numbers as they had in 1958. However in the section of the valley within the city, very few mosquitoes appeared in comparison to the number which had been present in 1958.

ACKNOWLEDGMENTS. This study was financed by a grant from the Parks Department of the City of Edmonton. Airspray Limited, Wetaskiwin, Alberta laid down the lindane barriers. Members of both the Department of Entomology, University of Alberta and of the Parks Department, City of Edmonton assisted in evaluating the density of mosquitoes. Some equipment was made available by J. A. Shemanchuk, Research Station, Canada Agriculture, Lethbridge, Alberta. Details of lindane barriers used for mosquito control in Texas and helpful suggestions were generously supplied by R. L. Pearce, Angleton, Texas, U.S.A. Our thanks are expressed to all.

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STUDIES ON ORGANOPHOSPHORUS-TOLERANCE IN *AEDES AEGYPTI*

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INTRODUCTION. Larval selection with malathion applied to the Penang strain of *Aedes aegypti* resulted in an increase in malathion-tolerance and a great increase in DDT-resistance (Brown and Abedi, 1960). The physiological mechanism of the tolerance proved not to be an increase in detoxication, but a decrease in absorption into the larvae (Matsumura and Brown, 1961a), which was shown not only with malathion but also with DDT. The purpose of this investigation was to ascertain whether selection with malathion, or with parathion, would induce similar tolerance increases in other strains of this species. Biochemical investigations were also made to determine whether there was any increase in detoxication of malathion or parathion, or any decrease in absorption of malathion, parathion or DDT.

MATERIAL AND METHODS. The susceptible strain chosen for selection originated from Kongolikan, Upper Volta, in the interior of West Africa. This strain had been maintained without exposure to insecticides by Dr. J. M. Doby, University of Rennes, France, who kindly supplied the stock to this laboratory in early 1961; its LC_{50} to DDT was .003 ppm, a record for susceptibility in *A. aegypti*. The DDT-resistant strain originated in Trinidad, in the southern Caribbean area, and had been maintained in our laboratory for 3 years, during which time its LC_{50} to DDT had slightly reverted to 0.25 ppm.

Substrains were submitted to larval

lection pressure at approximately the 90 rcent mortality level, applied to every neration; the F_5 was chosen as the lected generation for test. The malathion nployed was 99 percent pure (American yanamid Co.) and the parathion 98.8 rcent pure (Nutritional Biochemicals orp.). Larval susceptibility levels were termined by the standard WHO method. For the biochemical investigations, radio- tive malathion and parathion were syn- esized from P^{32} phosphoric acid. Radio- tive DDT was supplied by Tracerlab c., with the C^{14} atoms in the para posi- on of the phenyl groups. Methods to termine the breakdown of radioactive alathion and parathion into their hydro- tic products, and the absorption of these mpounds into the body, followed those scribed by Matsumura and Brown 916b), but with certain modifications. the *in vitro* experiments, the phos- atase products were removed first, by idifying to pH 2 before extraction with loroform. In the *in vivo* and *in vitro* periments a control figure to compensate r protein-absorbed malathion in the ueous fraction was obtained by adding radioactive malathion at the termination of a parallel experiment. The absorption of radioactive DDT was determined by the method described by Fast and Brown (1962).

RESULTS. Selection with malathion (Fig. 1) resulted in a steady increase in tolerance in successive generations, with little change in slope of the dosage-mortality line. By the 5th filial generation a 5-6-fold increase in malathion-tolerance had been reached (Table 1); the similar increase previously obtained with the Penang strain is included in the table for comparison. However, only a moderate cross-tolerance to DDT was simultaneously induced in these strains, in contrast to the strong DDT-cross-resistance previously noted in the Penang strain. On the other hand, the cross-tolerance to parathion was greater than that in the Penang strain; but the cross-tolerance shown to Sevin by these two strains was less than that in the Penang strain.

