CHAPTER 1

REVIEW OF LITERATURE
1.1 Introduction

The carbamates are the esters of carbamic acid and represent one of the three major groups of modern synthetic insecticides. Carbamates were first developed by the Geigy Corporation in 1951 but not made commercially available until 1956 (Baron, 1991). Research into carbamates began following a search for compounds other than organophosphates that had anticholinesterase activity. One of the known compound of this group was alkaloid physostigmine, found in the Calabar bean (*Physostigma venenosum*), which had been used for trial by ordeal in its native West Africa. The toxic constituent, physostigmine or eserine, was isolated one hundred years ago and identified in 1925 as the methylcarbamate ester of eseroline. Physostigmine is the only known naturally occurring carbamate ester. Successful development of carbamates as insecticides was initiated by the researches of Hans Gysin in Switzerland, and Robert Metcalf and co-workers in the United States (Hussel, 1990).

1.2 Carbamates

The carbamates are produced from carbamic acid and have a similar mode of action that of organophosphates as they also block the enzyme acetylcholinesterase (Thacker, 2002). This process of enzyme inhibition is called carbamylation (Oonnithan and Casida, 1968). The carbamylation process is relatively less stable, i.e., the enzyme is not blocked for so long.
Because of its action as a reversible cholinesterase inhibitor its use is preferred over other insecticides like organophosphates and chlorohydrocarbons, which are irreversible cholinesterase inhibitors. Cholinesterase depression is common to all the carbamate pesticide both in blood and tissues. The reversibility of acetylcholinesterase inhibition confers advantage to carbamates over organophosphates (Thacker, 2002). The general structure of carbamate is shown below

![General structure of carbamate](image)

In which $R_1$ and $R_2$ are hydrogen, methyl, ethyl, propyl or short chain alkyls and $X$ is phenol, naphthalene or other hydrocarbon rings.

The commonly used carbamates are given in table 1.

**Table 1**: The commonly used carbamates

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Common Name</th>
<th>Chemical Name</th>
<th>Trade Name</th>
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<tbody>
<tr>
<td>1.</td>
<td>Aldicarb</td>
<td>2-Methyl-2(methylthio) propionaldehyde-0-(methyl carbamoyl) oxime</td>
<td>Temik®</td>
</tr>
<tr>
<td>2.</td>
<td>Bendiocarb</td>
<td>2,2-Dimethyl-1,3-benzodiox-ol-4-yl N-methylcarbamate</td>
<td>Dycarb®, Tatton®, Seedo®</td>
</tr>
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<td>3.</td>
<td>Carbaryl</td>
<td>1-Naphthyl-N-methylcarbamate</td>
<td>Sevin®, Carbacide®</td>
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<tr>
<td>4.</td>
<td>Carbofuran</td>
<td>2,3-Dihydro-2,2-dimethyl-7-benzofuranyl N-methylcarbamate</td>
<td>Furadan®, Brifur®</td>
</tr>
<tr>
<td>5.</td>
<td>Carbosulfan</td>
<td>2,3-Dihydro-2,2-dimethyl 7-benzofuranyl [(dibutylamino) thio] N-methylcarbamate</td>
<td>Advantage®, Marshal®</td>
</tr>
<tr>
<td>6.</td>
<td>Dioxy carb</td>
<td>2-(1,3-Dioxolan-2-yl) phenyl-N-methylcarbamate</td>
<td>Electron®, Famid®</td>
</tr>
<tr>
<td>7.</td>
<td>Formentanate HCl</td>
<td>3-Dimethylaminomethylene-amino phenyl N-methylcarbamate</td>
<td>Carzol®, Dicarzol®</td>
</tr>
<tr>
<td>8.</td>
<td>Mecarbam</td>
<td>S-(N-ethoxycarbonyl-N-methyl carbamoylmethyl) o, o-diethyl phosphorodithioate</td>
<td>Afos®, Murfotox®, Pestan®,</td>
</tr>
<tr>
<td>9.</td>
<td>Methiocarb</td>
<td>3,5-dimethyl-4-(methylthio)-phenyl N-methylcarbamate</td>
<td>Draza®, Mesurol®, Slug Guard®</td>
</tr>
<tr>
<td>10.</td>
<td>Methomyl</td>
<td>S-Methyl-N-[(methyl carbamoyl) oxy]theoacetimidate</td>
<td>Lannate®, Lanox®</td>
</tr>
<tr>
<td>11.</td>
<td>Mexacarbate</td>
<td>4-Dimethylamino-3,5-xylyl-N-methylcarbamate</td>
<td>Zectran®</td>
</tr>
<tr>
<td>12.</td>
<td>Oxamyl</td>
<td>N-N-dimethyl-2-methyl carbamoyl oxyimino-2-(methylthio) acetamide</td>
<td>Vydate®</td>
</tr>
<tr>
<td>13.</td>
<td>Pirimicarb</td>
<td>2-(Dimethylamino)-5,6,2-isopropoxyphenyl N, N-dimethylcarbamate</td>
<td>Abol®, Afox®</td>
</tr>
<tr>
<td>14.</td>
<td>Propoxur</td>
<td>2-Isopropoxyphenyl-N-methylcarbamate</td>
<td>Baygon®, Propagan®</td>
</tr>
<tr>
<td>15.</td>
<td>Thiodicarb</td>
<td>Dimethyl-N,N-[thiobis(methyl imino)carbonyloxy]-bis(ethanimidothioate)</td>
<td>Larvin®, Nirval®</td>
</tr>
<tr>
<td>16.</td>
<td>Trimethocarb</td>
<td>4:1 mixture of the 3,4,5- and 2,3,5-isomers of trimethyl phenyl N-methylcarbamate</td>
<td>Broot®, Landrin®</td>
</tr>
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</table>
1.2.1 Carbaryl

