GENERAL INTRODUCTION
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The castor semilooper *Achaea janata* (Lepidoptera, Noctuidae) is a major pest of castor (*Ricinus communis*) and groundnut (*Arachis hypogea*), the two major oil seed crops of India. The annual loss to both oil seed crops by the semilooper larvae is quite substantial (Holihosur, 1985). The adult moths of castor semiloopers are also known to feed on fruits and cause heavy damage (Patterson, 1926; Rakshpal, 1945 and Ayyar, 1922).

Control of the pest by using natural enemies has been suggested. Hymenopteran egg parasite of genus *Telengomus* (Kundu et al. 1967), braconid parasite *Microplitis ophiansae* (Ayyar, 1921), *Bacillus thuringersis* spores (Kulshreshtra et al. 1965), *Bacillus cercus* (Holihosur and Tontadarya, 1975) and *Serratia marcescens* (Govindrajan, 1977) have been reported to be useful against castor semilooper larvae. However, it was not clear from these reports, whether these natural enemies could be used successfully under the field conditions. Insects are man's most fierce competitors for control of this planet (e.g., crop damage, vectors of diseases etc.). It is imperative that investigators probe into insect physiological mechanisms in search of vulnerable points so that control can be approached from a rational point of view.

Man's desire to control his environment has created many useful chemicals. The first synthetic-organic insecticides that appeared for public use were dinitro compounds and *Dieldrin* (cited by Matsumura, 1975 and O'Brien, 1967). Perhaps the most significant discovery leading to proliferation of new synthetic insecticides was that of DDT. The use of
DDT revolutionized the control of insect pests. Other chlorinated hydrocarbon insecticides such as BHC, toxaphene, chlordane, aldrin and dieldrin followed immediately thereafter. The second massive introduction of new insecticides was initiated by a German worker, Gerhard Schrader, a pioneer in the Chemistry and use of organophosphate (OP) insecticides. The number of OP compounds used for insect control today is unmatched by any other group of insecticides. The most widely used OP compounds include parathion, systox, malathion, diazinon and dichlorvos. Of recently, fenitrothion (Sumithion) has been shown to be the most promising OP insecticide against agricultural and forest pests (for review see NRC bulletin, 1975 and FAO/WHO bulletin 1970).

Fenitrothion [O,O-dimethyl-O-(3-methyl-4-nitrophenyl) phosphorothioate] is a broad spectrum insecticide used exclusively throughout the world for the control of agricultural and forest pests. It has been used extensively since 1969 as a replacement for DDT in the protection of forest against defoliators (NRC Bulletin, 1975; Tanton, Khan, 1978a, 1978b, 1978c).

These findings and other properties listed below prompted us to choose fenitrothion for investigating its effect on the various physiological processes which enhance the poisoning action of the insecticide. The castor semilooper larvae which are the major defoliators of castor and groundnut crops were used as the test organisms.

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\begin{array}{c}
\text{CH}_3 \\
\text{CHO}_3 \\
\text{CHO}_3 \\
\text{S} \\
\text{P} \\
\text{O} \\
\text{NO}_2 \\
\end{array}
\]

Fenitrothion
Fenitrothion was first reported in 1956 by Drabek and Pelikan (cited by NRC Bulletin, 1975). They noted that this compound was remarkably less toxic to mammals than other OP compounds such as methyl parathion and parathion which also inhibit cholinesterase. Fenitrothion was introduced as an experimental OP insecticide in 1959 by Sumitomo Chemical Co.

**Selective Toxicity:**

One of the most promising properties of the fenitrothion in contrast to other OP compounds is its greater toxicity to insects than to homeotherms (Birds and Mammals). It has been proposed that this selectivity is due to the ability of homeotherms to more readily detoxify fenitrothion through the oxidation of the methyl group of the aromatic ring (Douch et al. 1968). This hypothesis was based on the isolation of 5-hydroxy-2-nitrobenzoic acid from *in vitro* studies with rat liver fortified with NADP. On the other hand Miyamoto (1969) was of the opinion that the fenitrooxon (the metabolic product of fenitrothion, see Fig.2) compared to other phosphate compounds has a relatively poorer penetration into the brain thus producing lesser amount of acid precipitated phosphorus on which inhibition of cholinesterase activity depends. Hollingworth et al. (1967) has given a more plausible explanation. He suggests that many factors contribute to the difference in toxicity. These include differential cholinesterase inhibition, penetration, activation by conversion of P=S to P=O translocation and detoxification.
Metabolism of Fenitrothion in Insects:

Fenitrothion degradation by homeotherms appears to follow several routes (NRC Bulletin, 1975). The OP compounds which are commonly used as insecticides are phosphorothioates such as fenitrothion. This compound is converted to its oxygen analogue fenitrooxon in order to inhibit cholinesterase.

