to intensify resistance by enhancing the frequency of the AChE insensitivity factor and its integration with other mechanisms which were already present at various frequencies in the population.

The very limited increase in cross-resistance toward fenthion and dichlorvos indicates that resistance to organophosphates in the population studied is not a general phenomenon. This might be because no fenthion and only small amounts of dichlorvos had been used on agricultural crops in El Salvador (Georghiou 1972). A further explanation may rest on the pronounced dissimilarities in the molecular structure of these two compounds compared to that of parathion.

Various studies on resistance to organophosphates in mosquitoes have shown that either enhanced detoxification of the chemical, reduced penetration, or both, may be causes of resistance (Bigley and Plapp 1962, Stone and Brown 1969, Apperson and Georghiou 1975). However, in the present case, a reduced sensitivity of the target enzyme, AChE, to inhibition by OP and carbamate insecticides was shown to be the cause of resistance to parathion and propoxur (Ayad and Georghiou 1975). This insensitivity does not extend to fenoxon, a fact which is consistent with the high toxicity of fenthion to OP-R larvae. It is not clear at present whether the varying levels of cross-resistance to other organophosphates are the result of corresponding

Table 1. Effect of changes in parathion structure on cross-resistance levels of *An. albimanus* larvae

Structure		LC$_{50}$ (ppm)		Resistance Ratios[a]
		St. Susc.	OP−R	
I.	(EtO)$_2$–P(=S)–O–C$_6$H$_4$–NO$_2$	0.008	0.67	83.0
II.	(MeO)$_2$–P(=S)–O–C$_6$H$_4$–NO$_2$	0.02	0.82	50.9
III.	(EtO)$_2$–P(=O)–O–C$_6$H$_4$–NO$_2$	0.11	5.13	46.5
IV.	(MeO)$_2$–P(=O)–O–C$_6$H$_4$–NO$_2$	0.18	3.05	17.0
V.	($\underline{i}$-Pro)$_2$–P(=S)–O–C$_6$H$_4$–NO$_2$	19.44	226.50	11.0
VI.	(MeO)$_2$–P(=S)–O–C$_6$H$_3$(CH$_3$)–NO$_2$	0.02	0.52	27.0
VII.	(EtO)$_2$–P(=O)–O–C$_6$H$_3$(CH$_3$)–NO$_2$	0.49	26.80	54.2
VIII.	(EtO)$_2$–P(=S)–O–C$_6$H$_3$(CH$_3$)–S–CH$_3$	0.04	0.54	15.3
IX.	(MeO)$_2$–P(=S)–O–C$_6$H$_3$(CH$_3$)–S–CH$_3$	0.05	0.08	1.5
X.	(MeO)$_2$–P(=S)–O–C$_6$H$_3$(CH$_3$)–S(→O)–CH$_3$	0.69	1.90	2.8
XI.	(MeO)$_2$–P(=S)–O–C$_6$H$_3$(CH$_3$)–S(O)(O)–CH$_3$	0.31	1.50	4.9

[a] Resistance Ratio = $\dfrac{\text{LC}_{50}\ \text{OP−R}}{\text{LC}_{50}\ \text{St. Susc.}}$

Table 2. Effects of parathion selection on the spectrum of resistance of OP−R larvae of *An. albrimanus*

Compound	H.−P. strain[a] LC$_{50}$ (ppm)	Carb.−L strain[a] LC$_{50}$ (ppm)	OP−R strain LC$_{50}$ (ppm)	Resistance ratios[d] OP−R strain
Parathion	0.0031	0.11	0.67	216.1
Methyl parathion	0.0065	0.24	0.82	126.2
Malathion	0.085	1.7	9.05	106.5
Fenitrothion	0.025	0.24	0.52	20.8
Dichlorvos	0.11	0.21	0.38	3.5
Fenthion	0.023	0.027	0.082	3.6
Propoxur	0.39	81%[c]	30%[c]	—
Carbaryl	0.89	66.6	137.92	150.0
DDT	0.01	0.20	0.08	8.0
Dieldrin	0.003[b]	1.30	8.78	2926.7

[a] After Ariaratnam and Georghiou (1971).
[b] LC$_{50}$ of susceptible Gorgas strain.
[c] Maximum mortality with 1000 ppm.
[d] Resistance Ratios = $\dfrac{\text{LC}_{50}\ \text{OP−R strain}}{\text{LC}_{50}\ \text{H.−P. strain}}$

reduction in AChE sensitivity toward these inhibitors.

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