The most important carbamates to have been developed include carbaryl, carbofuran, methiocarb, pirimicarb, propoxur and aldicarb (Thacker, 2002). The first carbamate to be used was carbaryl. This was first synthesized in 1953 and introduced for use in crop protection in 1956 (EPA, 2003). In India carbaryl was introduced by UNION CARBIDE in the early 1960s (whose pesticide interests were taken over by RHONE-POULENC after Bhopal gas diaster). Currently, over 300 products containing carbaryl are actively registered with the EPA (PBPPD, 2001). Its low mammalian toxicity has meant that it has been widely used around the home as well as in agricultural production.

Carbaryl controls insect pests of field crops, forage, vegetables, fruits, nuts, shade trees, ornamentals, forests, lawns, turf and rangeland, as well as pests of domestic animals (Caroline, 1994; WHO, 1994). It is mostly used against caterpillar pests on apples, pests on citrus fruit, mangoes, bananas, strawberries, nuts, vines, olives, okra, cucurbits, peanuts, soyabees, cotton, rice, tobacco, cereals, beet, maize, sorghum, alfalfa, potatoes, ornamentals and forestry. Carbaryl is used to control a variety of pests, including moths, beetles, cockroaches, ants, ticks and mosquitoes (Tomlin, 2000). Carbaryl is used against ectoparasites of humans and animals, including against head louse on children (Whitehead, 1995). Carbaryl formulations include baits,
dusts, wettable powders, granules, and oil, molasses, and aqueous dispersions and suspensions (EPA, 2003).

The chemical name of carbaryl is 1-napthyl N-methylcarbamate (EPA, 2003). Its structure is shown in figure 1.

![Chemical structure of carbaryl.](image)

**Figure 1:** Chemical structure of carbaryl.

The common name carbaryl is in general use except in Eastern Europe, where aryl alum is used, and in the USSR, where the trade name Sevin® is used as a common name. Other trade names have included Atozan®, Caprolin®, Carbacide®, Carbamine®, Carpolin®, Cekubaryl®, Denapon®, Denopton®, Devicarb®, Dicarbam®, Gamonil®, Hexavin®, Karbaspray®, Karbotox®, Karbosep®, Mervin®, NAC®, Panam®, Rayvon®, Septene®, Sevinox®, Sevidol®, Tercyl® and Tricarmam®. The code designations are ENT 23969, UC 7744 and OMS 29. The CAS (chemical abstract service) registry number is 63-25-2 (Baron, 1991).
1.2.1.1 Physical and chemical properties

Carbaryl is a white to light tan solid with a mild phenolic odour (HSDB, 1997). It has the empirical formula, C_{12}H_{12}NO_2 and a molecular weight of 201.20. The melting point of carbaryl is 142 °C and a vapour pressure of less than $4 \times 10^{-5}$ mm Hg at 26 °C (Kidd and James, 1991). Carbaryl has low volatility and low air-water partition coefficient. Thus, only limited evaporation can be anticipated after treatment (Baron, 1991). The dimensionless air-water partition constant for carbaryl (Henry's law constant) was found to be $5.3 \times 10^{-6}$ (Schemburg et al., 1991). Lee et al. (1990) calculated that, 50 days after treatment, 0.63% of the carbaryl applied to soil could have been volatilized and 78.84% degraded.

