Table 1. Mean percentage difference in oxygen content between the vessels compared.

| Vessels compared | Change in $O_2$ content (%) | Standard error | No. |
|---|---|---|---|
| Right atrium and pulmocutaneous | 120 | 6.3 | 11 |
| Pulmocutaneous and aorta | 160 | 12.6 | 12 |
| Aorta and carotid | 130 | 11.6 | 10 |
| Carotid and left atrium | 130 | 14.2 | 9 |

to a 1-ml tuberculin syringe which had been flushed with heparin anticoagulant (1000 U.S.P. units/ml). All oxygen analyses were made on sub-samples of equal size by the method developed by Roughten and Scholander (*5*).

To minimize the possible disturbance of pressure relationships within the circulatory system, blood was withdrawn at a rate approximating the flow in the vessels, as estimated by bulging or constriction of the vessel wall. To determine whether withdrawal of blood was causing a change in the distribution of blood by the heart, blood was also withdrawn from the aorta both 1 cm from the conus and distally, just proximal to the juncture of the left and right aortas, a point at which an amount of blood equal to that withdrawn would already be contained in the vessel. These proximal and distal readings were equivalent, and since all samples taken in any artery were at least 1 cm from the conus, it was assumed that withdrawal of blood did not significantly alter the flow through any vessel. It is conceivable that different mixing patterns could occur at the higher blood pressures found in unpithed frogs. However, in the course of the experiment, frogs in prime breeding condition with a high oxygen titer in the blood and an estimated high blood pressure, and commercially obtained frogs with low oxygen titer and low estimated blood pressure, showed a similar pattern of distribution for the oxygenated blood from the left atrium.

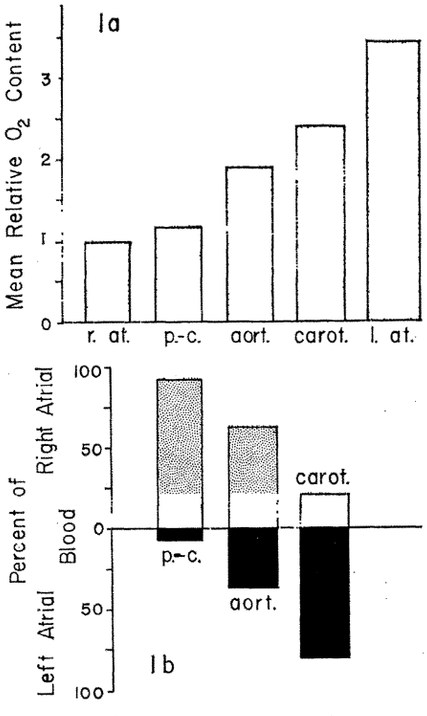


Fig. 1. Oxygen content in the vessels shown in *a* are multiples of the value for the right atrium; these values suggest mixing in the proportions shown in *b*.

So great a difference was found in the oxygen level of the blood in comparable vessels of different frogs that the results have been tabulated as a difference in oxygen content between different blood vessels of each individual frog (Table 1). On the basis of these data the oxygen contents of these vessels are compared directly as multiples of that in the right atrium (Fig. 1*a*).

If a separation of the blood streams within the ventricle is to be postulated, a consistent difference in the oxygen content of the vessels leaving the heart must be shown. The considerable difference in the oxygen content of the aorta and carotid arteries over that of the pulmocutaneous arteries indicates a directed and continuous, even though partial, separation of the atrial blood streams in the ventricle. On the other hand, since the oxygen content of the various arteries was always between the values for the two atria, some mixing of the blood in the ventricle must occur. If the difference in oxygen content between the vessels is considered, the degree of mixing between the left and right atrial stream can be quantified (Fig. 1*b*).

Blood entering the pulmocutaneous arteries is primarily right atrial, whereas that entering the carotid arteries, although mostly from the left atrium, contains some blood from the right atrium, showing that considerable mixing of the two streams has taken place. In the aorta, nearly two-thirds of the blood is from the right atrium and one-third from the left atrium. These results support the work by Simons (*3*) who stated that in anurans most of the left atrial blood enters the carotid and aortic arteries. Secondarily, Simons found an unequal distribution of oxygenated blood to the left and right aortic arches; unfortunately, these two vessels were not critically compared in the present study, since only the left side was sampled. Sharma (*4*) stated that in *R. pipiens* the aortic and carotid arteries receive only left atrial blood and the pulmocutaneous arches receive a mixed stream. The present study does not support this statement (Fig. 1*b*).

In *Rana pipiens* the carotids receive primarily left atrial blood which is highly oxygenated, whereas the pulmocutaneous vessels receive blood almost exclusively from the right atrium. Only the aorta receives blood subjected to considerable mixing (*6*).

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6. I thank Dr. Warren F. Walker for his advice and encouragement during the course of this work, and Oberlin College for providing the use of facilities and materials.

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## Effect of Malathion Analogs upon Resistant and Susceptible Culex Tarsalis Mosquitoes

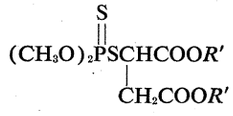
*Abstract.* The effects of minor variations in the malathion molecule upon the resistance of *Culex tarsalis* have been examined. By replacing the carboethoxy group by carbomethoxy, the 60-fold resistance to malathion is abolished. The results confirm the importance of carboxyesterase action in determining susceptibility to malathion.