Depending on their site of action, insecticides might use a variety of routes to reach their targets. Insecticide toxicity is known to be affected by a variety of factors like penetration rate, temperature, age, sex and nutrition (Brooks, 1976; De Vries and Georgiou, 1979; Sun, 1960; Champ and Dyte, 1977 and Scott and Georgiou, 1984). The most common mode of insecticide entry into insect body is through cuticle and ingestion followed by absorption through the walls of the alimentary tract. The mechanism of penetration of insecticides through insect cuticle has remained still open (for review see Brooks, 1976).
Many OP insecticides show positive correlation between temperature and toxicity in that toxicity increases as temperature increases. Conversely, pyrethroids and DDT have shown negative temperature correlation (Harris et al. 1978; Gammon and Holden, 1980). There are also reports to indicate that all pyrethroids do not act alike in the given temperature (Scott and Matsumura, 1983). Studies on the effect of temperature on the toxicity of insecticides is of particular importance in tropical countries like India, where the temperature fluctuation varies from 18°C to 40°C. Not many OP compounds have been investigated on their effect at different temperatures. The precise mechanism of influence of temperature on the toxicity has not been illustrated. However, it has been argued that temperature affects the rate of penetration of insecticide. Hence the influence of temperature on the toxicity of OP compound appears to be the most fascinating field of investigation.

There are a number of techniques which have been utilized to aid toxicological evaluation of insecticide action. The most commonly used techniques manometric or respirometric assay. Insects are highly aerobic. Because of their small size the entire insect can be placed in a Warburg flask and oxygen consumption can be determined. These results could be used to identify whether the insecticide under study would inhibit respiratory mechanisms. The insect central nervous system (CNS) is supplied with tracheae which facilitate a direct oxygen supply to nervous tissue. It is quite possible that the insecticides which inhibit respiratory mechanism may disrupt the normal functioning of CNS. It appears from these observations that study of
respiration following the insecticide treatment would give a valuable first approximate indication on the mode of action of insecticide.

In order to reach the site of action and exert its influence, the processes of absorption, distribution, biotransformation and excretion of insecticides are involved. The mechanism of penetration and absorption of insecticides through insect cuticle has still remained controversial and has been discussed in details by Brooks (1976) and Gerolt (1969, 1970 and 1972). Since the present experiments are designed to test the influence of insecticide on the overall physiology of the organism, we have not attempted to elaborate on this point.

Insect CNS compared to mammals appear to be more vulnerable to topical application of insecticides since the insect has large ratio of surface area to volume. Using labelled oils, Lewis (1962) showed that within 15 min of tarsal contact, adults of *Phormia terraenovae* acquired a monolayer of material over their entire body surface. Once the insecticide enters the insect hemolymph, it may be carried to all parts of the body in solution or bound to proteins or dissolved in lipid particles, depending on its physical properties. Due to the open circulatory system in insects, the circulating hemolymph bathes all the organs and it may be anticipated that the insecticide will be taken up by alimentary tract, CNS and other tissues. During distribution both nonenzymatic chemical conversions and enzymatic biotransformations, leading to more toxic molecules (bioactivation) or less toxic ones (detoxication) are likely to take place. Both the original
insecticide and its conversion products may be excreted as such following further conversion into water soluble conjugates. So enhanced excretion provides a means of protection for the organism against the toxicant. The further absorption of insecticide or its metabolic products by various tissue systems depend on their ability to cross the semipermeable membrane.

It is evident from these observations that membrane permeability or enzymatic conversions may profoundly alter the pharmacological behaviour of an insecticide (O'Brien, 1967 and Brooks, 1974 may be consulted for further details on this topic).

The insect nervous system continues to be the major target for insecticides and it is generally accepted that many of these compounds act either directly on the excitable membrane (Narahashi, 1976) or on enzymes involved in synaptic transmission (Matsumura, 1975). Basic research on the neurobiology of insect is conducted on a very limited number of convenient species and with leads from developments in the vertebrate area. At present new toxicants are discovered mainly by systematic screening and optimization of structure activity relationship for chemicals so discovered. Ideally, physiological and biochemical research, by such chemicals and their analogues as probes, should lead to a better understanding of both the biological target and action of the chemical. The insect nervous system consists basically of a dorsal anterior brain and a ventral nerve cord with segmental ganglia, from which nerves run to sense organs and muscle system and stomatogastric system which innervates the alimentary canal.
A number of studies have examined the action of insecticides on a range of insect nerve preparations and varying degrees of sensitivity have been reported (Narahashi, 1976). The precise mode of action is unclear although it would appear that there is a change in the ionic permeability and thereby a change in associated electrical events following insecticide application. Debate has centered around the possibility that some neurons may be more susceptible than others, and in particular attention has focussed upon the central versus peripheral nervous system (Clements and May, 1972).