Carbaryl disrupts the normal functioning of the insect nervous system and cause toxicity by contact or ingestion (Tomlin, 2000). It also disrupts nervous system by adding a carbaryl moiety to the active site of the acetylcholinesterase enzyme, which prevents it from interacting with acetylcholine (Klassen et al., 1996). The chemical neurotransmitter acetylcholine is used to relay nervous system signals across the nerve synapse. Acetylcholinesterase is the enzyme responsible for the breakdown of acetylcholine once it is released into the synapse. When the enzyme is inhibited, surplus acetylcholine builds up, resulting in nervous overstimulation. The carbaryl group is released from the active site of
acetylcholinesterase by spontaneous hydrolysis and restoring nerve function (Gray, 1996).

1.2.1.2 Absorption and distribution

The toxicity of carbaryl is greatly influenced by the vehicle and route of exposure and the importance of these factors vary among the insecticidal carbamates (Baron, 1991). Carbamates are readily absorbed during passage through the gastrointestinal tract, and absorption is so partly related to the vehicle in which they are administered (IPCS, 1994). The most important human exposure route is dermal, and those occupationally exposed, such as insecticide formulators and applicators and farm workers (Baron, 1991). The greatest risk to these individuals would be from working with carbamates under conditions of high temperature. Low-level exposure to residues in foods may occur wherever carbamates are used on edible commodities and where tolerances have been granted for such uses. Once absorbed, carbaryl was rapidly distributed to the tissues and organs. Metabolism and eliminations are relatively rapid, no evidence has been found for bioaccumulation of carbamates (Baron, 1991).

Penetration of carbaryl through rat skin depended on the solvent, being greater in acetone than in benzene or corn oil, rapid early penetration was by the parent compound (O'Brien and Dannelley, 1965). In a percutaneous absorption study with rats, about 57% of a continuously applied dose of $[^{14}C]$
carbaryl in acetone penetrated the shaved skin in 168 hr. The absorption rate was 0.18 μg/cm²/hr; t₁/₂ was 1.26 hr for absorption and 67 hr for elimination (Knaak et al., 1984). In mice, t₁/₂ for acute dermal penetration of [¹⁴C] carbaryl in acetone was 12.8 min, and the label was detected in the blood, tissues and excreta within 5-15 min after application. By 8 hr after application, 73.3% of the dose had appeared in the excreta, while 4.9% remained in the intestine and 2.6% in the liver; levels in other tissues and organs ranged from less than 0.1% to 0.6% (Shah et al., 1981). In rats, carbaryl was absorbed more rapidly from the intestine than from the stomach (Cambon et al., 1981). In mice, about 69% of an intubated dose of carbaryl was absorbed within 60 min. The t₁/₂ for absorption was 17 min. Within an hour, 16.9% of the dose appeared in the urine and 8.6% in exhaled CO₂ (Ahdaya et al., 1981).

The gastric intubation of rats with [¹⁴C] carbaryl, the percentage of the dose per gram of tissue ranged from less than 0.1% to nearly 0.4% after 1 hr; levels had significantly declined in liver and fat (Tanaka et al., 1980). The acute oral exposures of rats to carbaryl at 450-1500 mg/kg, residues were detected in tissues at 48 hr after dosing. In rats that died, minimum residue levels were 11.7 ppm in the liver, 5 ppm in the brain and 3.6 ppm in the heart (Mount et al., 1981).
1.2.1.3 General metabolism

As with other carbamates the principal metabolic pathways are hydroxylation, hydrolysis, and epoxidation, resulting in numerous metabolites subjected to conjugation, forming water-soluble sulfates, glucuronides, and mercapturates (Carpenter and Livestone, 1961; Dorough et al., 1963; Dorough and Casida, 1964; Knaak et al., 1965; Menzie, 1969; Bend et al., 1971). Hydrolysis of carbaryl results in the formation of 1-naphthol, carbon dioxide, and methylamine (Carpenter and Livestone, 1961; Sakai and Matsumura, 1971). Hodgson and Casida (1961) have reported the first evidence for carbaryl hydroxylation. Carbaryl is metabolized by a rat liver microsome system, requiring NADPH and oxygen, to form formaldehyde yielding derivative.

Animals metabolize carbaryl both by hydrolytic (hydrolysis and hydroxylation) and non-hydrolytic pathways. The principal metabolic pathways such as hydrolysis and ring hydroxylation metabolize carbaryl to 1-naphthol and hydroxylated naphthylmethylcarbamate, which form water-soluble sulfates, glucuronides and mercapturates. Carbaryl rapidly metabolized in mammals to 1-napthol, CO₂ and methylamine by hydrolysis (Baron, 1991). The hydroxylation resulted in 4-hydroxycarbaryl, 5-hydroxycarbaryl, N-hydroxymethylcarbaryl, 5,6-dihydro-5,6-dihydroxycarbaryl and 1,4-naphthalendiol. Carbaryl is soluble under normal
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storage conditions but is hydrolyzed rapidly at pH 10 or above (PIP, 1996). Biotransformation of carbaryl is basically similar in humans, rats, guinea pigs, monkeys and sheep; the major difference being the extent to which carbaryl was hydrolyzed to yield 1-naphthol. Much less hydrolysis occurs in monkeys or pigs than in humans (Knaak et al., 1968; Sullivan et al., 1972; Lin et al., 1975).

