Table 5.—Precipitation in inches as recorded at the Miami International Airport

Month	1962	1963
June	10.36	6.80
July	3.74	1.77
August	8.02	4.77
September	7.82	11.12

quito-borne disease epidemics or possibly when needed as a means of economy. The authors further urge that additional testing be done to determine the latitude and scope of aerial applications of insecticides for domestic mosquito control.

Table 6.—Overall general and *Aedes aegypti* breeding indices for Dade County, Florida.

Month	*Aedes aegypti*		General	
	1962	1963	1962	1963
June	3.8	3.0	5.9	4.7
July	1.2	3.2	1.8	4.2
August	1.3	3.0	2.0	3.6
September	2.25	5.5	3.6	6.1

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INSECTICIDE-RESISTANCE RESEARCH ON *A. AEGYPTI*

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In 1954, populations of *A. aegypti* in Trinidad were discovered to be DDT-resistant. These resistant larvae were found by Brown and Perry (1956) to detoxify DDT to DDE, but the detoxifying enzyme could not be demonstrated by Brown (1956) *in vitro*. The DDT-resistance character in the Trinidad strain was reported by Coker (1958) to be due to a single gene allele, and this was confirmed by Qutubuddin (1958). A DDT-tolerance developed in a Malayan strain was considered by Coker (1958) to be due to a different factor.

Ten years later, in 1964, DDT-resistant populations of *A. aegypti* are now known in many Caribbean islands, notably Jamaica and Hispaniola, and on the adjacent mainland of South America in northeastern Colombia, Venezuela and the Guianas. Moreover, dieldrin-resistance has been reported from Puerto Rico by Fox (1961), and subsequently discovered in Jamaica, the Virgin Islands,

the Grenadines and Curaçao. Strong DDT-resistance has been developed by laboratory selection of DDT-tolerant strains from New Orleans and Key West, Florida (Abedi and Brown, 1961a). Increased DDT-tolerance has been reported in *A. aegypti* from Saigon, Vietnam, and strong DDT-resistance has been developed in the laboratory in a strain from Penang, Malaya (Abedi and Brown, 1960). Both DDT-resistance and dieldrin-resistance have been developed by laboratory selection of material from Karachi, Pakistan. It would appear that *A. aegypti* resembles *Culex fatigans* in being capable of both types of resistance to most of the chlorinated hydrocarbon insecticides in any part of its range.

The mechanism of DDT-resistance in this mosquito is revealing itself in studies in our laboratory to be very similar to that in the house fly, which Sternburg, Kearns and Moorefield (1954) had discovered to be mainly due to detoxication

to DDE by the enzyme DDT-dehydrochlorinase. The DDT-resistant larvae from Trinidad and from Isla Verde, Puerto Rico were found by Chattoraj and Brown (1960) to produce at least 5 times as much DDE as susceptible strains; larvae of the DDT-resistant Penang strain however did not show a great increase since its normal counterpart, characterized by the comparative DDT-tolerance typical of Malayan *A. aegypti,* had a considerable DDE production to start with. Larvae of the American DDT-resistant strains responded to the presence of DDT by producing and excreting large amounts of peritrophic membrane, which the Penang DDT-resistant strain did not (Abedi and Brown, 1961b). Conversely this latter strain had developed a 25 percent higher content of phospholipid, which the American strains had not; however Fast and Brown (1962) found that this increased phospholipid could be developed by selection without increased DDT-resistance. It was expected that the Penang DDT-resistant strain might show decreased DDT absorption, but it did not.

Detailed examination of DDT-resistant larvae and their excreta by Abedi, Duffy and Brown (1963) revealed that DDE was the only DDT metabolite in *A. aegypti,* as in the house fly. The dehydrochlorination inhibitor DMC was found to be a DDT synergist for 6 DDT-resistant strains of *A. aegypti.* All these strains were susceptible to Dilan, which cannot be detoxified by dehydrochlorination. However, the DDT-resistant mosquito strains differed from resistant house flies in showing cross-resistance to o-chloro-DDT, which contains an extra chlorine on the benzene ring to introduce steric hindrance to detoxication.

It was eventually possible to demonstrate the DDT-dehydrochlorinase enzyme in *A. aegypti* larvae, by protecting it during the process of preparation with glutathione, nitrogen and low temperature. Kimura and Brown (1964) found the mosquito enzyme to have an optimum pH of 7.4, and to dehydrochlorinate DDD

faster and methoxychlor slower than DDT; but unlike the house fly enzyme, it could also dehydrochlorinate o-chloro-DDT. In a large number of *A. aegypti* strains of American origin, their DDT-dehydrochlorinase activity was found to be proportional to their DDT-resistance level. However in the Penang material, the dehydrochlorination of DDD but not of DDT was proportional to the resistance level.

