Introduction
"The struggle between man and insects began long before the dawn of civilization; has continued without cessation to the present time, and will continue, no doubt as long as human race endures. It is due to the fact that man and certain insect species consequently want same things at the same time. Man commonly thinks of himself as the conqueror of the Nature, but the effect that insects had thoroughly mastered the world and taken full possession of it long before man began to attempt".

- Forbes.

Today things are contrary to what Forbes said. Man's endeavour to counteract the ravages of these pests to himself and his lively assets, bore success when Muller (1939) accidentally discovered DDT. Since then rapid progress has been made in the chemical control of pests and within a short time he achieved a miraculous success. These chemicals in the broad sense of the term extend in to almost every phase of life.

Agriculture including commercial and household production of vegetables has long been dependent on the chemical control of pests from the time of seed planting, through growing crop to the warehouse and the kitchen shelf. Besides agriculture, these pesticides have also been proved successful in combating vector-borne diseases thereby saving not only thousands of lives but also billions of man hours. No doubt immediate success has been achieved through these pesticides. However, indiscriminate use of these chemicals posed a great threat to man and his environment by eliminating beneficial insects, causing ecological imbalance, becoming phytotoxic by destroying useful plants and entering in to the food chain causing toxicity to both target and non-target animals including human
beings. Further aggravating the problem, large amounts of pesticidal agents were disseminated by natural forces like wind, rain, flow of rivers and ocean currents to far away places on the globe from tropical forests to antarctic snows. Consequently their residues began to appear in food stuffs (Egan and Hubbard, 1975; Mukherjee et al., 1980; Passino, 1981), wild life (Kaphalia et al., 1981), human fat (Mukherjee et al., 1980; Rosival et al., 1980), human milk (Kalra and Chawla, 1981, Dillon et al., 1981) and human placenta and accompanying fluid (Saxena et al., 1980). This was manifested in high mortality and reduced reproductive potential in organisms such as birds and fish and disruption of ecosystems (Braun and Frank, 1980; Hall et al., 1980; Yockim et al., 1980; Lieberman and Alexander, 1981) and development of pesticide resistance in target and non-target species (Hama, Hiroshi, 1980; Motoyama et al., 1980; Conway and Comins, 1981). Because of unawareness and lack of appropriate training as to how to use pesticides, both the workers in the manufacturing plants and farmers in the fields get exposed to these harmful chemicals and suffer from a variety of health problems.

Pesticides are variously classified by different authors. Depending on the target pests on which they act, they are classified as insecticides, herbicides, rodenticides, molluscicides, fungicides, weedicides etc. Based on chemical nature, they are classified into 3 general groups: (a) Inorganic compounds including arsenicals, mercurialas, borates and fluorides, (b) Natural organic compounds like nicotine, pyrethrum, rotenone and derris, (c) Synthetic organic compounds like organochlorides, organophosphates and carbamates.

The first group of synthetic organic pesticides used on large scale were organochlorides. It was well established that the benefits, these
compounds conferred on mankind during early years of use in the fields of agriculture and public health management could not be ignored. However, during 1950's and 1960's reports of large residues of these long persistent pesticides were made. In addition, many pest species have shown resistance of varying degrees to these compounds. Hence, both developed and developing countries are in the process of restricting their usage, where suitable alternatives are available. So their period of utility is ending.

The problems confounded by man in the usage of persistent organochloride compounds were mitigated with the advent of the most versatile groups of insecticides namely organophosphates and carbamates. They proved to be valuable substitutes for organochlorine insecticides being less persistent either in the environment or within the organism. They are readily degradable into non-toxic compounds. Hence, these are the largest and most versatile group of pesticides in use at the present time.

Besides these three groups of insecticides (organochlorides, organophosphates and carbamates), we have another group of insecticides which are relatively recent in origin, namely pyrethroids. These compounds are fast gaining ground as promising insecticides due to their specificity of action and low mammalian toxicity. They are also highly degradable.

Despite the great concern over environmental pollution by the pesticides (Gupta and Gupta, 1980; Attri, 1981), we still continue to depend on these chemicals for agricultural productivity and disease control (Dennis et al., 1974; Viswesvariah, et al., 1975). In this modern era, perhaps we cannot dispense with pesticides completely since there is hardly no
alternative available at hand. Scientists and Researchers are very much interested in manufacturing and formulation of safe pesticides, to achieve the desired effects without affecting the non-target organisms. So we have to depend more on biodegradable pesticides and lessen our sole dependence on persistent pesticides.

Today, integrated pest management (IPM) has great relevance. Its aim is to achieve pest control by combining different approaches. These include biological, chemical, physical and economic methods (Metcalf, 1980; Flint and Van den Bosch, 1981). IPM uses a variety of techniques such as natural predators, pathogens, parasites, resistant hosts, weather and other environmental factors and appropriate chemical pesticides at correct timings.

After taking into consideration all the factors necessary for a chemical to be used as an ideal pesticide, pyrethroids seems to have a promising future. Synthetic pyrethroids are more powerful insecticides than the classical insecticides, viz., organochlorides, organophosphates and carbamates. In India, synthetic pyrethroids are the most explored group of insecticides and have replaced many of the conventional insecticides.

History and Chemistry of Pyrethroids:

Many plants contain toxic compounds and some are selectively poisonous to insects and have proved to be valuable as insecticides. Pyrethrum compounds have long been known as powerful insecticides and are effective against a wide variety of arthropods. Pyrethrum is obtained from the flowers of plants belonging to the family Asteraceae of the genus
Chrysanthemum. The most important species that possess high toxicity to insects are Chrysanthemum cinerariaefolium, C. coccineum and C. carneum.

Pyrethrum powder was first used around 1800, and by 1851, their use was world wide. Formerly, pyrethrum was utilized chiefly in the form of fine ground flowers. This material was called "insect powder", a term officially recognized as applying solely to powdered pyrethrum. Today, pyrethrum products include both dusts and sprays intended for use against a variety of household, veterinary and store product pests. Pyrethrum compounds are also known for their quick knockdown action or induction of temporary paralysis, and the amount required to produce mortality is relatively much greater than that for paralysis.

The pyrethrum compounds consist of four esters, which are the combinations of two different alcohols with two different acids (Staudinger and Ruzicka, 1924). The pyrethrum contains six insecticidally active components of which pyrethrin I and pyrethrin II are most active (Head, 1973; Elliott and Janes, 1978).

The pyrethrins are relatively unstable due to their low photostability and high degradability, so they leave practically no residues in the biosphere. In addition, they are relatively non-toxic to mammals but highly toxic to insects. Part of their relatively non-toxicity to mammals appears to result from a rapid metabolism (Soderlund and Casida, 1977 a, b). These favourable properties together with high cost of extraction from plants have stimulated the search for synthesis of more stable pyrethroids based on the structure of
the natural pyrethrins. Many changes were brought about in the basic structure of pyrethrins to prolong their effective life.