Selection with parathion (Fig. 2) resulted in a steady increase of the LC_{50}, without much change in slope for the Kongolikan strain, but with steepening

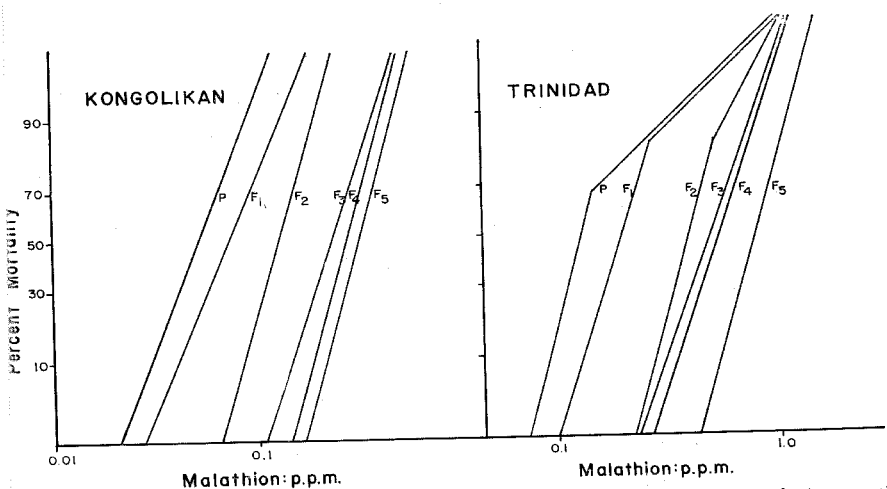

FIG. 1.—Dosage-mortality lines for larvae of the Kongolikan and Trinidad strains of *A. aegypti* lected with malathion for 5 generations.

TABLE 1.—Larval LC_{50} levels in p.p.m. of malathion-selected and parathion-selected strains as compared with their originals.

	Malathion	Parathion	DDT	Sevin	Pyrethrins	Na Arseni
Original strains						
Kongolikan	0.06	0.008	0.003	1.8	0.029	32
Trinidad	0.12	0.016	0.25	2.4	0.034	24
Penang	0.26	0.035	0.08	1.9	..	..
Strains after Malathion selection						
Kongolikan	0.30	0.022	0.021	1.2	0.020	46
Trinidad	0.73	0.030	0.54	3.9	0.034	33
Penang	1.43	0.055	2.56	9.1	..	..
Strains after Parathion selection						
Kongolikan	0.18	0.027	0.012	1.4	0.020	41
Trinidad	0.24	0.030	0.70	3.9	0.034	35
Resistance ratios* after Malathion selection						
Kongolikan	5.0	2.7	7.0	0.6	0.7	1.3
Trinidad	6.1	1.9	2.2	1.6	1.0	1.4
Penang	5.7	1.6	32.0	4.8	..	..
Resistance ratios after Parathion selection						
Kongolikan	3.0	3.4	4.0	0.8	0.7	1.3
Trinidad	0.9	1.9	2.8	1.6	1.0	1.5

* LC_{50} of the selected strain divided by that of the original strain.

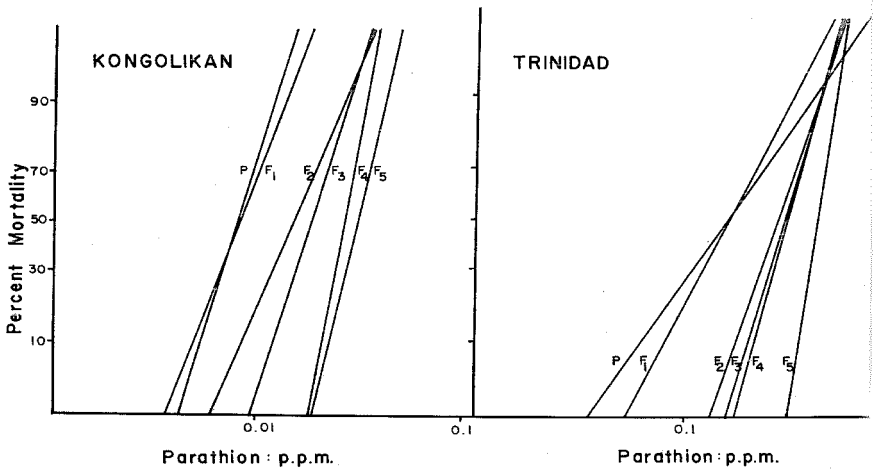

FIG. 2.—Dosage-mortality lines for larvae of the Kongolikan and Trinidad strains of *A. aegy* selected with parathion for 5 generations.