The mosquito enzyme was inhibited *in vitro* by DMC, and also by the compound dibutyl-p-chlorobenzenesulfonamide, known commercially as WARF-Antiresistant. An equal mixture of Antiresistant and DDT was found by Pillai, Abedi and Brown (1963) to be 10–50 times more toxic than DDT to the resistant strains. Moreover, selection of the Trinidad and Penang strains with this mixture resulted initially in a reduction of the DDT-resistance and Mixture-resistance levels, accompanied by a drop in the DDT-dehydrochlorinase content. But subsequently the resistance picked up again, so that by the 6th generation the Mixture-resistance had multiplied by 10 times, accompanied by a considerable increase in DDE production *in vivo.*

The analogue of DDT in which the hydrogen on the tertiary carbon atom had been replaced by its non-radioactive isotope deuterium could scarcely be attacked at all by the DDT-dehydrochlorinase enzyme of *A. aegypti.* This deutero-DDT, produced by Dr. D. J. Hennessy of Fordham University, was found by Pillai, Hennessy and Brown (1963) to be 50–100 times as toxic as DDT to the 9 DDT-resistant strains tested. Moreover 5 generations of selection with deutero-DDT increased the LC_{50} of a Trinidad susceptible strain by only 4 times, whereas selection with DDT for the same period increased the DDT-resistance by 1000 times. Now after 10 generations of selection these two figures are 20 and 2200 times respectively. Thus deutero-DDT is an effective remedial insecticide for

DDT-resistance in *Aedes aegypti;* it had proved ineffective against DDT-resistant house flies because it could be detoxified by the fly dehydrochlorinase. But for *Aedes aegypti* deutero-DDT offers a substitute that does not develop much resistance to itself, and that has the same formulation and safety characteristics as DDT itself. One catch, however, is that deutero-DDT selection considerably increases the DDT-resistance level.

The character DDT-resistance in all strains of *A. aegypti* studied is neither dominant nor recessive, the hybrids between resistant and susceptible mosquitoes being exactly intermediate, although the dosage-mortality lines of the 3 genotypes overlap considerably. With the aid of mutant-marker strains kindly provided by Dr. G. B. Craig of the University of Notre Dame, Brown and Abedi (1962) were able to find that the DDT-resistance of the Trinidad, Isla Verde and Penang strains was not linked with the marker *black-tarsus* on Chromosome III. Instead it was linked with two markers close together on Chromosome II, namely *spot* and *yellow* at crossover distances of 20–25 units from them. That the DDT-resistance was identical in all 3 strains was indicated by a special study in which they were first purified by sib selection of single broods, and then crossed together. The F_2 of these interstrain crosses showed no greater variance than the F_2 from the intrastrain crosses, and was identical with the F_1 variance, indicating that one and the same gene was involved in all. It should be mentioned that R. J. Wood of the University of Pavia has recently studied the DDT-resistance of the Trinidad strain and found it due to a main gene, which he considers is probably on Chromosome III, modified by a second gene and also by a sex-linked factor.

The genetics of dieldrin-resistance could be studied with greater precision, since not only was it clearly due to a single gene allele, but the F_1 hybrids are exactly intermediate and completely separable from the susceptible homozygotes by a larval diagnostic dosage of 0.08 p.p.m.

dieldrin. In the Isla Verde strain the dieldrin-resistance was found by Khan and Brown (1961), to be linked with the marker *yellow* on Chromosome II, so close to DDT-resistance that selection with either insecticide produced and maintained resistance to both. By means of 3-point crosses involving at least 3 mutants in each marker strain, Klassen and Brown (1964), found that the dieldrin-resistance gene was located at crossover distances of 15 from *Gold,* 19 from *yellow* and 22 from *spot.*

In due course it proved possible to obtain a DDT-susceptible dieldrin-resistant strain in addition to the Trinidad DDT-resistant and the Isla Verde doubly resistant strains. Thus crosses could be made introducing the two resistances in coupling and in repulsion, and the larvae from the Mendelian F_2 could be categorized with first a diagnostic dosage of dieldrin and 24 hours later one of DDT. Such experiments indicated the crossover between the dieldrin gene and the DDT gene to be approximately 4 percent. Since the crossover values of the DDT gene from the marker *yellow* were consistently greater than those from the dieldrin gene to *yellow* in the Isla Verde, Curaçao and Jamaican strains, it is evident that the order is *spot-yellow-gold-dieldrin-DDT.* However, Coker has recently reported results from the Trinidad strain showing *spot* to be closer to DDT than *yellow.*