Modifications brought about in both alcohol and acid side chains made them stable enough to be used for agricultural purpose (Williams and Brown, 1979). The first synthetic pyrethroid to be developed was allethrin (Schechter et al., 1949) and cyclethrin in 1950 (O'Brien, 1960). Allethrin was prepared by esterification of synthetic chrysanthemic acid with allethrolone. It has a strong insecticidal activity, but was photolabile. Afterwards, there were a series of changes, each contributing to make pyrethroids more potent and stable (Elliott, 1977). Pyrethrin I (Fig. 1a) contains all the basic characters for good insecticidal activity, later, substitution in the acid moiety and incorporation of 3-phenoxy alcohol (decamethrin) (Fig 1b) have improved the stability of the molecule against breakdown and metabolism.

Significant development towards stabilization was achieved with the discovery of phenothrin type alcohol substitution, where m-phenoxybenzyl alcohol moiety has few vulnerable sites for chemical and metabolic attacks.

The next breakthrough towards improving insecticidal activity was achieved by replacing the vinylmethyl moiety by halogens (permethrin) (Fig 1c) and also by addition of cyano group (cypermethrin) at the α-position of the alcohol moiety. Later workers have also discovered the possibility to substitute the cyclopropane ring of the acid side chain, (Fenvalerate) (Fig 1d). Though this particular modification did not appreciably increase the insecticidal activity, but made the synthetic process easier and economical.
FIG. 1

A) PYRETHRIN-1

B) DECAMETHRIN

C) PERMETHRIN

D) FENVALERATE
All these have been achieved without sacrificing mammalian safety and impact on wildlife and environment.

It is clear that the insecticidal activity of the pyrethroids depends on the structural configuration. The activity of a particular optically pure isomer is often far greater than that of its enantiomer. The cis isomers are generally more toxic than their corresponding trans isomers. The presence or absence of an α-cyano group also affects the activity of the pyrethroids. Pyrethroid insecticides without an α-cyano group act both on the central and peripheral nervous system and those with an α-cyano group act only on the central nervous system (Clements and May, 1977). The activity of the pyrethroid compounds also depends on the integrity of the molecule, since derivatives of either the alcoholic or acidic components are inactive (Elliott, 1977).

Synthetic pyrethroids are particularly useful against tissue borers, i.e., mostly Lepidopterous larvae damaging cotton, brinjal, bhindi, tomato etc. It is said that 45% of world's annual insecticide is used for the control of tissue borers alone. These compounds, like chlorinated hydrocarbons are lipophilic, but unlike chlorohydrocarbons these are not persistent as they are rapidly degradable.

Symptomology:

The categorization of poisoning symptoms is the first step in the analysis of the site and mode of action of insecticides. Explanation of poisoning symptomology in neurophysiological terms is difficult due to the complexity of the nervous system and the widespread actions of pyrethroids
on sensory, motor and central nervous systems. Furthermore, many of the overt poisoning symptoms are also probably side effects, not directly involved in toxicity. For example at higher temperatures, hyperexcitability increases, whereas mortality reduces (Adams and Miller, 1979).

The symptoms of poisoning of pyrethrum and many of the synthetic pyrethroids in insects include a sequence of initial hyperactivity, followed by incoordination and knockdown leading to paralysis and finally death (Clements and May, 1977; Leake, 1977; Gammon, 1978). Gammon et al., (1981) in cockroaches also noted differences in in vitro poisoning symptoms between the two classes of pyrethroids. Type-I compounds cause restlessness, incoordination and hyperactivity followed by prostration and paralysis, while type-II compounds cause a characteristic pronounced convulsive phase, that is within minutes of dosage, cockroaches become ataxic and incoordinated. There were also periods of convulsions, hyperactivity and sporadic, sustained contractions involving extension of the metathoracic legs.

Leake (1977) reported an interesting correlation between symptoms and aberrant electrical activity in central neurons of leech, Hirudo medicinalis, during S-bioallethrin poisoning. General incoordination was correlated in time with repeated depolarization in P cells and Retzius cells. Other studies have shown that the flight neurons of the housefly, Musca domestica remain highly active long after prostration and paralysis have set in (Adams and Miller, 1980).

In rats, type-I pyrethroids produce symptoms characterized by aggressive sparring behaviour, elevated startle response, whole body tremors
and prostration, whereas type-II pyrethroids induce burrowing behaviour, coarse tremors, clonic seizures, sinuous writhing and profuse salivation without lacrimation (Verschoyle and Aldridge, 1980). There is increasing evidence that type-II action involves primarily an action on the central nervous system of mammals, whereas type-I syndrome may have both central and peripheral systems (Barnes and Verschoyle, 1974).

Selectivity of Pyrethroids:

Pyrethroid compounds are the most selective and potent neurotoxicants and possess little or no action on mammalian nervous system and undergo rapid metabolism in mammals. Elliott (1977) analysed the selectivity ratios of rat oral LD$_{50}$/insect topical LD$_{50}$ (mg/kg). According to him, pyrethroids have greater selectivity ratio with-4500, followed by chlorinated hydrocarbons - 91, organophosphates-33 and carbamates-16.

The cis-isomers are generally more toxic than the corresponding trans-isomers. The trans-isomers of resmethrin, phenothrin and permethrin have very low mammalian toxicity despite their similar insecticidal potential to their cis-isomers. Cockroach cercal sensory nerves are highly sensitive to repetitive firing caused by compounds without an O-cyano group (Type-I action) but not with an O-cyano group (Type-II action).

At times, even small structural changes have a great influence on the relative potency and species selectivity of pyrethroids. For example, Lacewing larvae are highly tolerant to pyrethroids, especially deltamethrin, which for other insects is usually the most potent toxicant (Ishaaya and Casida, 1981). Larvae of the noctuid, *Plutella xylostella*, resistant to deltamethrin
and many closely related compounds are highly sensitive to the trans-isomer of 4D, a compound that is usually a relatively poor insecticide (Martel, 1980). Also, mites that survive against insecticidal doses of most pyrethroids are very sensitive to fenpropathrin and to analogs of fenvalerate and fluvalinate with a 4-t-butyl group in place of the 4-chloro substituent (Matsuo et al., 1980; Henrick et al., 1980).

Mode and Action of Pyrethroids:

The primary target of the pyrethrin group of insecticides is considered to be the nervous system, as judged by their quick action and neuromuscular disorders. The symptoms of pyrethrin poisoning follow the typical pattern of nerve poisoning i.e., excitation, convulsions, paralysis and death. Regular, rhythmic and spontaneous nerve discharges have also been observed in insect and crustacean nerve-muscle preparations poisoned with pyrethrins. The primary target of pyrethrins seems to be the ganglion of the insect nervous system (Roy et al., 1943) although some pyrethrin poisoning effects can also be observed in isolated legs. Electrophysiological studies revealed that pyrethrins cause repetitive discharges and conduction block (Yamasaki and Ishii, 1952).

