the encephalitis viruses are introduced into the area by infected arthropods or vertebrates, and do not exist in an endemic state. Several of the larger pools of *C. quinquefasciatus* collected from railroad box cars in 1960 have been shipped to the Texas State Department of Health Laboratory for virus studies, with negative results.

Anopheles quadrimaculatus were taken in numbers sufficient to indicate that there is some international dispersal of the species by railroad box cars. Even though there has been a decline to the point of disappearance of malaria in the United States and much of Mexico, sporadic cases continue to occur. Two cases were brought to our attention in Cameron County in September, 1960. The malaria potential is of concern to public health workers, especially in areas such as the Lower Rio Grande Valley of Texas, with large populations of *A. quadrimaculatus*.

Of interest was the recovery of but a single *Culex tarsalis*. The species is frequently intercepted on aircraft arriving in Brownsville from Mexico.

SUMMARY. Inspections of railroad box cars have been conducted upon their arrival in Matamoros from the interior of Mexico, just prior to their admittance into the United States. From February 1958, through December 31, 1960, 4,436 cars in 113 trains were examined and 3,162 adult mosquitoes were collected alive. They were primarily *Culex quinquefasciatus*, with nine other species represented by small numbers.

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BIOCHEMICAL STUDY OF A MALATHION-TOLERANT STRAIN OF *AEDES AEGYPTI*

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A malathion-tolerant strain has been developed by larval selection with malathion applied to a colony of *Aedes aegypti* from Penang, Malaya (Brown and Abedi, 1960). This strain attained a 5-fold increase in malathion-tolerance, but simultaneously acquired a cross-resistance to DDT and DDD in excess of 30 times the normal.

Malathion-resistance, as first studied in the house fly, has been found to involve an increase in the detoxification of malaoxon (March, 1959), and a concomitant decrease in the enzyme aliesterase (Oppenoorth, 1959). Later, the strong and specific malathion-resistance developed in a strain of the mosquito *Culex tarsalis* has been found to involve a marked increase in carboxyesterase activity detoxifying malathion, and a smaller increase in phosphatase activity detoxifying malaoxon (Matsumura and Brown, 1961). The aim of this study was to ascertain whether these or other biochemical mechanisms were associated with the malathion-tolerance of the Penang strain of *Aedes aegypti*.

The malathion-tolerant Penang strain

was tested in the F_{11} generation, after having been maintained without selection pressure since the F_8 generation, the last to be reported by Brown and Abedi (1960). The LC_{50} for the F_{11} was 0.65 p.p.m. malathion, as compared with 0.31 for a susceptible Guelph strain. The LC_{50} level to malaoxon was 4.9 p.p.m., as compared with 2.6 p.p.m. for the normal strain, and 1.3 p.p.m. for the susceptible Guelph strain. Thus the malathion-tolerant Penang strain in the F_{11} was twice as tolerant as the normal to both malathion and malaoxon.*

The aliesterase activity was assessed according to the method of Oppenoorth (1959), using methyl n-butyrate as the substrate. Measured as the cu. mm. CO_2 produced per gm. tissue per minute, the activity proved to be 11.1 ± 2.8 for the malathion-tolerant strain, and 8.6 ± 3.2 for the normal strain. This difference is without statistical significance, the t_7 being 0.59.

The phosphatase activity and carboxyesterase activity in homogenates of either strain were assessed *in vitro* according to the method of Matsumura & Brown (1961), using radioactive malathion as the substrate. The results with the three strains (Table 1) indicate the malathion-tolerant and the normal strains showed virtually identical activities in these two detoxifying enzymes; both Penang strains showed slight differences from the Guelph strain in the enzyme activities, but they were not quite significant statistically.

Since no difference in detoxifying activity was evident, an experiment was performed to investigate the absorption of malathion by larvae of the different strains *in vivo*. Groups of 25 larvae were exposed to 1 p.p.m. radioactive malathion in 25 ml. water for 1 hour; they were then removed to 25 ml. distilled water for 1 hour, and the radioactivity remaining in the larvae and excreted into the water was assessed according to the method described by Matsumura and Brown (1961). The results (Table 2) show that all strains ingested similar amounts of malathion, but they also reveal that the malathion-tolerant Penang strain excreted more and retained much less of the insecticide within the body. In fact, the amount actually retained (and thus absorbed internally) in the malathion-tolerant strain was less than one-twelfth as much as in the other two strains. The difference was highly significant statistically, the t_7 values being respectively 3.48 and 3.97.

Since the malathion-tolerant strain was characterized by having also gained a high cross-resistance to DDT, the strains were similarly compared for their absorption and retention after exposure to 1 p.p.m. radioactive DDT. It was found that the malathion-tolerant strain retained (absorbed internally) only one-quarter as much DDT as the normal Penang strain. The lower absorption in the malathion-tolerant strain was highly

TABLE 1.—Phosphatase and carboxyesterase activities in malathion-tolerant and susceptible strains of *Aedes aegypti*

Strain	Phosphatase products		Carboxyesterase products	
	Percent*	t_9**	Percent*	t_9**
Malathion-tolerant Penang	10.0±1.3	0.42	2.5±0.2	0.71
Normal Penang	9.3±0.9	..	2.3±0.2	..
Susceptible Guelph	6.2±1.3	1.96	2.8±0.4	1.11

* Percentage of malathion radioactivity converted to hydrolytic products.
** Statistical comparison with normal Penang strain.

* This strain gradually reverts toward susceptibility when maintained without selection pressure.

TABLE 2.—Malathion and metabolites excreted and retained internally: micrograms per 100 larvae

Strain	Excreted into clean water			Retained in the body
	Malathion (+ malaoxon)	Phosphatase products	Carboxyesterase products	
Malathion-tolerant Penang	1.50±.77	5.81±1.45	1.75±.79	0.14±.13
Normal Penang	2.00±.72	2.93± .23	0.11±.11	1.77±.47
Susceptible Guelph	0.75±.52	3.46±1.12	0.88±.52	2.12±.52

significant statistically not only at 1 p.p.m. ($t_5 = 3.50$) but also on exposure to 5 p.p.m. DDT ($t_5 = 5.05$).

It is therefore evident that the malathion-tolerance of the Penang strain of *Aedes aegypti*, in contrast to the malathion-resistance studied in *Culex tarsalis*, derives not from increased detoxification but from decreased absorption of malathion. This decreased absorption is even more marked in the case of DDT, to which the cross-resistance is much greater than to malathion itself. Therein the malathion-tolerant strain resembles a DDT-resistant strain developed in the Penang stock by DDT pressure, which was found to absorb and retain only one-third as much DDT as the normal strain.

SUMMARY. Larvae of a malathion-tolerant strain of *Aedes aegypti*, developed by laboratory selection of stock from Penang, Malaya, were found not to differ from the normal in activity of the detoxifying enzymes phosphatase or carboxyesterase, nor in aliesterase. They did differ in showing only one-twelfth as much absorption and retention of malathion as larvae of the normal strain. Moreover, the high cross-resistance shown by this strain to DDT was accompanied by only one-quarter as much absorption and retention of DDT as the normal.

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