SUMMARY OF SYMPOSIUM

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It was rash to accept the chairman's invitation to summarize this symposium, for it has turned out to contain so many and diverse points. Fortunately, however, the order of the successive contributions makes sense, starting with the underlying biochemical and genetical causes of resistance, and then proceeding from the northeastern States where the problem is hesitantly developing, thence to Florida where organophosphorus-resistance is eventually being added to organochlorine resistance, and finally to California where every mosquito resistance problem that we can now think of has definitely developed.

Dr. Perry has shown us what research in the past 10 years has revealed about the biochemical mechanisms of resistance, and that a mosquito gets to be resistant essentially because it can break down the insecticide. With DDT, the detoxication mechanism removes hydrochloric acid from the molecule, and the enzyme that does it is thus a dehydrochlorinase. This dehydrochlorination has been found to occur in DDT-resistant culicines just as definitely as in resistant house flies. In anophelines the picture is not so clear, but probably Dr. Perry will agree that dehydrochlorination accounted for part of the DDT-resistance in the Turkish *Anopheles atroparvus* that he studied.

Attempts to counter this resistance in mosquitoes by adding to the DDT some dehydrochlorinase inhibitor, such as DMC or WARF-Antiresistant, proved effective at first but after a few generations of this treatment the mosquitoes developed a resistance to the DDT-synergist mixture. Substitution of DDT with compounds similar in molecular configuration but far less open to detoxication has proved more successful. Deutero-DDT, less detoxicable because the hydrogen in the center of the molecule is replaced by its isotope deuterium, is effective against DDT-resistant mosquitoes, and better than DDT against the susceptible ones. The compound CP-47412, containing cyclopropane instead of ethane as the central spine of the molecule, is perhaps even more successful. Nevertheless, the remedial insecticides for resistant strains are still usually

discovered by the empirical hit-or-miss method of screening tests, although our biochemical knowledge of resistance indicates where we have the greatest chance of finding them, and can provide explanations of why the good ones are effective.

Much has been learned about the genetics of DDT-resistance, of dieldrin-resistance, and of OP-resistance in mosquitoes, as Dr. Klassen has described to us. The stimulating idea of Dr. Perry that the insecticides may themselves directly induce the detoxifying enzyme, coupled with his unoptimistic corollary that thus all insecticides will evoke resistance in mosquitoes, is one that I remember the biochemist F. P. W. Winteringham injecting into the symposium on insecticide-resistance held at the International Congress of Zoology in 1958 celebrating the Darwin-Wallace centennial. But fortunately those who have looked for evidence of the direct induction of resistance of insects during their life-cycle as a result of exposure to the insecticide always failed to find it, from Campbell 40 years ago with sodium arsenate on silkworms, through those of Beard with nicotine and pyrethrins on waxmoth larvae, to those of Hadaway with DDT, BHC and diazinon on house flies. We have tried ourselves to obtain evidence of this post-adaptation with dieldrin on *Aedes aegypti* larvae, but the results never showed it conclusively. Neither Harrison with house flies nor Luers with Drosophila could induce DDT-resistance in strains which lacked the specific pre-adaptations or genetic factors, even if they tried to select for as many as 150 generations. On the other hand, we know that strains of either of these insects that carried the genetic factors or genes for DDT-resistance rapidly responded to selection and became resistant to DDT. In scholarly terms, resistance is the result of Darwinian selection of Mendelian factors.

So we do not have to resign ourselves to the prospect that resistance will inevitably develop to all insecticides in every species of mosquito; instead by detailed study of specific cases we can try to discover hedges, edges and angles that form some basis for strategy and tactics in chemical control. Genetic studies of selected laboratory strains, supplemented by field experience, indicate to us how long a given insecticide may be expected to remain effective. Resistance to dieldrin, other cyclodiene insecticides, and BHC will probably develop fairly quickly and decisively, since the single gene necessary for dieldrin-resistance can express itself fully without waiting for the accumulation of supporting genes. On the other hand the main gene for DDT-resistance does need supporting alleles, at least from our experience with *A. aegypti;* thus it usually develops more slowly, and is liable to revert to susceptibility if DDT selection is withheld before the strain has accumulated the supporting alleles. Organophosphorus-resistance requires the assembling, as a result of selection, of many genes of minor effect before the main gene can produce a true resistance, and this may take many years to develop.