Staatz et al., (1982) studied the effects of permethrin and deltamethrin on the CNS of mice. They found that pyrethroid-induced signs of toxicity appeared more quickly when toxins were given directly to the CNS through intracerebroventricular injection than when they were administered through peripheral route. They therefore, concluded that in mice the main site of action of pyrethroids is the CNS. This was also proved in frog by effectively antagonizing the signs of toxicity of deltamethrin with
diazepam, the action of which is known to be largely central (Cole and casida, 1983).

Pyrethroids exert widespread potent actions on both peripheral and central nervous elements in arthropods. These actions have been studied at various levels, from extracellular recordings of the arthropod ventral nerve cord activity (Gammon, 1979) to single cell intracellular recordings (Leake, 1977) and ultimately to membrane ionic channels (Narahashi and Lund, 1980). Preparations containing sensory elements (Clements and May, 1977; Miller and Adams, 1977; Osborne and Hart, 1979), motor units (Adams and Miller, 1980; Salgado et al., 1983) and neurosecretory cells (Orchard and Osborne, 1979; Orchard, 1980) have been shown to be sensitive to nanomolar concentrations of pyrethroids.

Gammon et al., (1981) after extensive survey on various pyrethroid analogs and their effects on cercal sensory nerves of the American cockroach concluded that there are two types of pyrethroid actions. Type-I compounds, devoid of \( \text{OC-cyano} \) group induce repetitive firing in a cercal sensory nerve following a single electrical stimulus. This can be observed in the sensory neurons, interneurons and motor neurons (Takeno et al., 1977; Van den Bercken and Vijverberg, 1980; Vijverberg et al., 1982; Lund and Narahashi, 1983). While type-II action characteristic to compounds with \( \text{OC-cyano} \) group, supresses the appearance of repetitive discharges. This phenomenon was also demonstrated in the abdominal nerve cord of American cockroach, where cypermethrin at \( 10^{-7} \)M concentration exhibited the ability to inhibit or mask the ability of S-bioallethrin to induce its characteristic repetitive discharges.
Moreover, Type-I pyrethroids act largely through a mechanism identical to that of DDT. The most prominent reasons cited for this conclusion was the cross-resistance exhibited by Kdr-type DDT-resistant insects to type-I pyrethroids (Scott and Matsumura, 1983), negative temperature correlated lethal actions (Scott and Matsumura, 1981), sensitivity of insect sensory neurons (Gammon, 1980), appearance of repetitive discharges (Narahashi et al., 1977) and their effect on the operation of the sodium gate (Lund and Narahashi, 1982).

It has also been well established that the main and primary target site of pyrethroids is the sodium channels of nerve membranes. Individual sodium channels are modified by pyrethroid molecules to give rise to prolonged openings during depolarizing stimulation without changing the conductance in their open state. This leads to a marked prolongation of sodium current, which in turn causes the depolarizing after potential. When the depolarizing after potential reaches the threshold for excitation, after discharges are triggered. Repetitive activity thus initiated causes neuromuscular disorders like hyperexcitation, ataxia, paralysis etc. By comparison of isomers of tetramethrin, it was concluded that there are three sites to which the pyrethroid can bind. They are trans, cis agonistic site and a negative allosteric site (Narahashi, 1984).

Histopathology:

Biochemical changes were closely associated with the pathological changes of animal tissues (Dikshith et al., 1975). Histological changes in fish, rainbow trout exposed to permethrin were reported (Kumaraguru et al., 1982). They observed alterations in gill epithelium, like clubbing of epithelial cells
and fusion of adjacent secondary lamellae, while the other tissues like intestine, kidney and liver are least affected. This speaks of the tissue specificity of permethrin.

However, histological changes are also observed in gill, intestine, liver and kidney of fish exposed to fenvalerate (Radaiah, 1988). Necrosis of renal cells, denaturation of the liver cells (El-Zalbani and Soliman, 1981; Feng et al., 1982), hypertrophy of hepatoparenchyme cells (Kadota et al., 1976) were also reported in rat and mice administered with pyrethroid insecticides.

Biochemical changes:

Earlier biochemical studies on the pyrethrins dealt with disruption of several enzymes and pathways of intermediary metabolism (Casida, 1973). Pyrethrins and synthetic pyrethroids are reported to inhibit various ATPases including neural Ca$^{2+}$ ATPase and Ca-Mg$^{2+}$-ATPase (Clark, 1981; Clark and Matsumura, 1982). In general type-I pyrethroids are relatively better inhibitors of Ca$^{2+}$-ATPase than type-II Pyrethroids, while Ca-Mg$^{2+}$-ATPase is more sensitive to type-II than type-I compounds.

Ahmed et al., (1987) reported inhibition of AChE in different areas of rat brain on different days after injection of decamethrin intraperitoneally. They observed maximum inhibition of AChE on the 3rd day in cerebellum, on the 4th day in the midbrain, on the 5th day in cerebrum and on the 8th day in all the spinal cord areas as well as in pons and medulla. There are also reports of localized changes in brain glucose utilization in rats administered with deltamethrin (Cremer et al., 1980).
Nagy et al., (1983) reported an increase in total lipids in rats administered with pyrethroid compounds. Remarkable changes in fish muscle enzymes like pyruvate kinase, dehydrogenases (LDH, SDH and MDH) were also observed under permethrin exposure (Anastasi and Bannister, 1980). Since blood takes part either directly or indirectly in all the biochemical process of the body, it is but natural to expect alterations in it, when exposed to pesticides. Therefore, some marked biochemical changes in the blood of rats such as hyperglycemia (Lock and Berry, 1981; Cremer and Seville, 1982), elevation in blood glutamate and pyruvate level (Ahmed et al., 1984) and increase in lactate content (Cremer and Seville, 1982) were reported upon exposure to pyrethroid compounds.

### Influence of Temperature:

The effective dose of a pesticide is usually temperature dependent, whether one considers behavioural effects or toxicity. The site and mode of action of an insecticide could vary with temperature. For example in cockroach, temperature plays a key role in defining the site of action of allethrin (Gammon, 1978, 1979). He observed repetitive firing in the CNS and also in sensory and motor axons at 32°C following LD95 dose of allethrin. However, at 15°C where allethrin is almost ten times more toxic, repetitive firing is observed in sensory and motor axons, instead of rapid nerve blockage as expected from earlier in vitro studies (Narahashi and Anderson, 1967; Wang et al., 1972).

Gammon (1978, 1979) also reported that the repetitive firing in the CNS is positively temperature correlated, while in sensory and motor neurons it is negatively correlated. The influence of temperature on the toxicity of
four pyrethroids, viz., cypermethrin, fenvalerate, d-phenothrin and bioallethrin to 3rd instar larvae of *Aedes aegypti* showed that the toxicities of all pyrethroids are in the range of 1.35 to 3.63 fold greater at 20°C than at 30°C (Cutkomp and Subramanyam, 1986).