ope in the Trinidad strain, which reached -3 times the normal by the 5th filial gen-ration. It induced a slight cross-tolerance › malathion in the Kongolikan strain, ut not in the Trinidad strain. There was ight cross-tolerance to DDT, but none › Sevin. No cross-tolerance was evi-enced against pyrethrins or sodium ar-nite after parathion or malathion selec-on.

Homogenates of the selected and orig-al strains in phosphate buffer at pH 7.8 ere incubated with radioactive malathion r 30 minutes at 23° C., and the per-ntage conversion to phosphatase and rboxyesterase products was determined. he results (Table 2) show that the tive malathion or parathion for 1 hour, transferred to clean water for 1 hour, and then their bodies and the ambient water were asssesed for malathion (and mala-oxon) and hydrolysis products. The results (Table 3) show that the selected strains produce no more phosphatase or carb-oxyesterase products than the original strains. On the other hand, the malathion-selected larvae have evidently absorbed considerably less malathion into their bodies than larvae of the original strains; this difference is significant for the Trin-idad stock, and becomes significant for the Kongolikan stock when the mala-thion concentration is increased to 10 p.p.m. No difference in absorption, how-

TABLE 2.—Phosphatase and carboxyesterase activities in homogenates of selected and resistant strains: percent of insecticide converted *in vitro*.*

Strains	Phosphatase products		Carboxyesterase products	
	Percent	t_7	Percent	t_7
alathion-selected material incubated with 250 micrograms malathion				
Kongolikan original	0.74±.01	0	0.16±.01	0.43
Kongolikan selected	0.76±.01		0.14±.01	
Trinidad original	1.24±.05	0	0.18±.01	0
Trinidad selected	1.24±.05		0.18±.01	
rathion-selected material incubated with 25 micrograms parathion				
Kongolikan original	0.20±.16	0.46	Nil	
Kongolikan selected	0.35±.28		Nil	
Trinidad original	0.76±.14	0.52	Nil	
Trinidad selected	0.87±.16		Nil	

* Average of 4 experiments.

alathion-selected material does not differ om the original materal in either phos-natase or carboxyesterase activity; more-er the parathion-selected material does ot have significantly more phosphatase tivity than the original, and of course produces no carboxyesterase products om parathion.

Larvae of the selected and original rains were exposed to 1 p.p.m. radioac-

ever, is shown between the parathion-selected and normal strains.

Larvae of the six strains were exposed to 1 p.p.m. radioactive DDT for 1 hour, transferred to clean water for 1 hour, and the amounts of radioactivity in the bodies and ambient water were determined. The results (Table 4) show that there is no difference in absorption between the para-thion-selected and the original strains, nor

TABLE 3.—Excretion and retention of radioactive malathion (or parathion) and metabolites: micrograms per 25 larvae.*

Strains	Percent mortality	Excreted into clean water		Retained in the body	
		Solvent-extractable insecticides**	Water-soluble products***	Amount	$t_{\frac{1}{2}}$
Malathion-selected strains exposed to 1 p.p.m. malathion					
Kongolikan original	2	.07±.02	.09±.02	.18±.04	
Kongolikan selected	1	.03±.01	.07±.02	.14±.02	0.8
Trinidad original	0	.07±.02	.14±.03	.19±.02	
Trinidad selected	0	.02±.01	.05±.01	.08±.01	4.9
Malathion-selected strains exposed to 10 p.p.m. malathion					
Kongolikan original	97	.66±.04	.17±.06	1.82±.28	
Kongolikan selected	81	.66±.17	.12±.02	1.12±.10	2.3
Trinidad original	79	.07±.02	.13±.02	1.12±.19	
Trinidad selected	13	.05±.01	.07±.02	0.26±.04	4.4
Parathion-selected strains exposed to 1 p.p.m. parathion					
Kongolikan original	3	.26±.18	.26±.12	.18±.04	
Kongolikan selected	1	.11±.03	.11±.09	.16±.01	0.5
Trinidad original	4	.20±.05	.55±.23	.31±.05	
Trinidad selected	0	.23±.14	.60±.15	.23±.05	1.1

* Average of 4 experiments.
** Malathion plus malaoxon (or parathion plus paraoxon).
*** Phosphatase and/or carboxyesterase products.

between the Kongolikan malathion-selected and the original strain. However, the Trinidad malathion-selected strain absorbed less than half as much DDT as the original strain.