As Dr. Perry has told us, our knowledge of the biochemical basis of DDT-resistance is matched by our ignorance of the physiological cause of dieldrin-resistance. Perhaps this question will become academic before it is settled, since the cyclodiene insecticides are going out of favour for mosquito control not only because of the development of resistance to them but also due to their greater hazard to wildlife. At least we know that the acquisition of dieldrin-resistance, or of DDT-resistance, does not involve any cross-resistance to malathion and other OP compounds. Conversely, the acquisition of malathion-tolerance in *Culex fatigans* does not involve DDT-resistance or dieldrin-resistance; however a strong cross-tolerance to DDT is induced by malathion selection of some strains of *A. aegypti,* and by fenthion selection of *C. fatigans.*

From Dr. Sutherland's paper, it is clear that the resistance problem in the north-eastern states has reached a stage no further developed than in Canada, where DDT can still be used with effect against the northern mosquitoes. Wherever the

treated areas support only a small proportion of the mosquito population, as in northern forests or eastern salt-marshes, the incipient resistance that develops may be diluted by the surrounding susceptible gene-pool, and come to nothing if that insecticide is withheld for a year or two. Only where the populations are isolated and of modest size can resistance surely develop in such salt-marsh mosquitoes; thus the river-mouth population of *Aedes cantator* at Moncton, New Brunswick, Canada has become considerably DDT-resistant and strongly dieldrin-resistant. Certain small isolates of *Culex pipiens* in the northeast are going the same way.

When we come around to Florida we approach the question of OP-resistance, which is the most important problem of the foreseeable future. Mr. Gahan tells us that OP-tolerance in *Aedes taeniorhynchus,* first suspected from susceptibility tests made around Cocoa Beach in 1952 but subsequently considered not to amount to anything, now amounts to 10-20-fold in several parts of the State and involves observed control failures with malathion. He would call it "resistance," thus making the point that this term applies to cases where a control procedure becomes ineffective, it being a practical matter and the role of resistance tests on the insects themselves being merely to confirm or deny it. Malathion-tolerance induced by laboratory selection of *Aedes aegypti* has turned out to be due to reduced adsorption of the insecticide and not to detoxication, thus making Dr. Perry's point that such a mechanism can give only a moderate tolerance and not a true resistance. On the other hand, the malathion-resistance developed by *Culex tarsalis* and the parathion-resistance developed by *Aedes nigromaculis,* both in California, have each proved to be associated with increased detoxication. The enzymes responsible for detoxication of OP compounds are all types of esterases, mainly carboxy-esterase for malathion in *C. tarsalis* and phosphatase for parathion in *A. nigromaculis.* Such enzymes are produced in OP-resistant house flies by the resistance allele converting an aliesterase into one or other of these detoxifying esterases, but this enzyme conversion has not yet proved to be the case in mosquitoes.

In the house fly there appeared to be two types of OP-resistance, one to malathion and the other to parathion and diazinon. It is true that the malathion-resistance in *Culex tarsalis* did not extend to any of the other OP insecticides, but this type of resistance is unimportant in California and has in fact all but disappeared. As Dick Peters has told us, the important problem is the resistance of *Aedes nigromaculis,* which first appeared in Kings County to parathion in 1958, to methyl parathion in 1962 and to fenthion in 1965. Collections made here in 1963 showed a 4,000-fold resistance to parathion and a 20-fold cross-resistance to methyl parathion and fenthion, while the cross-resistance to malathion was 10-fold. Although Pat Gillies has provided evidence that malathion selection induces the most resistance to malathion and the least to parathion, methyl parathion and fenthion, nevertheless it increased the LC_{50} levels to these insecticides by some 10 times. So although there are indications of there being two types of OP-resistance here, the separation is insufficient to allow a substitution of one to correct for resistance to the other, as has been done between DDT and dieldrin against anopheline mosquitoes in Java.

Nevertheless, investigations of cross-resistance spectra among the OP compounds are well worth while, especially to find out which of the excellent new ones would be the best to use. There are quite a few that are more larvicidal than fenthion, or safer for wildlife and man, or both. These include Bromophos and Sumithion which are safer, CD-7438 which has greater residual activity, Dursban which is more effective, and ABATE which is even more effective and safer. Although ABATE suffers less from cross-resistance than Bromophos, it usually suffers more than Dursban, so that with DDT-resistant and/or OP-resistant strains

it often happens that Dursban is slightly better, though both are remarkably good. We now await the result of selecting laboratory strains of mosquitoes with Dursban or ABATE. Obviously these compounds can absorb quite a lot of tolerance and still will be effective at dosages within the margin of safety to wildlife and higher animals. They have bought us quite a bit more time in this race against the development of resistance.

And so experience, research, development and testing have enabled us to live with resistance. But now the study of OP-resistance is much more complex and far less clear-cut than the organochlorine-resistances we formerly investigated. However it is up to us to persevere, for had it not been for the biochemical and genetical research already done we would not have even remotely understood what we were dealing with in this all-important problem of developed insecticide-resistance.