However, the effects of temperature on allethrin poisoning are complex. The ability of allethrin to initiate repetitive after discharges increases as the temperature is increased. However, nerve-blocking action, which is most likely responsible for the characteristic paralysis produced by pyrethroids is enhanced at low temperatures in both cockroach (nervecord) and squid (axon) nerves.

**Synergism:**

Usually the action of pyrethroids, paralytic or lethal can be effectively augmented by various synergists like piperonyl butoxide, sulfoxide, propylisone, tropital etc. Synergists, not only help in minimising the release of toxicant into the atmosphere but also helps in achieving better results at lower doses.

Piperonyl butoxide, the most important pyrethroid synergist and a classical Mixed Function Oxidase (MFO) inhibitor (Casida, 1970; Hodgson and Philpot, 1974; Testa and Jenner, 1981) increases the toxicity of all pyrethroids to houseflies and some other insects. At high doses it synergizes pyrethrín-I by 300-fold and deltamethrin by 10 fold to houseflies. It has also been reported to synergize the ip toxicity of Cis-resmethrin and deltamethrin by 3 to 25 fold to mice (Soderlund and Casida, 1977 b).
There are also reports on enhanced knockdown action of most pyrethroids in resistant diamond black moth larvae by synergists like piperonyl butoxide, 5,5,5-tributyl phosphorotrithioate and chlordimeform (Chen et al., 1985). Even organophosphate compounds like monocrotophos and Acephate are reported to synergize the toxicity of cypermethrin to white fly under glasshouse conditions by 5 to 50 fold (Ishaaya et al., 1987). Another O.P. insecticide, ethion also synergizes the toxicity of deltamethrin to a DDT-resistant strain of cattle tick (Martel, 1980; Nolan et al., 1977).

Degradation of pyrethroids:

Pyrethroids being natural products are easily metabolised in the biological systems. Previously it was assumed that hydrolysis at 3-phenoxybenzyl alcohol group as the main degradation mechanism (Martin and Crosby, 1971). However, studies with Labelled C$^{14}$ pyrethroids proved that the pyrethroid degradation is mainly through oxidation.

Pyrethroids are metabolized by ester hydrolysis and oxidation through esterases and oxidases at methyl, methylene, alkanyl or aryl substituents. The metabolites thus obtained are generally excreted as alcohols, phenols or carboxylic acid and their glycine, sulfate, glucuronide or glucoside conjugates by insects, birds and mammals. Atleast 89 metabolites are identified from cis-and trans-permethrin alone in various species and systems. Some important metabolites of permethrin are: 4-hydroxy permethrin in housefly adults, rainbow trout, chickens, rats, goats and cows (Gaughan et al., 1977, 1978; Ivie and Hunt, 1980; Glickman et al., 1981), 2-hydroxypermethrin in rats (Gaughan et al., 1978) and 6-hydroxypermethrin in houseflies (Shono et al., 1978).
The mode of metabolic detoxification is dependent on such structural features as the cis or trans configuration of the acid moiety and the presence or absence of the cyano substituent in the alcohol moiety. The cyano-phenoxy benzyl pyrethroids are known to oxidize more slowly than the other compounds. Metabolic pathways vary little with species, but differences appear in the rates of esterase and oxidase attack and the sites of oxidation and types of conjugates formed. Mixed function oxidase (MFO) system is said to be involved in the detoxification of most of the pyrethroids in mammals and at least some pyrethroids in insects and fish.

Mikami et al., (1984) reported that the degradation of fenvalerate was primarily through hydrolysis at the ester bond and its degradation products had no tendency to accumulate in soils. Takahashi et al., (1980) described the photodegradation pathways for fenvalerate in water and on soil surface (Fig. 2).

Resistance:

Despite precautions to minimize the spread of resistance, its development may eventually make it uneconomical to use pyrethroids in some control situations for which they are currently very effective. Pyrethroid resistance of various types have been reported in houseflies, mosquitoes and cotton pests (DeVries and Georghiou 1980; Fullbrook and Holden, 1980; Omer et al., 1980; Priester and Georghiou, 1980).

Several insect species resistant to DDT are reported to be cross resistant to pyrethroids (Elliott et al., 1978; Sawicki, 1978). Resistance to
FIG. 2: PHOTODEGRADATION PATHWAYS FOR FENVALERATE (TAKAHASHI et al., 1980)
permethrin and fenvalerate has been reported in *Heliothis virescens* from the Imperial valley, California (Twine and Reynolds, 1980).

Of several types of resistant factors, Kdr factor, i.e., resistance to knockdown is found to be the most important in pyrethroid resistance in houseflies (Elliott *et al.*, 1978). The Kdr form of resistance has been demonstrated to be due to site insensitivity factor (Miller *et al.*, 1979; Osborne and Hart, 1979). Site insensitivity has also been demonstrated through electrophysiological studies in Japanese strain of houseflies as significant factor for resistance to pyrethroids and DDT (Ahn *et al.*, 1987). More recently a super-Kdr strain has also been isolated from a multiresistant housefly strain (Sawicki, 1978). This strain has considerably higher resistance to pyrethroids than the Kdr strain.

**Present Study:**

In recent years pesticide toxicology is one of the much sought-after areas for many researchers, in view of its implications in public health and well being of wildlife, domestic animals and man. As a result, pesticide toxicology research continues to be an important thrust areas for present and future investigators.

Although, much information on the biological effects of organochlorine, organophosphate and carbamate insecticides is available, it was felt that investigations on pyrethroids and their recent entry into the arena of pesticide world are not as extensive as on other groups of pesticides. Hence, fenvalerate, a synthetic pyrethroid was chosen for the present investigation, in view of its widespread usage for the control of all types of insect pests,
including those which have already developed resistance to chlorinated hydrocarbons, organophosphates and carbamates. Besides this, fenvalerate, is proved to be remarkably toxic to pests with a relative low toxicity to mammals and other vertebrates. It is also readily degradable in physical and biological environment posing less residue problem. Since nervous system is said to be the target site of action for all pyrethroids, the present investigation is concentrated on the central nervous system of cockroach. The rationale behind selecting the cockroach, *Periplaneta americana* as an experimental model is to understand the action of fenvalerate on a target animal. Since, biochemical changes are said to have a direct impact on the physiological status of the animal, studies on some aspects of protein and carbohydrate metabolism have taken up for investigation in the present study. The changes in electrical activity of the nervous system were also recorded in order to bring about a good correlation between physiological, biochemical and behavioural aspects in the cockroach under fenvalerate intoxication.
Material & Methods
SECTION - I

A. PROCUREMENT AND MAINTENANCE OF THE TEST SPECIES:

The insect model selected in the present investigation is the cockroach, Periplaneta americana. It is extremely ancient in origin, and survived against the mass extinction that destroyed so many forms of life at the end of cretaceous period, 65 million years ago, including the fabulous dinosaurs as well as the humble ammonites and trilobites. Cockroach is nocturnal in habitat and found in kitchens but prefers more roomy accommodation, such as bakeries, restaurants and sewage, where there is plenty of food and warmth. It is omnivorous and feeds on a variety of objects like clothes, shoes, books besides human food.