The Synapse - A Target Site For Insecticide:
Our knowledge of the structure and function of insect synaptic contacts has unfortunately lagged behind than in other animal groups, notably the vertebrates, crustaceans and molluscs. Insects do not differ fundamentally from these other animal groups in their basic neurophysiology and neurochemistry. Yet by virtue of their highly complex behaviour patterns coupled with their ability to fly, insects have placed great demands upon their nervous system which compared with vertebrates contains much smaller number of sensory, motor and interneurons. Thus complex activities tend to be coordinated by a few nerve cells, so much so that some activities may be controlled by the only one or two interneurons. This reliance by insect upon a few key neurones is a phenomenon which makes them particularly vulnerable to insecticides that disrupt synapses since synaptic activity lies at the heart of nerve cell interactions and coordination.
A synapse is the region where neural communication takes place. Furthermore, it is the site where the electrical and chemical activity of neurones is inexorably interlinked and is clearly open to interruption in a variety of ways. Some insecticides such as DDT and pyrethroids, directly perturb the intermission of action potentials. Both these insecticides induced multiple spiking and pyrethroids, in addition also block axonal transmission. Both OP and carbamates seem to interfere with neurochemistry of synapses.

The best understood action of insecticides is that of acetylcholinesterase (AChE) inhibition by carbamates and OPs. Inhibition increases the level of acetylcholine in the synaptic cleft, thus potentiating synaptic conduction which leads to repetitive or multiple spiking in the post-synaptic element. Multiple discharges at cholinergic synapses also result from poisoning with dieldrin and Y-HCH presumably by augmenting extracellular acetylcholine levels.

Another aspect of the neurophysiology of the insects nervous system was the ionic movement across the nerve membrane. It is known that the electric potential difference across the membrane is generated on account of differential distribution of sodium (Na+) and potassium (K+) ions (Hodgkin, 1951; Hodgkin and Huxley, 1952). Under normal conditions the concentration of K+ ions of the nerve cells far exceeds that outside. At the same time, the internal concentration of Na+ is much lower than that outside. The mechanism by which the nerve membrane performs the process of polarization and depolarization (action potential) depends upon the movement of these ions. It is generally accepted that the movement of Na+, K+ across the membrane is controlled by the membrane bound adenosine triphosphatases.
(ATPases). The "sodium pump" which requires for its operation Na\(^+\) and Mg\(^{2+}\) within and K\(^+\) outside is known to be handled by a specialized enzyme, Na\(^+\) K\(^+\) ATPase. The Na\(^+\) K\(^+\) ATPase is inhibited by cardiac glycoside ouabain.

The insect nervous system unlike vertebrates has no distinct protective covering (the myelin sheath and the insect has open circulatory system. These two factors made the insect CNS as the most susceptible and vulnerable tissue to the toxic action of chemicals. Several organochlorine insecticides have been shown to inhibit membrane bound ATPases (Koch et al. 1969; Matsumura and Patil, 1969; Desai et al. 1974; Cukomp, 1980; Mourelle et al. 1985). Surprisingly, the effect of OP compounds on the ATPase has not been investigated in a single insect. It has been shown that the OP compounds are highly lipophilic and the rate of penetration through the biological membrane is faster than organochlorine and carbamate insecticides (Shah and Guthrie, 1970, 1971 and Shah et al. 1972). It could be anticipated then that OP compounds may interfere with other membrane functions especially in ionic movements. This is one area where OP compounds offer a challenging field of investigation.