Since the addition of WARF-Antiresistant to DDT selected for greater DDT-susceptibility and lower dehydrochlorination in the first 2–3 generations before resistance subsequently rose to greater heights than originally, an investigation was made by Pillai and Brown (1965) on the genetics of the Mixture-resistant strains ultimately produced from the Trinidad and Penang strains. The character of resistance to the DDT-Antiresistant mixture was found to be linked with Chromosome II with crossover percentages of 24 and 28 respectively from the marker *spot,* indicating the main DDT-resistance gene already known. Whereas in the Trinidad strain the character showed al-

most completely independent assortment with the Chromosome-III marker *black-tarsus,* in the Penang strain the 41 percent crossover figure with this marker indicated that genetic material on Chromosome III was involved. When crosses were made between the Mixture-resistant strains and the purely DDT-resistant strains of the Trinidad and of the Penang stock, it was found that the F_1 was more resistant than both of the parents, and so was the F_2. Since this increase in resistance was not found when two purely DDT-resistant strains were crossed, it would appear that the Mixture-resistant strains had contributed modifier gene alleles additive to and different from those in the purely DDT-resistant strains.

Investigations have been made by Brown and Abedi (1960) of the likelihood of *Aedes aegypti* larvae developing resistance to the organophosphorus compounds malathion and parathion. Only a modest tolerance could be produced, without any increase in slope of the dosage-mortality regression line, and reverting when selection was relaxed. Malathion pressure applied for 5 generations to the Trinidad and Penang strains, and one from Kongolikan, Upper Volta, West Africa, increased the malathion LC_{50} by 5–6 times. Parathion pressure applied for the same period on the Trinidad and Kongolikan strains increased the parathion LC_{50} by 2–3 times. Cross-tolerance was shown between the two OP compounds, which extended in most cases to carbaryl (Sevin). All the OP-selected strains showed a notable cross-resistance to DDT, amounting to 32 times in the malathion-selected Penang strain.

All 3 strains developed by malathion selection were found by Matsumura and Brown (1961, 1963) to show a reduced absorption of malathion, and the Trinidad and Penang strains showed decreased absorption of DDT as well. Both the parathion-selected strains showed reduced absorption of parathion, but since their tolerance level was small it was not surprising to find that statistical significance was lacking for this interstrain difference. Neither the malathion-selected nor the parathion-selected strains showed any increase in detoxifying ability of the phosphatase type, nor did the malathion-tolerant *A. aegypti* strains show the increase in carboxyesterase activity characteristic of malathion-resistance in *Culex tarsalis.*

The malathion-tolerance developed in the Penang strain shows simple additive inheritance, the hybrids being intermediate in tolerance. This character, which is probably mainly polyfactorial at this early stage of development, showed independent assortment with the sex-chromosome I, and in the males it assorted independently with Chromosome III also. The malathion-tolerance showed some linkage with *yellow* and with the dieldrin gene on Chromosome II, the crossover percentages being 39 and 33 respectively. However in the females Chromosome III did have an effect, there being some linkage with the marker *black-tarsus* with 38 percent crossing over. It would seem that main genes will not appear for OP-resistance until strains of *A. aegypti* have been under selection pressure for the great number of generations found necessary to produce OP-resistance in the house fly.

These studies on insecticide-resistance in *Aedes aegypti* have shown that the mechanism of DDT-resistance is detoxication by DDT-dehydrochlorinase as in the house fly, and have discovered deutero-DDT to be a remedial insecticide that cannot be dehydrochlorinated by the mosquito enzyme. The studies have also shown that the DDT-resistance gene is located very close to the dieldrin-resistance gene on Chromosome II, and the dieldrin-resistance is such a precise character that it has aided in the mapping of several mutant genes on that chromosome. They have also revealed that organophosphorus-resistance in *A. aegypti* does not come as a specific entity like the malathion-resistance of *Culex tarsalis;* proper understanding of OP-resistance in *A. aegypti* will probably await the ultimate production of good resistant strains, which may take longer in this species than in the important vector *Culex fatigans.*

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COMING EVENTS IN ILLINOIS

The third biennial short course in mosquito control, sponsored by the Illinois Mosquito Control Association and the U. S. Public Health Service, will be held January 26, 27 and 28, 1965 at the South Cook County Mosquito Abatement District, 155th and Dixie Highway, Harvey, Illinois.

The Illinois Mosquito Control Association will hold its 10th Annual Meeting on March 1 and 2, 1965 at the new Ramada Inn, Route 66, Springfield, Illinois.

Anyone wishing to attend either or both of these meetings may contact Eugene M. Bellont, Secretary, at the South Cook County Mosquito Abatement District for further information.