DISCUSSION. The malathion-selected strains showed a modest increase in tolerance, approximately 5 times in 5 generations, which was similar to that observed in the Penang strain when it also had been submitted to 5 selections (Brown and Abedi, 1960). The gradual and steady movement of the dosage-mortality line without change in slope also suggest an analogy with vigor tolerance, or at least a polyfactorial genetic origin for the malathion-tolerance. This is borne out by the lack of increase in phosphatase or carboxyesterase hydrolysis, observed als for the Penang strain (Matsumura an Brown, 1961a); it stands in marked co trast to the great increase in carboxyestera activity induced in malathion-resista *Culex tarsalis* by a single gene alle (Matsumura and Brown, 1961b). Th significant decrease in absorption of mal thion, particularly marked in the Trinida strain, had also been observed in th Penang strain (Matsumura and Brow 1961a); it is difficult to regard it as mon factorial in genetic origin.

The parathion-selected strains showe only slight increases in parathion toleranc in fact no greater than that induced b malathion selection. Moreover, no signi cant differences could be found in par

TABLE 4.—Excretion and retention of radioactive DDT: micrograms per 25 larvae.*

Strains	Excreted into ambient water	Retained in the body	
		Amount	t**
ongolikan original	0.012±0.007	0.211±0.023	..
ongolikan Malathion-selected	0.032±0.004	0.197±0.011	0.55
ongolikan Parathion-selected	0.036±0.016	0.196±0.027	0.42
rinidad original	0.022±0.010	0.157±0.016	..
rinidad Malathion-selected	0.037±0.022	0.068±0.005	5.33
rinidad Parathion-selected	0.026±0.010	0.160±0.019	0.12

* Average of 4 to 7 experiments.
** Statistical comparison with original strain.

ion hydrolysis or absorption. Nevertheless the dosage-mortality lines moved readily towards this slight tolerance; evidently the genetic factors available for defenses against parathion are weak and non-specific.

The malathion-selected Trinidad strain, which cross-tolerance more than doubled its LC_{50} for DDT, absorbed less than half as much DDT as the original. On the other hand the selected Penang strain, which became highly cross-resistant to DDT (Brown and Abedi, 1960) also absorbed about half as much DDT as the original strain (Fast and Brown, 1962). The selected Kongolikan strain, in which 7-fold increase in cross-tolerance still left it relatively susceptible to DDT, showed no decrease in DDT absorption. The parathion-selected strains, which developed no cross-tolerance to DDT, showed no differences in absorption of DDT. These results suggest that the increases in DDT-tolerance induced in mosquito larvae by selection with organophosphorus compounds are associated with decreases in the absorption rate for DDT, but the relationship may be complicated by other resistance mechanisms such as detoxication of DDE (Chattoraj and Brown, 1960).

SUMMARY. Selection with malathion applied to larvae of a Caribbean and a West African strain of *A. aegypti* resulted in a 5-fold increase of tolerance in 5 generations. This tolerance was associated with a decrease in absorption of malathion by the larvae, and was not accompanied by any increase in detoxication by phosphatase or carboxyesterase hydrolysis. There was only a moderate cross-tolerance to DDT, which in the Caribbean strain was associated with a decrease in absorption.

Selection with parathion applied to these strains resulted in a 2-3-fold increase in tolerance, no greater than the cross-tolerance to parathion induced by malathion selection. There was no increase in detoxication by phosphatase hydrolysis and no decrease in absorption. There was a slight cross-resistance to DDT, but no decrease in DDT absorption.

This work was performed on a grant-in-aid from the National Institutes of Health.

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