INTRODUCTION

The insect toxicology is concerned with the pathological principles involved in the killing of insects with insecticides. It is obvious that, when an insect is affected by a toxic chemical agent, a pathological state results. This may be readily discernible in the gross appearance of the insect and its organs, or it may be confined to the derangement or destruction of cellular elements. Such reactions as convulsions, spasms and paralysis are usually the result of direct action upon the insect nervous system, particularly upon the central nervous system. An insecticide for example, may bring about an initial exciting stimulation or irritation which is followed by stupefaction and immobility, muscle paralysis and finally death. The cause of death from most of the ‘nerve affecting insecticides’ is not known with certainty. There is general agreement among most of the workers on the importance of acetylcholinesterase in the normal functioning of the insect nervous system and that its absence causes death. It has been shown that organophosphorus insecticides are active inhibitors of cholinesterase enzymes both in vitro and in vivo, and that there is a correlation between their ability to inhibit cholinesterase in vivo and their insecticidal activity. Definite proof of this theory, however, has never been furnished and some year ago doubt as to its correctness has been expressed by many workers. Various esterases instead, have been implicated in the toxic action of organophosphorus insecticides to insects (Casida, 1955; Hopf, 1952,
Roeder et al. (1947) showed that di-isopropyl-fluorophosphonate (DFP) interferes characteristically with the transmission of nerve impulses across certain synapses in the nerve cord of *P. americana*. Chadwick and Hill (1947) injected cockroaches with DFP, hexaethyl-tetra-phosphate (HETP) and eserine and found that, on a molecular basis, HETP was the most toxic. The three substances inhibited cholinesterase *in vitro* in nerve cord, but HETP was less active than the others. Injection with eserine produced reversible inhibition of cholinesterase, whereas DFP inhibition was irreversible. Working with parathion and 32 related compounds, Metcalf and March (1949) showed a general relationship between the *in vitro* anticholinesterase activity with the bee brain homogenates and the *in vivo* toxicity to house flies and honey bees. They further demonstrated in honey bees a correlation between symptoms and *in vivo* brain cholinesterase inhibition. *In vivo* nerve cholinesterase inhibition has also shown good correlation with mortality (Chadwick and Hill, 1947) and symptomology (Chamberlain and Hoskins, 1951) in cockroaches poisoned with organophosphates. Malathion produced a marked cholinesterase inhibition in poisoned cockroaches and houseflies but the enzyme had nearly recovered before death of the insect (O’ Brien, 1956). The correlation between *in vitro* anticholinesterase activity and insect toxicity is quite good for diethyl substituted phenyl phosphates (Fukuto and Metcalf, 1956). Studies with
phosphoramides and phosphorothioates are less easily interpreted due to active impurities in the insecticides and the *in vivo* increase in anticholinesterase activity through oxidation reactions (Casida, 1956). Much is known about the mechanisms of inhibition of cholinesterases, both *in vivo* and *in vitro*, and there is little doubt that the inhibition of acetylcholinesterase by organophosphorus compounds is closely associated with their insecticidal action (Winteringham and Lewis, 1959). However, the importance of cholinesterase (ChE) inhibition as the sole lesion in insect poisoning has been challenged.

Roeder (1948) states that normal synaptic function in *P. americana* is dependent on the presence of a certain level of cholinesterase. Yet acetylcholine and related substances had no effect on transmission through the ganglia and added little, if anything, to the action of cholinesterase inhibitor (HETP, DFP and eserine). Hopf in 1952 gave some evidence which threw doubt on the validity of the widely held theory that the action of phosphoric insecticides was to inhibit acetylcholinesterase in the insect nervous system. According to him, the phosphorus insecticides which are thought to inhibit a general esterase are not specifically connected with cholinesters. Injections into the locust of various cholinesters and of prostigmine produced no symptoms. Eserine was considerably less toxic than it is to mammals. Adrenaline and tubocurarine had no effect whatever and the toxic action of tetraethylpyrophosphate (TEPP) could not be influenced by injection of
atropine, tubocurarine or acetylcholine (ACh). A tentative suggestion was made by Hopf (1952) that the action of TEPP (and other substances) was likely to be a general esterase which happened to split acetylcholine but might fulfil a quite different function in the insect nerve tissue.