During the past two decades considerable attention has centred around the biochemical conjugations of certain naturally occurring body constituents such as sterols, steroid hormones, glycoproteins and bile acids. Since the conjugation products are usually water soluble, readily extractable substances and their formation generally results in decrease in toxicity, physiological significance of conjugation mechanism was initially considered to be for the sole purpose of detoxication. However, this hypothesis has been
challenged from time to time by various investigators (Fishman, 1970; Hutson et al., 1972; Mandel, 1971). Among several conjugates, glutathion conjugation appears to be of great significance in OP toxicity. The biochemistry of glutathion conjugation has been reviewed by a number of workers (Boyland and Chassid, 1969; Wood, 1970; Motoyama and Dauterman, 1980). Glutathion dependant transferase reactions are characterized by a direct enzymatic attack on the substrate and their importance in the metabolism of a large number of OP insecticides has been demonstrated in a few insect species. The importance of glutathion S-transferase in OP resistance has been recognized only during the last few years. Although the enzyme has been known to be involved in insecticide metabolism for some time (Fukami and Shishide, 1963, 1966), its importance as a possible resistance mechanism seems to have been neglected due to the fact that the products of its action on OP compounds are identical to those formed by other enzymes. It is evident from these observations that the detailed investigation especially with reference to the induction of glutathion S-transferase by the OP insecticides at different dose levels is warranted.

**Release of Neurohormones With Respect to Insecticidal Action:**

It is well recognized now that the insect nervous system is the major if not the sole target site for insecticides (Narahashi, 1976). Neurosecretory cells may therefore also be affected and indeed some insecticides can induce release and Reynolds, of neurohormones (Granett and Leeling, 1972; Maddrel[l, 1972; Casida and Maddrel[; 1971; Samaranayaka, 1977 and 1974 and Norman, 1980). Electron microscopy of secretory hyperactivity in neurosecretory cells as a result of poisoning with lindane has been described (cited by Norman, 1980).
The neurosecretory cells of *Carausius* and *Rhodnius* are highly sensitive to low concentration of pyrethroids. The giant axons of *Periplaneta americana* are sensitive to allethrin and pyrethrin. Insect neuroendocrine organs are exposed to the hemolymph and as such are readily accessible to insecticide molecules that enter the hemolymph.

Orchard and Osborn (1979) have shown that insect neurosecretory cells possess all the electrical properties of other neurons and their electrical activities are modified by low concentrations of insecticides (Orchard, 1980). It is possible from these observations that regardless of their effect on the CNS insecticides may act directly on neurosecretory system and provoke hormone release. In *Rhodnius* insecticide induced paralysis was found to induce the release of diuretic hormone and cuticular plasticization factor (Cagsida and Maddrell, 1971, Maddrell and Reynolds, 1972). Abnormal release of hyperglycaemic and adipokinetic hormones were found to have occurred in locusts at paralytic stage of poisoning (Samaranayaka, 1974 and 1978). In *Periplaneta americana*, Orr and Downer, (1988), have shown that both carbohydrates and lipid levels of the fat body and hemolymph showed significant changes following lindane treatment at prostration stage of poisoning. It may be argued from these studies that the initial action on the primary target site results in secondary and tertiary effects and finally death of the organism. Hyperactivity of CNS is believed to be the result of the action of the insecticide on the primary site. Electrical activity of neurosecretory cells results in the release of neurohormones. Neurohormones play a role in an enormous variety of metabolic, physiological and behavioural events.
The liberation of a plethora of these controlling factors, many at untimely stages of insects life cycle would lead to startling imbalance of a variety of body functions. As a result of the hyperactivity of neurosecretory cells there would be a multiple of secondary and tertiary effects and these may well play a large part in the eventual poisoning of the insect.

Surprisingly, the majority of these experiments are confined to a few insecticides like pyrethroids, DDT, lindane and endosulfan. OP compounds have not been investigated in this respect. Indeed the excellent properties of fenitrothion, like selective toxicity, high penetration rate would offer an interesting chemical to test its effect on the other organ systems like digestive system, neuroendocrine system and finally on the fat body metabolism.

There is no doubt that in recent times OP insecticides are gaining more importance among all the types of insecticides due to their certain novel qualities. Fenitrothion appears to be the most recommended one among OP insecticides. It is evident from the literature that OP compounds have been studied more thoroughly from one angle i.e. their action on neurochemistry especially with reference to inhibition of cholinesterase. However, their secondary and tertiary effect(s) have not been identified. I thought therefore that it will be rewarding to investigate the various physiological disturbances caused by fenitrothion treatment to the lepidopteran insect, the *Achaea janata* a serious pest of two oil seed crops of India. In this investigation, I have
chosen only the 5th instar larvae, since 1) this stage causes heavy damage to the crops and 2) the size of the larva is quite big and it is possible to collect adequate quantity of tissues for biochemical investigation. The various facets of the present investigation are presented in the form of chapters and are detailed under 'contents'.

The results obtained in the present investigation are based on the facilities available in the Department. Hence the results obtained are more suggestive than definitive. Further studies involving more advanced techniques are needed to confirm the results. However, it is pointed out that this forms a good basis for further studies.