Carbaryl has been shown to be metabolized in vitro by cells from both animal and plant sources and the main product was 1-naphthol (Chin et al., 1979). Partial metabolism of carbaryl to CO₂ was demonstrated in vitro (Palut et al., 1970). Most mammals given naphthyl-labelled carbaryl excreted 68-74% of the dose in the urine and 2-11% in the feces within 24 hrs of administration (Knaak et al., 1968; Sullivan et al., 1972). The metabolism of up to 85% of carbaryl occurs within 24 hrs after administration (EPA, 1987).

The hydrolysis of carbaryl to 1-naphthol was probably the critical step in the house flies (Eldefrawi and Hoskin, 1961). The housefly metabolites of 1-naphthol and naphthalene have been characterized (Terriera et al., 1961). The administration of ¹⁴C-labelled carbaryl to house flies yielded ¹⁴C dioxide (Dorough et al., 1963). Flies appeared to metabolize carbaryl through an initial hydroxylation and form an unstable intermediate from p-nitrophenyl dimethylcarbamate which may be the N-methyl derivative of the compound (Hodgson and Casida, 1960, 1961). In no case the complete metabolic
pathway of a carbamate insecticide in an insect has been elucidated (Baron, 1991).

1.2.1.4 Biochemical effects of car bamates

Bordy et al. (1983) found that at 24 hr post dosing with methmoyl, a carbamate, at 40 mg/kg/day by oral intubation, caused significant increase in serum alkaline phosphatase, glutamate oxaloacetae transaminase, serum triglycerides, phospholipids, free fatty acids and cholesterol in rats. A single oral 500 mg/kg dose of carbaryl or seven doses of 71 mg/kg/day increased the activities of acid phosphatase, aspartate amino transferase (ASAT) and alanine amino transferase (ALAT) in the liver and kidney but did not affect the activities of alkaline phosphatase, lactate dehydrogenase or succinate dehydrogenase (Kiran et al., 1985).

Acid and alkaline phosphatases activities increased significantly after pyrethrum treatment in both brain and ventral nerve cord with ganglia whereas acetylcholine esterase decreased rapidly in newly emerged Schizodactylus monstrous (Banerjee et al., 1984). Activity levels of proteases were significantly elevated due to sub-lethal dose of carbaryl in bliser beetle, Mylabris pustulata (Bharathi and Govindappa, 1985 a). In beetles exposed with carbaryl, there was depletion in the levels of all the four nitrogenous end products in the malpighian tubules such as free ammonia, glutamine, uric acid and urea, indicating that the tubules were actively extracting the excretory
products from blood into the pellets (Bharathi and Govindappa, 1985 b). The activities of ALAT and ASAT were markedly elevated in the hemolymph. These changes were similar to those under stress conditions (Bharathi and Govindappa, 1985 c). After exposure all digestive enzymes were inhibited in the foregut but activated in the midgut after the exposure. The effects of short term exposure to the pesticide were reversible (Bharathi and Govindappa, 1986).

Excessive utilization of lipids occurs under toxic impact of insecticides like carbaryl and lindane in the scorpion, *Heterometrus fulvipus* (Rajyalakshmi and Reddy, 1991). Blister beetles exhibited lipid oriented metabolic pattern during pesticide exposure. There was a significant depletion of total lipid occurred both during short-term and prolonged exposure to sub-lethal dose of carbaryl (Bharathi and Govindappa, 1985 d). An elevation in the levels of ASAT and ALAT was observed in maternal and embryonic tissues of *H. fulvipus*, with carbaryl and lindane treatment after 48 hrs. Reports are available regarding the toxic effects of pesticides such as organophosphates, organochlorine and carbamate groups on the physiology and biochemistry of some fresh water fishes (Webb and Brett, 1973; Arunachalam *et al.*, 1980; 1985; Palanichamy *et al.*, 1986; Vasanthi and Ramaswamy, 1987).
1.2.1.5 Toxicity of carbaryl

1.2.1.5.1 Acute toxicity of carbaryl

Exposure to a single high concentration is likely to elicit an immediate (acute) response that is qualitatively different from repeated exposure (chronic) to the same chemical at much lower concentrations, for example, a single exposure to 1 mg of carbaryl to a rat will result in the killing of large number of liver cells and death due to liver failure within 5-6 days; exposure to a few nanograms of carbaryl for several months does not kill liver cells but does result in liver cancer (Shank, 2004).