While these results were being prepared for publication, Lord and Potter (1951) reported that they had found an enzyme in extracts of Tenebrio mollitor which splits o-nitrophenylacetate (NPA) and ethyl butyrate but not acetylcholine, and which could be inhibited by TEPP. Van Asperen (1958) demonstrated the presence of an aliphatic esterase (Ali-E) in the thorax of houseflies, which was distinct from the ChE found in the heads. Although the Ali-E was inhibited in vitro by DDVP at the same concentration as ChE, in vitro studies showed the thoracic Ali-E to be inhibited faster and to a greater degree than ChE. According to Winteringham and Lewis (1959), other effects, such as the stimulated oxygen consumption, liberation of toxins other than acetylcholine (ACh), the temporary or ineffective nature of pyridine-2-aldoxime methiodide and atropine in reversing the action of anticholinesterases, are not readily explainable in terms of acetylcholinesterase inhibition.

The results of Plapp and Bigley (1961), however, showed that inhibition of Ali-E did not appear to be correlated with the toxic action of the insecticides. Ali-E activity was always inhibited before symptoms of poisoning were observed and was usually recovering from the early massive inhibition at death.
Cholinesterase inhibition, on the other hand, was always closely related to the symptomology of poisoning. This enzyme was only slightly inhibited by sublethal dosages of both insecticides, whereas a 50% to 70% inhibition occurred at LD-50 treatment levels. Maximum inhibition was always correlated with the time of knockdown. Another interesting pattern was noted. With both insecticides Ali-E activity was usually inhibited to the same degree regardless of the amount of insecticide. Thus, lethal dosages caused no more inhibition than LD-50 or sublethal dosages. The inhibition of ChE activity was always more closely related to the amount of insecticide used. Sublethal dosages caused little or no inhibition, LD-50's caused partial inhibition and massive amounts caused almost complete inhibition.

Ashurst (1959) suggested that the perineurium controls active ionic regulation between the nervous system and the blood. However, there is no direct evidence to support the idea that the ionic balance is upset when peripheral ChE is inhibited, although the question is intriguing. Treherne and Smith (1965) in their experiments with 14 C-labeled acetylcholine (ACh) showed that peripheral ChE of the cockroach nerve cord was responsible for hydrolyzing excess of ACh. The exact function of the peripheral enzyme as it relates to death of the insect is not known.

In a recent paper (Farnham et al., 1966) it was reported that ChE tends to reappear in the thoracic ganglion of flies that recover from paralysis. Also, they showed that ChE activity was always inhibited in paralysed flies, but no direct connection
was made between reactivation of ChE and death. These results correspond to those of Molloy's data (1961) in which flies that showed severe poisoning symptoms and were examined at long intervals after treatment (24 hr) had more active ChE in the central nervous system than flies that were examined after a shorter interval of time after treatment. Ramade in 1965 suggested that the site of action of a toxicant may be a function of the type of insecticide. In the work of Brady and Sternburg (1966), who studied in vivo ChE inhibition and poisoning symptoms of houseflies, the results indicated that the amount of ChE inhibition at knockdown appeared to be inversely proportional to the reactivity of the OP compounds. Booth and Metcalf (1970) stated that the topical application of organophosphorus (OP) and carbamate insecticides showed that the brain of the fly was a secondary site of action for the toxicants, while the peripheral regions of the thoracic ganglion were the primary areas of ChE inhibition. Inhibition with the OP compounds seemed to penetrate into the neuropile of the ganglia with time. No penetration further than the perineurium occurred with the carbamates. Reactivation was noted in dead flies with both the OP and carbamate compounds. The possible importance of the peripheral ChE of the thoracic ganglion is considered in conjunction with symptomology of poisoning.

The above literature reveals that the studies on the mode of action of these organophosphorus insecticides have been primarily limited to the biochemical methods which have provided quantitative information. Very little is known about the effects
of these organophosphorus toxicants at some localized site which might be responsible for knockdown and/or death of the insects. In addition, there can be anomalous reactions that could occur through the mixing and crushing of the tissue and as such cannot be estimated or might give inaccurate quantitative data (Booth and Metcalf, 1970). Hence histochemical techniques can be used to alleviate these disadvantages as no maceration of insect tissue occurs in the preparations.

Taking into consideration the conflicting ideas about the mode of action of organophosphate insecticides, a more extensive study of the insect nervous system and its relation to insecticides at cellular level is needed. Hence the present project has been undertaken to study the effects of an organophosphate 'malathion' on the central nervous system of the adult cockroach, Periplaneta americana. Morphological and cytological studies have been made on the nervous system of the normal and treated individuals. In addition, alterations in carbohydrates, lipids, nucleic acids, phosphatases, and esterases contents are studied in the treated individuals at cellular level by employing modern cytochemical techniques.