distribution of acetylcholinesterase and cholinacetylase is the fact that the former is not present in the supernatant or soluble subfraction M_3.

These results (*5*) are indicative of a fine compartmentalization of enzymes and active substances within the nerve-ending complex (*6*).

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Dieldrin Susceptibility: Partial Restoration in Anopheles Selected with a Carbamate

Abstract. *Selection of dieldrin-resistant* Anopheles albimanus *Wied. and normal* Culex pipiens quinquefasciatus *Say with* m-*isopropylphenyl methylcarbamate for 21 and 30 generations, respectively, resulted only in vigor tolerance to this and other aryl methylcarbamates. However, it caused substantial restoration of dieldrin- and DDT-susceptibility in* Anopheles *by increasing the frequency of homozygous susceptible phenotypes from 10 to 83 percent.*

One of the most serious problems confronting the malaria eradication program of the World Health Organization is the selection of suitable insecticides to replace DDT and dieldrin where these have been rendered ineffective by the development of resistance in the vector mosquitoes. Malaria is transmitted exclusively by anopheline mosquitoes, and of the 85 described species of *Anopheles* at least 28 (*1*) are known to have developed resistance to one or both of these insecticides. The organophosphorus insecticide malathion has been tentatively suggested as a substitute (*2*), but resistance to this compound has already appeared in field populations of *Culex tarsalis* Coq. in California (*3*) and in Puerto Rican populations of *Aedes aegypti* L.; the latter is also resistant to DDT and dieldrin (*4*). *Aedes nigromaculis* Ludl. has shown increased tolerance to the organophosphorus insecticide parathion in California (*5*). This situation emphasizes the need for thoroughly testing potential new insecticides, not only for toxicity to resistant strains but also for the degree of resistance that they can induce.

Research on new insecticides in this laboratory has revealed that several carbamic acid esters, such as *m*-isopropylphenyl methylcarbamate, and *m-sec*-butylphenyl methylcarbamate are highly toxic to *Culex pipiens quinquefasciatus* Say and *Anopheles albimanus* Wied., even more so than DDT, dieldrin, malathion, parathion and fenthion (*6*). Selection of house flies (*Musca domestica* L.) with *m*-isopropylphenyl methylcarbamate resulted in high levels of resistance to this material and to related aryl methylcarbamates (*7*); the resistance is due primarily to ability of flies to metabolize the carbamate at a rate commensurate with its penetration into the insect (*8*).

The activity of carbamates in mosquitoes is peculiarly different from the activity in house flies, in that several compounds highly toxic to the house fly are ineffective against mosquitoes, and vice versa (*9*). Many carbamates are synergized to a remarkable degree by piperonyl butoxide in house flies (*10*) but only slightly in mosquitoes (*6*). These observations suggest the presence of distinctly different defense mechanisms against carbamates in mosquitoes, and prompted the authors to explore the potentialities of resistance development in mosquitoes through selection with *m*-isopropylphenyl methylcarbamate.

The mosquitoes used were a laboratory strain of *Culex pipiens quinquefasciatus* of normal susceptibility to insecticides, and a strain of *Anopheles albimanus* from Panama (*11*) consisting of dieldrin-susceptible, -hybrid and -resistant phenotypes in a ratio of approximately 10 : 44 : 46. This latter strain also showed approximately tenfold tolerance to DDT at the LC_{50} level. The selection technique consisted of first exposing for 24 hours all 4th-instar larvae of each generation to a solution of *m*-isopropylphenyl methylcarbamate (*12*) in tap water at concentrations which produced 80 to 95 percent mortality, and then propagating the survivors. About 3000 larvae were used in each generation; selection extended over 30 generations of *Culex* and 21 generations of *Anopheles*.

At the end of selection, larval susceptibility to *m*-isopropylphenyl methylcarbamate was 2 times as low in *Culex* and 2.7 times as low in *Anopheles* as at the beginning (Fig. 1). Susceptibility to the related methylcarbamates *o*-isopropylphenyl, *o*-isopropoxyphenyl, *m-sec*-butylphenyl, *m-tert*-butylphenyl and 4-methylthio-3,5-xylyl, remained virtually unchanged (less than two times at the LC_{50}), except for Sevin (1-naphthyl methylcarbamate) which was 5.2-fold less effective against *Culex* than initially. There was no change in the slopes of the dosage-mortality regression lines of the selected strains compared with those of the parental strains, suggesting that the small reduction in susceptibility to *m*-isopropyl-

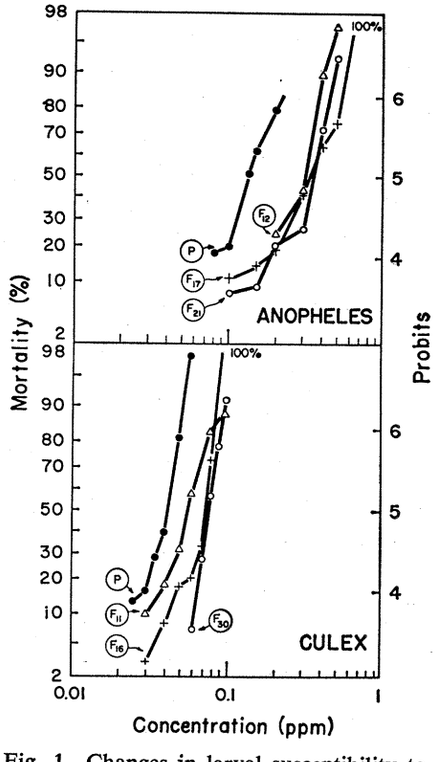

Fig. 1. Changes in larval susceptibility to *m*-isopropylphenyl methylcarbamate in *Anopheles* and *Culex* in the course of selection with this compound (*21*).