Cockroaches were collected in and around Tirupati and were acclimated to the laboratory conditions (25 to 30°C) for one week in wire-mesh cages before using them for the experiments. They were fed with bread and rice ad libitum. Feeding was stopped 1 day before the commencement of the experiment to avoid any metabolic variations due to diet. Adult male cockroaches were used in the present study to avoid reproductive cycle induced changes in females.

B. SELECTION OF PESTICIDE:

Sumicidin (R) (Fenvalerate) (S-5602, OMS-2000), a Synthetic pyrethroid compound, technical grade, 93.7% (W/V) supplied by Rallis India Ltd., was used.
Some important physico-chemical properties of Fenvalerate

<table>
<thead>
<tr>
<th>Sl.No.</th>
<th>Property</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Common name</td>
<td>Sumicidin, Fenvalerate (BIS)</td>
</tr>
<tr>
<td>2.</td>
<td>Chemical name</td>
<td>$\alpha$-Cyano-3-phenoxy benzyl-isopropyl-p-chlorophenyl acetate</td>
</tr>
<tr>
<td>3.</td>
<td>Structural formula :</td>
<td><img src="image" alt="Structural formula" /></td>
</tr>
<tr>
<td>4.</td>
<td>Empirical formula</td>
<td>$C_{25}H_{22}O_3\text{NCl}$</td>
</tr>
<tr>
<td>5.</td>
<td>Molecular weight</td>
<td>419.9</td>
</tr>
<tr>
<td>6.</td>
<td>Appearance</td>
<td>Yellowish oily liquid at 23°C</td>
</tr>
<tr>
<td>7.</td>
<td>Density</td>
<td>1.17 g/ml at 23°C</td>
</tr>
<tr>
<td>8.</td>
<td>Refractive index</td>
<td>MD 1.5655 at 21.5°C</td>
</tr>
<tr>
<td>9.</td>
<td>Partition coefficient</td>
<td>$1.03 \times 10^{-5} (n\text{-octyl alcohol}/H_2O)$ at 23°C</td>
</tr>
<tr>
<td>10.</td>
<td>Vapour pressure</td>
<td>$2.8 \times 10^{-7}$ mm Hg at 25°C</td>
</tr>
</tbody>
</table>

The above technical information was provided by the Rallis India Ltd., Bangalore, India.
C. SELECTION OF SOLVENT:

Most of the pesticides that accumulate in the animal systems are characterized by their extremely low solubility in water. Hence, the researchers have to rely on organic solvents, such as acetone, ethanol, benzene etc., to solubilize these chemicals. However, there is a claim that some of the organic solvents used at the concentrations to dissolve the pesticide cause toxic effects to the physiological state of the animal. In the present study, acetone was chosen as the solvent after due trials with other solvents. It was also confirmed that this solvent had no noticeable effects on the mortality and physicochemical parameters studied.

D. TOXICITY EVALUATION OF FENVALERATE:

The dosage-mortality experiments were conducted to evaluate the toxicity of fenvalerate on the cockroach for carrying out physiological as well as biochemical studies. Lethal doses were determined by "Probit method" proposed by Finney (1964). To determine the lethal and sublethal doses, the cockroaches were treated with different concentrations of fenvalerate. This was done in two ways (a) by applying fenvalerate externally on the dorsal side of the abdomen (topical application) and (b) by injecting fenvalerate into the body of the cockroach (in vivo).

(i) Topical application:

The cockroaches were divided into different batches of 20 animals each. Each batch was exposed to a particular concentration of fenvalerate ranging from 5 μg to 8.5 μg. The application was done with the help of a Hamilton Syringe and the chemical was smeared uniformly on the dorsal surface of the abdomen holding the wings apart. Enough care was taken to
ensure that the chemical does not spill over during and after application. Control cockroaches were applied with acetone alone without fenvalerate. It was ascertained that the smeared sample evaporated before transferring the cockroaches to their respective cages. Each batch was maintained in a separate cage, and fed with rice and bread ad libitum. After 48h, the number of animals died or alive was scored. After repeating each experiment for 5 times, the average mortality at each dose was taken to determine the LD$_{50}$. The per cent kill observed for each dose was calculated and converted to probit kill by means of a probit table. A graph was plotted between Fenvalerate concentration and probit kill. LD$_{50}$ was the concentration where 50% of the test animals were killed.

ii) Injection:

As in topical application, cockroaches were divided into different batches (20 per each batch) and injected with different concentrations of fenvalerate ranging from 0.8 $\mu$g to 2.0 $\mu$g. Injection was done into the abdomen from the lateral side with a fine sterile syringe, inserting it through the joints between 5th and 6th tergal plates. The chemical was injected at the same locus throughout the course of the investigation. While injection was done, median line was avoided to avert the possible damage to the median abdominal nerves. After 48h, the number of animals died or alive was scored. Control animals were injected with acetone alone without fenvalerate. After calculating the per cent kill, graphs were plotted as in topical application.
Besides topical application and injection, in vitro experiments were also conducted by adding different concentrations of fenvalerate directly to the tissue homogenates.

iii) Selection of sublethal concentration:

It is well known that acute exposure to higher doses is always lethal, causing death. Death may ensure under lethal exposure before the behavioural manifestations of toxicity could be noticed. Observation of these behavioural changes is possible during sublethal doses. Although the animal looks normal due to the apparent development of tolerance during the course of time, continuous exposure may result in several behavioural abnormalities. The cockroaches under lethal intoxication (Fenvalerate) remained motionless, and then tumbled down with their ventral side up and continued in this moribund condition without recovery. Thus for studies with sublethal doses, the concentrations were chosen so as to induce all the behavioural responses as under lethal dose but to allow a recovery of the animals to their normal posture within a short time following exposure. So in the present investigation 1/10th fraction of LD$_{50}$ (0.69 µg/g body weight) under topical application and 1/12th fraction of LD$_{50}$ under injection (0.11 µg/g body weight) were selected as sublethal concentrations.

iv) Time concentration Dependency:

The effect of pesticide is mainly related to the time of exposure and its concentration. In view of this, experiments were conducted using both sublethal and lethal doses, and a time course study was also made to evaluate the effect of the pesticide in relation to the time of exposure.
Experiments were carried out at 3, 6, 12, 24 and 48h after sublethal and lethal doses for both injection and topical application of fenvalerate.

Besides in vivo and topical studies, in vitro experiments were also carried out to evaluate the direct effect of fenvalerate on some selected enzyme systems. For in vitro studies, the concentration of fenvalerate selected varied from 5 to 200 μ moles, viz., 5, 10, 25, 50, 75, 100, 150 and 200 μ moles.

