The Aliphatic Esterases of Organo-Phosphate Resistant Houseflies

Adult houseflies, *Musca domestica*, which are susceptible to organophosphorous (OP) insecticides possess fairly active ali-esterases which are present in the muscles of the head and thorax, and appear to function as intestinal enzymes in the abdomen[1]. The ratio of activity in these 3 parts of the body are respectively 1:2:5. On the other hand, houseflies which are resistant to OP insecticides show very low ali-esterase activity which can be from $^1/_5$–$^1/_{10}$ that of susceptible. Very few exceptions to the rule have been found[2] so that a test for ali-esterase activity is in general use in the biochemical diagnosis of resistance. The esterase which is missing in resistant strains of houseflies has been called ali-esterase (a) which seems to be of little biological significance[3] and is believed to have been replaced by a detoxifying enzyme[4]. It is worth mentioning that the methods used in assessing ali-esterase activity, whether manometric, titrimetric or colorimetric, depended on measurements of the products of hydrolysis of the substrates used.

The following experiments have been made in order to find if this marked distinction in ali-esterase activity between resistant and susceptible strains is a true biochemical difference or, an artifact resulting from the shortcomings of the methods used in enzyme assay.

The SK strain of houseflies which is very resistant to OP and chlorinated hydrocarbon insecticides, was used. Its ali-esterase activity as measured by the rate of hydrolysis of methyl and ethyl butyrate is $^1/_5$ that of control (WHO standard susceptible).

Experiment I. Flies were injected intra-thoracically with various doses of phenyl acetate, methyl and ethyl butyrate. These substrates form water emulsions and show an optimum concentration for in vitro experiments of 9×10^{-3}, 4.8×10^{-2} and $3\times10^{-2}M$ respectively. The blood volume of houseflies is related to body weight and a volume of 0.17 µl/mg body weight has already been found[5]. Accordingly, the flies used throughout the experiments which weighed 18 mg, have a blood volume of 3.06 µl. The concentrations of the injected substrates were represented as final molar concentrations. For the above substrates, the optimum concentrations will be reached by an injection of 0.003, 0.016 and 0.021 µl respectively. The relation between log substrate concentration and percentage of mortality is illustrated in Figure 1 from which it is obvious that there is no difference in this respect between the 2 strains. Furthermore, toxicity of phenyl acetate happened at from 8 times the optimum concentration onwards while that for methyl and ethyl butyrate at approximately 10 fold their optimum concentration.

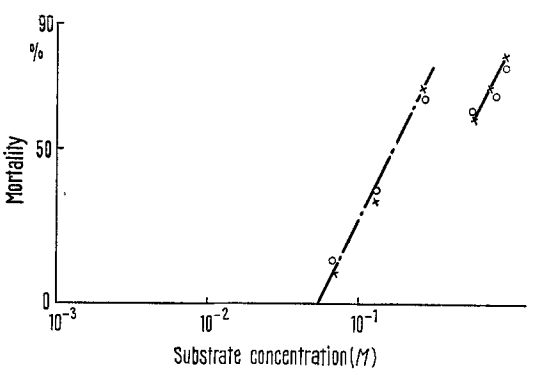

Fig. 1. Relation between substrate concentration (M) and toxicity. —·—, phenyl acetate; —, methyl butyrate; ×, OP resistant; ○, susceptible.

Experiment II. Flies were fumigated with ethyl and methyl butyrate in a 60 cm³ fumigation chamber at room temperature. Both esters proved very toxic fumigants and the CT factor mortality relationship is represented by Figure 2 from which no difference between the 2 strains can be revealed.

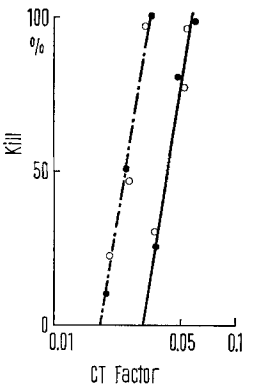

Fig. 2. Log CT factor plotted against % Kill. —·—, methyl butyrate; —, ethyl butyrate; ●, susceptible; ○, resistant.

Experiment III. 0.15 µl of methyl butyrate was injected into both strains of flies. A volume of 2 µl blood was removed at intervals of 5, 10 and 15 min and assayed for the ester in a Unicam spectrophotometer. It has been found that there is no trace of the ester in the blood of resistant flies, 10 min after the injection was made while the susceptible blood showed traces of what might be the ester or butyric acid. Both show the same maximum absorption peak at 210 nm.

The fact that the biochemical difference between the 2 strains with respect to the level of ali-esterase activity is not reflected in the failure of the OP resistant strain, or, the supremacy of the susceptible flies to metabolise these esters in vivo, indicate that the ali-esterases in both strains must be equally active, but, perhaps, act differently. It may be reasonable to assume that while these esters are metabolized in susceptible flies to alcohol and acid, the corresponding enzyme in resistant flies combine with them to form inactive complexes. Indeed, it may be that the latter pathway is more efficient than the former as indicated by the supposed involvement of the resistant ali-esterase in the detoxification of OP esters.

Résumé. Des mouches domestiques résistantes et non-résistantes à l'organophosphate ont répondu de façon semblable à des traitements variés d'esters aliphatiques. On suggère que l'activité aliestérasique très faible des mouches résistantes ne résulte que des imperfections de la méthode d'essai.

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