The acute toxicity, expressed as the LD$_{50}$ varies considerably according to species, formulations and vehicles. The oral LD$_{50}$ of carbaryl to rat ranges from 200 to 850 mg/kg and from 100 mg/kg to 650 mg/kg in mice. Cats are sensitive to carbaryl with an LD$_{50}$ of 150 mg/kg, whilst pigs and monkeys are less susceptible having an LD$_{50}$ greater than 1000 mg/kg. Based on the LC$_{50}$ values assessed in the laboratory studies to banana rhizome weevil showed that carbaryl was found to be least toxic than aldicarb, carbofuran and phorate in increasing order (Visalakshi et al., 1986). The acute toxicity of carbaryl to rat of both sexes was $> 500$ mg/kg (LD$_{50}$). The symptoms of acute intoxication are typical of acetylcholinesterase inhibition. The acute oral, dermal and inhalation exposure of rats and rabbits to carbaryl at doses ranging from 450 to 1500 mg/kg resulted in transient
acetylcholinesterase inhibition in the brain, plasma and erythrocytes ranging from 30% to greater than 65%. At the higher doses, other blood parameters were also affected (Mount et al., 1981; Kossakowski and Lysek, 1982).

Bostanian et al. (2000) used carbaryl to manage aphids, maggots and leaf cutting caterpillars in the apple orchard. Ahmad et al. (2002) revealed that carbaryl has much intrinsic toxicity against the oblique-banded leaf roller, *Christoneura rosaceana* than the eighteen other insecticides used and exhibited a very low level of resistance against it. The experiments on insecticide susceptibilities of cat fleas showed that, of the eleven strains tested only two field strains developed tolerance against carbaryl. Sean et al. (1982) showed that technical grade carbaryl was toxic to clerids only at high doses (LD$_{50}$=287 μg/g of body weight and LD$_{90}$=2242.1 μg/g of body weight) by topical application. Carbaryl at 2 pounds of toxicant per acre gave evidence of a lasting residual control of cotton leaf predator (*Bucculatric thurberiella*), salt marsh caterpillar (*Estigmene acrea*), and cotton bollworm larvae (Shorey et al., 1962). A 5% sevin provided effective control of the corn earworm, *Heliothis zea* (Shorey et al., 1962).

Ball and Su (1979) studied the toxicity of carbaryl to female *Diabrotica virgifera* by topical application. Lawrence et al. (1973) reported the topical treatment of carbaryl on *Chrysopa rufilabris* on duration of larval, pupal and adult survival and 50% WP of carbaryl gave the lowest percent of
survival and had unfavourable effects on \textit{C. ruflabris}. Visalakshi \textit{et al.} (1986) showed that carbaryl controls rice swarming caterpillar, \textit{Spodoptera mauritia}. The insecticide toxicity on the diamond black moth described a significantly high larval mortality with carbaryl after 72 hrs of treatment (Hill and Foster, 2000). Effect of carbaryl on eggs, larvae and adults of the green lace-wing, \textit{Chrysopa scelester}, was studied by Krishnamoorthy (1985) and found that at 0.10\% caused 100\% adult mortality. He has also observed that carbaryl is highly toxic to first instar larvae and adults but low to medium toxic to second and third instar larvae of the green lace-wing. Lecrone and Simlonitz (1980) studied the toxicity of carbaryl to green peach aphid, \textit{Myzus persicae}, and \textit{Coleomegilla maculata} and \textit{Chrysopa oculata}. Toxicity of carbaryl insecticide to \textit{Amblyscius fallacis} and \textit{Typhlodromus pyri} was studied by Watve and Leink (1976) and found that \textit{A. fallacis} was extremely susceptible to carbaryl while \textit{T. pyri} was highly tolerant. The recommended field rate/100 gal water of carbaryl is 2.016 kg and the \textit{LC}_{50} value for \textit{A. fallacis} is 0.2 kg. Residual toxicity of carbaryl was observed in first instar larvae of spotted bollworm and was found to be most persistent and effective which gave 37.93\% mortality on the 15\textsuperscript{th} day after treatment (Patil and Pokharkar, 1977). Carbaryl provides extensive protection of \textit{Ponderosa} pine trees against western pine beetle attack (Smith \textit{et al.} 1977). Tsai and You (1962) obtained excellent control of spotted bollworm with 0.1\% carbaryl. Unequal response of Douglass fire tussock moth, \textit{Orgyia pseudotsugata} was found among four
populations to the pesticide carbaryl and the range of $LD_{50}$ value was 14.1 to 172.0 $\mu$g/g body weight (Stock, 1979).