E. FACTORS LIKELY TO INFLUENCE TOXICITY:

The following factors are likely to contribute to the variations in toxicity of a given pesticide. Measures were taken in the present study to nullify them to the extent possible.

i) Species specificity:

Since the toxicity of the pesticide vary for several species of a single genus, only one species of cockroach, Periplaneta americana was chosen for the present investigation.

ii) Size of Cockroaches:

The size of the test species is one of the important factors contributing to the differential toxicity. The susceptibility is known to differ with size of houseflies and mosquitoes (Page et al., 1949). The LC_{50} values obtained for fish, Tilapia mossambica also varied with size (Ayyanna, 1988). So in the present study adult cockroaches of equal size, weighing about 1 ± 0.1 g were used throughout.
iii) Sex of the Animals:

The earlier studies on lepidopteran insects revealed that no significant differences in mortality occurred between males and females treated with pyrethroids (Klein et al., 1983). However, reproductive cycle of females may induce some changes in normal metabolism. So males were chosen for present investigation.

iv) Temperature:

The toxicity of pesticides is known to alter with fluctuations in temperature (Chadha et al., 1964; Herzberg et al., 1980). The toxicity of fenvalerate is known to increase with decrease in temperature, i.e. negative temperature coefficient of insecticidal action was observed (Narahashi, 1971a,b). Hence, the toxicity studies were conducted at moderate cool conditions (29°C) and high temperatures during summer were brought down to normal level by using humidifiers.

v) Nutritional Status:

It also contributes to differential toxicity of test chemicals (Das and Garg, 1981). So cockroaches were fed regularly with rice and bread ad libitum for one week before using them for experiments.

F. PREPARATION OF CONCENTRATIONS:

A stock solution of 100 mg/ml of fenvalerate was prepared in acetone and the appropriate amounts were aliquoted. This stock solution was used to prepare various concentrations of the chemical by diluting it with distilled water. All the required concentrations were prepared just before the experimentation.
G. SELECTION OF TISSUE FOR BIOCHEMICAL ANALYSIS:

Since synthetic pyrethroids are highly potent neurotoxicants all studies were concentrated exclusively on the central nervous system (CNS) of cockroach. Moreover, the pyrethroids having α-cyano group are well known for their action on the central nervous system. Since the insecticide selected (Fenvalerate) in the present study contains α-cyano group, CNS was the appropriate tissue for the analysis of the biochemical parameters and also for physiological studies. The CNS consisted of the brain, suboesophageal ganglion and all the thoracic and abdominal ganglia with their connectives. All the behavioural, biochemical and physiological observations were made at some selected time intervals viz., 3, 6, 12, 24 and 48h after exposure to fenvalerate.

SECTION - II

BIOCHEMICAL ASSAYS

1. Total Proteins:

Total protein content was estimated by the method of Lowry et al., (1951).

1% homogenate of the CNS was prepared in 10% trichloroacetic acid and it was centrifuged at 1000 g for 15 minutes. The supernatant was discarded and the residue was dissolved in 2ml of 1 N sodium hydroxide. From this, 0.1 ml was taken and 4 ml of alkaline copper reagent and 0.4 ml of Folin phenol reagent (diluted with distilled water in 1 : 1 ratio) was added. After 30 minutes the developed colour was read at 600 nm in a spectrophotometer against a reagent blank.
The amount of proteins present in the sample was calculated using a standard prepared from bovine albumin, and the values were expressed as mg/g wet weight of the tissue.

2. Soluble proteins:

Soluble proteins were estimated by the method of Lowry et al., (1951).

1% homogenate of the CNS was prepared in 0.25 M sucrose solution and centrifuged at 1000 g for 15 minutes. The supernatant was used to estimate the sucrose soluble protein fraction. Required amount (ml) of 10% trichloroacetic acid was added to the supernatant and centrifuged for 15 minutes at 1000 g. The supernatant was discarded and the residue was dissolved in 2 ml of 1 N sodium hydroxide. To 0.1 ml of this, 4 ml of alkaline copper reagent solution was added followed by 0.4 ml of 1:1 Folin phenol reagent. After 30 minutes the developed colour was read at 600 nm in a spectrophotometer against a reagent blank.

The amount of soluble proteins present in the sample was calculated with the help of a bovine albumin standard, and the values were expressed as mg/g wet weight of the tissue.

3. Free amino acids:

Free amino acid content was estimated by the method of Moore and Stein (1954) as described by colowick and Kaplan (1957).

1% homogenate of CNS was prepared in 10% trichloroacetic acid and centrifuged for 15 minutes at 1000 g. To 0.5 ml of the supernatant, 2 ml of
ninhydrin reagent was added and kept in boiling water bath for 6 1/2 minutes and then cooled. The contents were made upto 10 ml with distilled water. The intensity of colour developed was read at 570 nm in a spectrophotometer against a reagent blank. The free amino acid content was expressed as μ moles of tyrosine equivalents/g wet weight of the tissue.

4. Total Carbohydrates:

Total carbohydrate content (Anthrone positive substances) was estimated by the method of Carroll et al., (1956).

1% homogenate of the CNS was prepared in 10% trichloroacetic acid (TCA) and centrifuged at 1000 g for 15 minutes. To 0.4 ml of supernatant, 5 ml of anthrone reagent was added and the contents were boiled for 15 minutes and then cooled. The colour developed was read at 620 nm in a spectrophotometer against a blank containing TCA and anthrone reagent in same proportion. The values were expressed as mg of glycogen/g wet weight of the tissue.

5. Glycogen:

The glycogen content was estimated by the method of Carroll et al., (1956).

4% homogenate of the CNS was prepared in 10% trichloroacetic acid and centrifuged for 15 minutes at 1000 g. From the supernatant, 0.5 ml was taken and 5 ml of 95% ethanol was added and the contents were allowed to stand overnight in the refrigerator. After the completion of precipitation, the contents were centrifuged at 1000 g for 15 minutes. The supernatant was
discarded and the residue was dissolved in 1 ml of distilled water. To this, 5 ml of anthrone reagent was added and boiled for 15 minutes and then cooled to room temperature. The intensity of the colour developed was read at 620 nm in a spectrophotometer against a reagent blank.

The glycogen content was calculated and the values were expressed as mg of glycogen/g wet weight of the tissue.

6. Estimation of Lactate:

Lactic acid was estimated by the method of Barker and Summerson (1951) as modified by Huckabee (1961).

10% homogenate of the CNS was prepared in cold 10% trichloroacetic acid and centrifuged at 1000 g for 15 min. 0.8 ml of the supernatant equivalent to about 80 mg of the tissue was taken into a centrifuge tube. To this, 1 ml of 20% copper sulphate solution was added and the contents were made upto 10 ml with distilled water. To this 1 g of powdered calcium hydroxide was added and the tubes were shaken vigorously until the contents were dispersed uniformly. Then the tubes were kept for an hour with intermittent shaking and later centrifuged. 1 ml of the supernatant was transferred into a clean dry test tube and 0.05 ml of 4% copper sulphate solution was added followed by 6 ml of sulphuric acid. The contents were mixed well by shaking and kept in boiling water bath for 6 minutes and cooled. When the contents were sufficiently cooled, 0.1 ml of P-hydroxydiphenyl was added directly into the solution and the precipitate was kept at room temperature for 30 minutes. Later the contents were placed in boiling water bath for 90 sec followed by cooling, and the colour developed
was read at 560 nm against a blank in a spectrophotometer. The lactic acid content was expressed as $\mu$ moles of lactic acid/g wet weight of the tissue.