The insecticide malathion [O,O-dimethyl *S*-bis(carboethoxy) ethyl phosphorodithioate] is toxic to most insects and of very low toxicity to mammals. It has been shown that this is due to the more rapid degradation of malathion in mammals, due to a carboxyesterase which hydrolyzes the $COOC_2H_5$ (carboethoxy) group (*1*). Recently Matsumura and Brown (*2*) have shown that when a strain of *Culex tarsalis* mosquito becomes resistant to malathion, it does so as the result of an increase in a carboxyesterase. Confirmation of the importance of carboxyesterase comes from the demonstration that when it is inhibited in vivo by treatment with compounds such as EPN

(O-ethyl O-*p*-nitrophenyl phenylphosphonothiorate) or TOCP (tri-*o*-cresyl phosphate), the toxicity of malathion to mammals or to resistant *Culex* or houseflies is greatly increased (*2*, *3*).

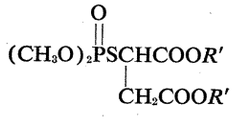
Since resistant *Culex* owe their resistance to malathion to their ability to degrade the carboethoxy group (*2*), it seemed possible that, if the ethyl group were replaced by other alkyl groups, the resistance mechanism could be overcome. This would occur only if the carboxyesterase were specific for the ethyl substituent.

Table 1 shows data for malathion analogs



with various COO*R′* groups. The $LC_{50}$ (in parts per million) for the various compounds for the susceptible (*S*) and the resistant (*R*) full-grown larvae (Fresno strain) was determined by the World Health Organization standard method (*4*). The resistant strain is 60 times less sensitive than the susceptible strain when *R′* is ethyl (that is, for malathion), but when *R′* is methyl, the two strains are equally susceptible; resistance has been abolished ($R/S = 1$). The implication is that the carboxyesterase in the resistant strain cannot hydrolyze the carbomethoxy group. To judge by the *R*/*S* ratio, it seems that this carboxyesterase has some activity toward other COO*R′* groups, particularly when *R′* is *n*-propyl or *n*-butyl, but has much less activity when *R′* is isopropyl or pentyl.

Table 1 also shows data for malaoxon



with various COO*R′* groups. Once again the resistant strain is far less sensitive than the susceptible strain when *R′* is ethyl (that is, for malaoxon) but shows no resistance when *R′* is methyl; and again, resistance is high when *R′* is *n*-propyl. However, in this case, when *R′* is *n*-butyl, the carboxyesterase is relatively inactive.

Table 1 shows degrees of resistance to other compounds containing the carbomethoxy or carboxyamide group. In no case was resistance found—a result that confirmed the earlier conclusion that the carboxyesterase cannot degrade the carbomethoxy group and suggested that it is also inactive with respect to the carboxyamide group. However, it must be pointed out that with the compounds morphothion and the isomers of "thionophosdrin" and phosdrin there has been evidence only with the *trans*-crotonate isomer of "thionophosdrin" that the carboxyesterase is important in degradation (*5*).

Table 1. Toxicity ($LC_{50}$ in parts per million) and toxicity ratios (*R*/*S*) of malathion analogs and of various other compounds to susceptible and resistant strains of *Culex* mosquito larvae.

| Compound | Toxicity | | *R*/*S* |
|---|---|---|---|
| | Susceptible | Resistant | |
| *Malathion analogs* | | | |
| *R′* | | | |
| Methyl | 0.13 | 0.13 | 1.0 |
| Ethyl | 0.025 | 1.5 | 60.0 |
| *n*–Propyl | 0.09 | 3.1 | 34.4 |
| Isopropyl | 0.19 | 0.86 | 4.5 |
| *n*–Butyl | 0.4 | 11.0 | 27.6 |
| Pentyl | 0.66 | 6.4 | 9.7 |
| *Malaoxon analogs* | | | |
| *R′*: | | | |
| Methyl | 0.5 | 0.48 | 1.0 |
| Ethyl | 0.08 | 0.66 | 8.3 |
| *n*–Propyl | 0.39 | 3.0 | 7.7 |
| Isopropyl | 0.62 | 1.0 | 1.6 |
| *n*–Butyl | 2.2 | 3.9 | 1.8 |
| *Morphothion (O,O–dimethyl S–morpholinocarbamoylmethyl phosphorodithioate)* | 2.0 | 1.8 | 0.9 |
| *"Thionophosdrin" (O,O–dimethyl–1–carbomethoxy–1–propen–2–yl phosphorothionate)* | | | |
| *Cis*–Crotonate isomer | 3.0 | 3.0 | 1.0 |
| *Trans*–Crotonate isomer | 1.2 | 0.36 | 0.3 |
| *Phosdrin (O,O–dimethyl–1–carbomethoxy–1–propen–2–yl phosphate)* | | | |
| *Cis*–Crotonate isomer | 0.07 | 0.07 | 1.0 |
| *Trans*–Crotonate isomer | 0.1 | 0.06 | 0.6 |

When one compares for various COO*R′* the ratio of the $LC_{50}$'s for the malaoxon or P(O) derivatives and the malathion or P(S) derivatives, one finds that the values are about constant for susceptible insects: 0.26, 0.31, 0.23, 0.31, and 0.18 [the pentyl, for which only the P(S) is available, being neglected]. The P(S) compounds are therefore consistently more toxic. Since the actual toxicant in every case is the P(O) derivative, these data imply that more insecticide gets into the insect with P(S) compounds than with P(O) compounds. This is probably due to the more lipophilic character of the P(S) compounds, which helps them to "partition" from water into the larvae. For the resistant insects the ratios vary widely, being 0.27, 2.3, 1.03, 0.86, and 2.8, respectively. Why is it that when *R′* is ethyl or *n*-butyl, the P(O) compounds are more toxic than the P(S) to resistant insects? A possible explanation is that the carboxyesterase in the resistant insects has a higher affinity for COO*R′* in P(S) compounds when *R′* is ethyl or *n*-butyl.

These results show that insects develop resistance to insecticides of this class by a mechanism specific enough so that minor variations in the insecticide molecule can suffice to overcome resistance (*6*).

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