1.2.1.5.2 Chronic toxicity of carbaryl

The toxicity of carbaryl to common prawn (Palaemon serratus) was studied for 29 days in the adults and the induction thresholds for inhibitory effects of acetylcholinesterase were determined (Bocquene et al., 1991). Carbaryl with sub-lethal dose showed some effects on protein metabolism of fresh water fish (Rao et al., 1987; Reddy and Bashamohideen, 1987). Rath and Mishra (1980) have reported a reduction in protein content in Tilapia mossambica with chronic exposure to pesticide media. The protein content was reduced in Oreochromis mossambicus, Mystus vittatus and Channa striatus which were reared at carbofuran media for different duration of exposure (Palanichamy et al., 1989). The acid and alkaline protease activities were increased in muscle, liver gill and intestine of M. vittatus (Palanichamy et al., 1989). There was significant inhibition of acetylcholinesterase activity observed in the selected tissues of Metapenaeus monoceros after chronic exposure (Reddy et al., 1990). The residual toxicity studies of some of the commonly used insecticides in the first instar larvae of spotted bollworm revealed that out of the insecticides tested, carbaryl was observed to be the most persistent and effective that gave 37.93% mortality on the 15th day after treatment (Patil and Pokharkar, 1977). In female western corn rootworm, sub-
lethal dosage of carbaryl stimulated oviposition and extended longevity (Ball and Su, 1979).

1.2.1.6 Effects of carbaryl on non-target organisms

Carbaryl is classified by the World Health Organisation as moderately hazardous (WHO, 1992). The toxicity varies considerably according to species and formulations. The mice, rats and cats were very sensitive with a low range of carbaryl. The pigs and monkeys were less susceptible to carbaryl. Toxic effect of carbamide groups of pesticides on the fresh water fishes were reviewed (Webb and Brett, 1973; Arunachalam et al., 1985; Palanichamy et al., 1986). The toxicity of carbaryl in adult prawn, *P. serratus*, was studied in the adults and it inhibits acetylcholinesterase (Bocquene et al., 1991). Carbaryl was toxic to *Macrobrachium malcolmsonii* (Bhavan and Geraldine, 2002). Toxic effect of carbaryl in the respiratory movements of an air breathing fish, *C. striatus* exposed to sevin was also studied (Anbu and Ramaswamy, 1991). Carbaryl is very highly toxic to shrimp, crab and oysters (EPA, 2002).

According to EPA report carbaryl can range from highly to slightly toxic to freshwater fish on an acute basis and is moderately toxic to ocean and estuary fish. Salmon, trout, and perch are the most sensitive species and are killed by concentrations between 250 and 970 ppb (EPA, 2002). Carbaryl is used in grasshopper baits, might cause harm to the small mammals who share
grasshopper habitat and carbaryl exposed mice ran more slowly and were more apt to cannibalize their offspring than unexposed mice (Punzo, 2003). Relyea and Mills (2001) studied the interactions between carbaryl predatory salamanders and tree frog tadpoles and showed 60% mortality of tadpole exposed to 50 ppb and adding a second stress to the tadpoles, carbaryl induced mortality to 97%. The toxic concentration of carbaryl at 50 ppb harmed the survival of tadpoles (Rohr et al., 2003).

Carbaryl fits into integrated pest management programs, because it is relatively non toxic to coleopterous predators *Enoclerus lecontei* and *Enoclerus sphengeus* using residual film and topical application method but highly toxic to western pine beetle *Dendrotonus brevicornis* (Sean et al., 1982; Robertson and Gillette, 1978; Greene, 1983). The toxicity of chemical insecticides to parasitoids and predators at reduced dosages in increasing order of toxicity was malathion < carbaryl < toxaphene < methyl parathion (Wilkinson et al., 1975). The estimated LC$_{50}$ (AI g/Acre (0.405 hectar) of carbaryl for 8 species of parasitoids and predators are 18.2, 286.0, 54.5, 18.2, 376.8, 13.6, 68.1, 1362.0 for *Compoletis sonorensis*, *Chelonus blackburni*, *Brachymeria intermedia*, *Meteorus leviventris*, *Varia ruralis*, *Hippodamia convergens*, *Chrysopa carnea* and *C. carnea* larvae respectively (Wilkinson et al., 1975).
The broad spectrum of effectiveness of carbaryl against many agricultural pests has been recognized for several years and has been confirmed by the results of Shorey et al. (1962). Carbaryl is also lethal to many non-target insects, including bees and beneficial insects (Kidd and James, 1991). EPA's databases show that numerous bee kill incidents also have been reported in several states (EPA, 2003). With regard to beneficial insects, the insecticide used reduced populations of parasitic Hymenoptera. Carbaryl was known to be highly toxic for honey bees. When ingested, LD$_{50}$ of carbaryl was found to be 0.18 µg/ bee (Alvarez et al., 1970) and the contact LD$_{50}$ for carbaryl in adult honey bee was 1.3 µg/ bee (Stevenson, 1978).