7. Estimation of pyruvate:

Pyruvate content was estimated by the method of Friedman and Haugen (1942).

2% homogenate of the CNS was prepared in 10% Trichloroacetic acid and centrifuged at 1000 g for 15 minutes. To 2.0 ml of the supernatant, 0.5 ml of 2,4-dinitrophenyl hydrazine was added and the tubes were kept for 5 minutes at 25°C and 3 ml of 2.5 N sodium hydroxide solution was added. After 10 minutes the developed colour was read at 540 nm in a spectrophotometer against a reagent blank. The standard graph was prepared by using sodium pyruvate. The values were expressed as $\mu$ moles of pyruvate/g wet wt. of the tissue.

8. Assay of Aminotransferases:

The activity of AAT and AIAT was determined by the method of Reitman and Frankel (1957) as described by Bergmeyer and Bernt (1965).

10% homogenate of the CNS was prepared in 0.25 M ice cold sucrose solution. The homogenates were centrifuged at 1000 g for 15 minutes. The supernatant thus obtained was used for aspartate and alanine amino transferase assay.
a) Aspartate aminotransferase (AAT) (L-aspartate 2-oxoglutarate aminotransferase : E.C. 2.6.1.1) Activity:

The reaction mixture of 2.0 ml contained: 100 \( \mu \) moles of phosphate buffer (pH 7.4), 100 \( \mu \) moles of L-aspartic acid, 2 \( \mu \) moles of \( \alpha \)-ketoglutarate and the supernatant as enzyme source. The contents were incubated at 37°C for 30 minutes. The reaction was stopped by addition of 1 ml of 2,4-dinitrophenyl hydrazine solution (0.001 M) prepared in 0.1 N HCl. This is called ketone reagent. After 20 minutes, 10 ml of 0.4 N sodium hydroxide was added to all the tubes. The colour developed was read at 545 nm in a spectrophotometer against a reagent blank. The enzyme activity was expressed as \( \mu \) moles of pyruvate formed/mg protein/h.

b) Alanine aminotransferase (A1AT) (DL-Alanine 2-Oxoglutarate aminotransferase : E.C. 2.6.1.2) Activity:

The reaction mixture of 2 ml contained: 100 \( \mu \) moles of L-alanine, 100 \( \mu \) moles of phosphate buffer (pH 7.4), 2.0 \( \mu \) moles of \( \alpha \)-ketoglutarate, and supernatant as enzyme source. The reaction mixture was incubated at 37°C for 30 minutes. The reaction was stopped by adding 1 ml of 2,4-dinitrophenyl hydrazine solution prepared in 0.1 N HCl. The remaining procedure was the same as that of aspartate aminotransferase.

9. Assay of Dehydrogenases:

a) Succinate dehydrogenase (SDH) (Succinate Acceptor oxido reductase : E.C. 1.3.99.1) Activity:

SDH activity was estimated by the method of Nachlas et al., (1960).
4% homogenate of the CNS was prepared in 0.25 M cold sucrose solution and centrifuged at 1000 g for 15 minutes. The supernatant was used for enzyme assay. The reaction mixture of 2 ml contained: 40 \( \mu \) moles of sodium succinate, 100 \( \mu \) moles of phosphate buffer (pH 7.2) and 4 \( \mu \) moles of INT (2-P-iodophenyl)-3-(P-nitrophenyl)-5-phenyl tetrazolium chloride) and, 0.2 ml of supernatant as the enzyme source. The reaction mixture was incubated at 37\(^\circ\)C for 30 minutes and the reaction was stopped by adding 5 ml of glacial acetic acid. Zero time controls were maintained by adding 5.0 ml of glacial acetic acid prior to the addition of homogenate. The formazan formed was extracted overnight in 5 ml of cold toluene. The intensity of colour developed was read at 495 nm against reagent blank in a spectrophotometer. The activity was expressed as \( \mu \) moles of formazan formed/mg protein/h.

b) Malate dehydrogenase (MDH) (L-Malate NAD oxidoreductase : E.C. 1.1.1.37) Activity:

Malate dehydrogenase activity was estimated by the method of Nachlas et al., (1960) with slight modifications.

2% homogenate of the CNS was prepared in 0.25 M cold sucrose solution and centrifuged at 1000 g for 15 minutes. The supernatant served as the enzyme source. The reaction mixture of 2 ml contained: 40 \( \mu \) moles of sodium malate, 100 \( \mu \) moles of phosphate buffer (pH 7.2), 0.1 \( \mu \) moles of NAD, 4 \( \mu \) moles of INT and 0.6 ml of supernatant. The remaining part of the procedure was same as described for SDH. The activity was expressed as \( \mu \) moles of formazan formed/mg protein/h.
c) Lactate dehydrogenase (LDH) (L-Lactate NAD oxidoreductase: E.C. 1.1.1.27) Activity:

Lactate dehydrogenase activity was estimated by the method of Srikanthan and Krishnamurthy (1955) with slight modifications.

2% homogenate of the CNS was prepared in 0.25 M cold sucrose solution and centrifuged at 1000 g for 15 minutes. The supernatant served as the enzyme source. The reaction mixture of 2.0 ml contained: 40 \( \mu \) moles of sodium lactate, 100 \( \mu \) moles of phosphate buffer (pH 7.2), 0.1 \( \mu \) moles of NAD, 4 \( \mu \) moles of INT and 0.5 ml of supernatant. The subsequent steps followed were the same as described for SDH. The activity was expressed as \( \mu \) moles of formazan formed/mg protein/h.

d) Glutamate dehydrogenase (GDH) (L-glutamate NAD oxidoreductase: E.C. 1.4.1.3) Activity:

Glutamate dehydrogenase activity was estimated by the method of Lee and Lardy (1965) with slight modifications.

5% homogenate of the CNS was prepared in 0.25 M cold sucrose solution and centrifuged at 1000 g for 15 minutes. The supernatant was used as the enzyme source. The reaction mixture of 2 ml contained: 40 \( \mu \) moles of sodium glutamate, 100 \( \mu \) moles of phosphate buffer (pH 7.2), 0.1 \( \mu \) moles of NAD, 4 \( \mu \) moles of INT and 0.4 ml of supernatant. The subsequent steps followed were the same as described for SDH. The activity was expressed as \( \mu \) moles of formazan formed/mg protein/h.
SECTION - III

ELECTROPHYSIOLOGICAL STUDIES

Experimental Set-up:

The Ventral Nerve Cord (VNC) of cockroach, *Periplaneta americana* was chosen for electrophysiological studies in the present investigation. The VNC was easily accessible for manoeuvre with electrodes and its responses could be elicited and maintained for about 2 h after the preparation was made. This relatively short period was considered reasonably good, judging by the experimental set-up used in the present study, where continuous perfusion and removal of the Ringer medium was not possible. The responses elicited from VNC were considered as an index of the responses of the nervous system of the animal in general.