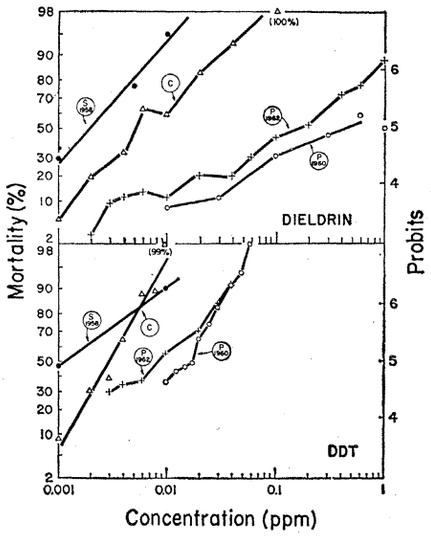

Fig. 2. Changes in larval susceptibility to dieldrin (top) and DDT (bottom) in *Anopheles: S-1958,* susceptible strain; *P-1960,* dieldrin-resistant derived from *S-1958; C,* selected from *P-1960* with *m*-isopropylphenyl methylcarbamate for 21 generations; *P-1962,* same as *P-1960* at the end of selection *C* (*21*).

phenyl methylcarbamate may be due primarily to vigor (*13*). If a specific gene for resistance to the carbamate had been present in the parental *Culex* or *Anopheles* populations, we believe that it would have manifested itself well within the number of generations involved in these selections. It is generally agreed that the time factor in the development of resistance may be considerably reduced if the strain under selection is already resistant to another insecticide (*14*). Therefore, the failure of dieldrin-resistant *Anopheles* to respond to selection with the carbamate may be of considerable practical significance in the utility of this or other closely related compounds in malaria eradication.

When the response of the carbamate-selected strains to non-carbamates was tested, it was found that while *Culex* was as susceptible to malathion, DDT, and dieldrin as originally, and *Anopheles* as susceptible to malathion as originally, the latter species showed almost complete loss of resistance to dieldrin and DDT (Fig. 2). The resistance level of the parental *Anopheles* strain to these compounds showed only negligible regression during the same period. Figure 2 shows that a concentration of 0.02 ppm dieldrin, which is required to kill all susceptible larvae, produced 10 and 20 percent mortality in the parental strain at the beginning and at the end of selection, respectively, and 83 percent in the selected strain. Complete mortality of the selected strain is obtained with 0.1 ppm dieldrin, a concentration which produces only 43 percent mortality in the parental strain. The DDT results show a shift of the dosage-mortality line of the selected strain to the range of the susceptible strain, and a considerable increase in slope resulting from the elimination of individuals at the lower and upper range of the curve. A concentration of 0.001 ppm DDT which kills 47 percent of the susceptible strain kills 9 percent of the carbamate-selected, while 0.01 ppm DDT which kills 36 percent of the parental and 90 percent of the susceptible, now kills 99 percent of the carbamate-selected strain.

The monofactorial type of inheritance of resistance to dieldrin in *Anopheles albimanus* (*15*) raises the possibility that the larvae obtained from the parental strain for the F_1 selection may have been predominantly homozygous (*rr*) for susceptibility to dieldrin. However, in view of the fact that the *rr* individuals represented only 10 percent of the parental strain, and the sample for the first selection was so large (1600 larvae), the chances of obtaining 83 percent *rr* larvae, as found in the selected strain, were very remote. The possibility of contamination is also excluded since no susceptible strain of *Anopheles* is maintained in this laboratory. The *RR* and *Rr* phenotypes have shown good biotic potential in the parental strain as evidenced by the rather small change in the relative frequency of phenotypes over a period of 21 generations (*16*), and thus biotic factors cannot account for the drastic reduction in frequency of *R* gene in the selected strain.

A reasonable hypothesis might be that the observed regression is a consequence of the selection by the carbamate, either as a true expression of negatively correlated toxicity, or as a result of some deleterious effect on the biotic potential of *RR* genotypes, such as reduction of oviposition or egg hatch, so that *rr* genotypes eventually predominate.

Several cases of negative correlation of activity of toxicants in certain DDT-resistant houseflies have come to light mainly through the work of Ascher (*17*), but were not found applicable to all DDT-resistant strains of this insect (*18*). The negative correlation between susceptibility to phenylthiourea and resistance to DDT in *Drosophila melanogaster* as described in Ogita's classical work (*19*) did not hold true for houseflies (*18*). Recently Cole and Clark (*20*) noted that Sevin and *m*-isopropylphenyl methylcarbamate were more toxic to two DDT-resistant strains of body lice (*Pediculus humanus humanus* L.) than to a susceptible strain. However, selection pressure by Sevin on 30 generations of one of the DDT-resistant strains did not produce a regression in DDT resistance or change the level of susceptibility to Sevin (*20*). In spite of the many failures in this direction, it is generally agreed that discovery of pairs of negatively correlated insecticides is at present the only practicable solution of the resistance problem.

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$$q^2rr: 2q(1-q)Rr: (1-q)^2 RR$$
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