1.2.1.7 Biochemical effects of carbaryl

1.2.1.7.1 Effects on protein

The acute oral administration of carabaryl to rats at doses ranging from 50 to about 500 mg/kg affected the levels of a variety of enzymes, amino acids, neurotransmitters of other substances in the blood and brain (Baron, 1991). Effects reported include a decrease in serum protein levels, blood free amino acids and brain acetylcholinesterase concentrations and changes in free amino acids metabolism in the liver and brain. Rath and Mishra (1980) have reported a reduction in protein content in *T. mossambica* when exposed to pesticide media. Effects of carbaryl on protein metabolism in some freshwater
fishes have been reported (Rao et al., 1987; Reddy and Bashamohideen, 1987; Palanichamy et al., 1989)

A decrease in cellular protein in the Hela cells was noted (Blevins and Dunn, 1975). Carbaryl showed considerable protein-binding ability in cultured human embryonic lung cells (Murakami and Fukami, 1983). Miller et al. (1979) demonstrated that carbon derived from carbaryl binds to microsomal proteins. Human serum albumin reacted in vitro with the ester group of carbaryl and catalyzed the hydrolysis and liberation of 1-naphthol. This reaction is similar to an "esterase type" action (Casida and Augustinsson, 1959) called carbamylation. Enzyme mediated binding of carbaryl to rat hepatic microsomal protein occurred in vitro in the presence of NADPH and oxygen (Neskovic et al., 1978). In a protein binding study with rats, carbaryl in the serum was bound primarily to albumin and partly to globulin and lipoprotein in the cytosol fraction of the intestinal mucosa (Tanaka et al., 1981). Carbaryl inhibited the synthesis of DNA, RNA and proteins in cultured rat and human embryonic lung cells (Lockard et al., 1982; Murakami and Fukami, 1983). The level of proteins showed an initial increase followed by a drastic decline in late larval stages, of the midgut tissue of S. mauritia with carbaryl administration (Nair, 1995).
1.2.1.7.2 Effects on amino acids

The sub-chronic oral administration of carbaryl to rats at 95 mg/kg/day for 30 days slightly decreased erythrocyte alanine levels (Jeleniewicz and Szczepaniak, 1980). Effect of carbaryl on tryptophan metabolism in rats was studied by Ashraf et al. (1990). Carbaryl administered to rats for 3 months at a daily intragastric dose of 60 mg/kg/day decreased the levels of tryptophan in the blood and decrease in amino acid concentration in the brain tissue and liver which later became normalized in both the tissues (Podolak-Majczak and Tyburezyk, 1984).

The radiolabelled metabolic products of carbaryl were covalently bound to amino acid residues of microsomal proteins that accounts to 99.2-99.7% of the bound radioactivity (Miller et al., 1979). Carbaryl binds to free amino acids of the blood (IPCS, 1994). Boyd and Boulanger (1968) reported an increased susceptibility to carbaryl toxicity in Albino rats fed a protein-deficient diet. An increase in the ratio of amino acid nitrogen to creatinine in the urine after carbaryl treatment may represent a decrease in the ability of the proximal convoluted tubule to reabsorb the amino acids (Knaak et al., 1968). Carbaryl inhibited the incorporation of 3H-uridine and 14C-labelled aminoacids into RNA and proteins in cultures of Hela cells and the effect was dose dependent (Myhr, 1973). Human serum albumin reacted in vitro with the
ester group of carbaryl. There was a temporary reduction in the ability to reabsorb amino acids at the highest dose (Wills *et al.*., 1968).

**1.2.1.7.3 Effects on glucose**

Disturbances have been reported in the carbohydrate metabolism and protein synthesis and detoxification function of the liver in mammals. Carbaryl is a weak inducer of hepatic microsomal drug-metabolizing activity (WHO, 1994). A single application of carbaryl at 30 mg/rat produced transient hypoglycemia at 20 hr followed by hyperglycemia at 44 hr and carbaryl inhibited lactate gluconeogenesis, and to some extent, gluconeogenesis from fructose pyruvate and alanine. Glycerol glucogenesis was unaffected (Orzel and Weiss, 1966). Intraperitoneal doses of carbaryl as low as 5 mg/kg produced a hyperglycemic response in intact or hypophysectomized rats. Hyperglycemic responses have also been reported in rabbits and dogs administered with carbaryl (Weiss *et al.*, 1964; 1965). Orzel and Weiss (1966) found a rise in blood glucose correlated with the onset and duration of tremors and the degree of brain ChE inhibition in rats that were treated intraperitoneal with 5 and 25 mg carbaryl/kg. The authors suggested the hyperglycemic effect was due to increased secretion of epinephrine. Hyperglycemia is thought to result from cholinergic stimulations as it is found in acute intoxications with organophosphorous compounds (Kaloyanova, 1963). In isolated rat hepatocytes, carbaryl was reported to inhibit
gluconeogenesis, reduce lactate dehydrogenase and aspartate aminotransferase activities, and enhance glucose-6-PO$_4$ activity (Parafita and Otero, 1983, 1984 a, b). The carbohydrates of midgut tissue of S. mauritia showed an initial increase in the first few days of carbaryl administration followed by a drastic decline in late larval stages (Nair, 1995).