The electrophysiological set-up used consisted of paired platinum hook electrodes both for stimulation and recording of the surface potentials, a Grass S 44 stimulator for delivering square wave pulses to the nerve, a Grass P5 pre-amplifier for feeding the compound action potentials, where the size of the potential is proportionate to the number of axons activated, a Tektronix 502 A dual beam oscilloscope for the final display of the potentials. The photographic recordings were made using a Grass C4R kymograph Camera.

Large and active male cockroaches were used for the electrophysiological investigations. The animal was pinned with its dorsal side up, on a waxed petridish. It was quickly dissected to expose the VNC. Care was taken to ensure that the gut contents did not spill over the cord during
the dissection. The cord was isolated from the brain by cutting between the suboesophageal ganglion and the first thoracic ganglion. It was also isolated from the peripheral inputs by severing the cercal nerves and all other peripheral nerves. The cord nevertheless remained within the animal adhering to the connective tissue and was not taken out of the animal. This facilitated the hooking of the cord on to the electrodes for recording the electrical activity. The stimulating electrodes were placed between the 5th and 6th abdominal ganglion and the evoked electrical potentials were recorded with the help of recording electrodes placed between the 3rd thoracic ganglion and the 1st abdominal ganglion.

Following dissection, the VNC was allowed to recover from the shock effects by flooding with the Ringer medium for at least 10 minutes before the potentials could be initiated. The Ringer medium contained: NaCl 128 mM, KCl 4.7 mM, CaCl$_2$ 1.9 mM and NaHCO$_3$, 8.6 mM. The preparation was viable without any loss of activity for about 2 hours at room temperatures (25°C - 30°C). Within each experiment the temperature varied by 0.5-1.0°C. This fluctuation was not found to cause any change in evoked potentials of the cord, as judged from control recordings made over a length of 2 hours. Care was taken to see that the electrodes caused minimal stretch to the cord. Precautions were taken against interference by the Ringer medium. The cord was perfused with the Ringer medium continuously, except at the time when the potentials were recorded, which could be accomplished in 1 or 2 minutes.

**PREPARATION OF THE TEST SOLUTION:**

Stock solution of fenvalerate was prepared using acetone as the solvent. Just before testing its effects on the electrical activity of the VNC,
the stock solution was diluted with the Ringer medium as per the requirements. The effects of different concentrations of fenvalerate ranging from $1 \times 10^{-7}$ to $1 \times 10^{-3}$M were examined in in vitro experiments in the present study. The concentration of the organic solvent in the test solution in any case did not exceed 1%. This concentration did not cause any noticeable effects on the evoked potentials of the VNC, as concluded from preliminary standardization with acetone alone.

Study of the Actions of Test Solutions:

The actions of fenvalerate on action potentials of the VNC were studied in 2 different ways, viz., (a) In vitro perfusion of the VNC. (b) topical application on the animal.

In vitro application was done, after recording the control response, by perfusing the VNC with the test solution for 2-3 minutes. In each experiment the effects of perfusion with fenvalerate followed by washing with the Ringer (5 minutes) were observed and recorded.

In studies with topical application, lethal and sublethal doses of fenvalerate were applied to the animals externally as described earlier in this section. In these experiments the control recordings were obtained by perfusing the cord with the haemolymph collected from that animal during dissection, since perfusion with Ringer might alter the response. The changes in the action potentials of the VNC were recorded at 3h, 24h and 48h following topical application of fenvalerate. The parameters chosen for investigation were threshold voltages, amplitude, duration, and latency of the compound action potential.
SECTION - IV

VALIDITY OF EXPERIMENTAL PROCEDURES

1. Aliquots for Assay:

Aliquots were selected for the assay such that the initial rates were approximately as near as possible, yet providing sufficient product to fall in a convenient range for spectrophotometric measurement.

2. Enzyme units:

The soluble protein content of the tissue homogenate (enzyme source) was estimated using Folin-phenol reagent (Lowry et al., 1951). This was used for the expression of enzyme activity. Enzyme activities were expressed in standard units, i.e., \( \mu \) moles of product formed or substrate cleaved/mg protein/hour.

3. Substrate Requirements:

All the enzyme activity levels were determined at saturating substrate concentrations i.e., in zero order.

4. Lambert-Beer Law:

All most all the products of the reactions were measured by using the colorimetric procedures, in which, the optical density (absorbance) of the resulting coloured complex was proportional to the concentration of the reaction products.

Thus standard graphs were prepared for each colorimetric estimation between concentration of the substance (either product or substrate) and the
optical density from which the activities of enzymes or quantities of substances were calculated.

5. Enzyme nomenclature:


6. Assay of dehydrogenases by using INT:

Tetrazolium salts constitute unique classes of oxidation, reduction indicators in the study of dehydrogenases. The advantage of using tetrazolium salts as electron acceptors are:

a) the tetrazolium salts give a stable colour on reduction.
b) they are highly insoluble in aqueous solution.
c) they can be reduced both aerobically and anaerobically.
d) they have high redox potential which makes the reduction easier.
e) they are easily permeable through membranes.

Various tetrazolium salts receive electrons from various sites of electron transport system (Nachlas et al., 1960). This is due to the inherent difference in the redox potentials of various tetrazolium salts. The introduction of P-nitrophenyl group in N2 phenyl region was observed to increase the efficiency of the dye by increasing its redox potential. Karmarker et al., (1959) reported that INT was superior to most of the tetrazolium salts as an electron acceptor for the assay of dehydrogenases.
7. Statistical treatment of the data:

The mean, standard deviation (SD) and test of significance or student's "t" test was calculated following the method of Pillai and Sinha (1968). The formula used for calculating SD was

\[ E x^2 - \left( \frac{E x}{n} \right)^2 / n \]

\[ \frac{\text{------------------------}}{n - 1} \]

Where, \( x^2 \) is the sum of square of deviations from the mean.

\( n \) number of individual observations.

The significance of the deviations from normal was calculated by calculating student's "t" test by using the following formula.

\[ t = \frac{m_1 - m_2}{\sqrt{\frac{SD_1^2}{n_1} + \frac{SD_2^2}{n_2}}} \]

\[ \frac{\text{------------------------}}{\sqrt{n_1 + n_2 - 2}} \]

Where \( m_1 \) is the mean of first set of observations

\( m_2 \) is the mean of second set of observations.

\( SD_1^2 \) and \( SD_2^2 \) are squares of standard deviations of the first and second sets of observations.

\( n_1 \) and \( n_2 \) are number of observations of the first and second sets.