1.2.1.8 Effects of carbaryl on organ systems

Carbaryl in the adrenals of rat induced the histopathological alternations (Baronia et al., 1992). Carbaryl has been established to be a neurotoxicant and effective poisonous chemical when added into mammalian body (Padilla and Hooper, 1992; Takahashi et al., 1994). Administration of the maximum tolerated dosage of carbaryl to mice for about 18 months did not increase the incidence of tumors (Innes et al., 1969). Carbaryl in the diet of rats at 400 ppm for 2 years did not affect the incidence of tumors (Carpenter and Livestone, 1961). Triolo et al. (1982) reported that carbaryl at 1000 ppm in the diet of mice for 20 weeks did not cause tumors. The dietary exposure of 20 ppm carbaryl to chicken for three months suppresses the immunity (Singh et al., 2007).

Marked vacuolation of the epithelium of the proximal tubules of kidney of rats and monkeys receiving very large doses of carbaryl were reported by Serrone et al. (1966). A 30-day exposure of rats to carbaryl at 10 ppm in their drinking water produced treatment related liver histopathology.
and slight decrease in platelet count and activity of clotting factor VII (Lox, 1984).

The effects of carbaryl on the nervous system are primarily related to cholinesterase inhibition and are usually transitory. The effects on the central nervous system were studied in rats and monkeys. In a small study on pigs, carbaryl was reported to produce a number of neuromuscular effects (150 mg/kg body weight in the diet for 72-82 days). No evidence of demyelination was observed in the brain, sciatic nerve, or in spinal cord sections examined microscopically. Carbaryl has been reported to effect coagulation. There have been no reports of confirmed induction of mitotic recombination and gene conversion in prokaryotes and eukaryotes in vitro. Negative results were obtained in tests for gene mutations and chromosome damages. Chromosomal damage with high dosage of carbaryl has been reported in vitro in human, rat and hamster cells. Carbaryl has been shown to induce disturbance in the spindle fibre mechanism in mammalian cells in vitro (IPCS, 1994). Delescluse et al. (2001) suggested that carbaryl provoked strong DNA-damaging activity in the human lymphoblastoid cell line. Carbaryl has special toxicity to somatic and germ cells in animals (Siboulet et al., 1984; Pant et al., 1995; 1996), however, others reported the contrary results (Osterloh et al., 1983; Bigot-Lasserre et al., 2003).
Effects of carbaryl on endocrine system

It is known that pesticides can cause certain type of cancers, birth defects, sterility problems, genetic mutations and behavioral changes. In recent years researchers have also begun to investigate the effects of pesticides on the human endocrine system because of the evidence that some pesticides may be responsible for altering the gender of species. Hormones are chemical messengers that regulate all biological processes in animals. These processes include blood sugar levels, growth and function of the reproductive system, and the development of the brain and nervous system (EPA, 2005). Hormones are mostly produced by endocrine glands like the ovaries, testes, pituitary gland and thyroid gland. Carbaryl's ability to disrupt hormones and the endocrine system was first demonstrated only a decade after its marketing began when researchers from the USSR Academy of Medical Sciences described its effect on the endocrine glands of rats (Shtenberg and Rybakova, 1968). Researchers from Tulane University showed that carbaryl inhibited the activity of two sex hormones, estradiol and progesterone, in human cells (Klotz et al., 1997). Carbaryl was identified as an endocrine disruptor (EPA, 2005). Chemicals that interfere with the hypothalamic-pituitary-testicularaxis have dramatic impact on male reproduction (Shank, 2004). Inhibitors of testosterone synthesis and inducers of phase I metabolism to accelerate the removal of circulating testosterone (e.g., several organochlorine compounds) decrease male fertility. Genotoxic
compounds interfere with normal meiosis by damaging DNA and chromosomes and inhibiting nucleic acid synthesis; mutations in the DNA and abnormal chromosomes can generate incompetent sperm or no sperm at all. Reproductive functions in both the male and female are under hypothalamic-pituitary control (Klassen, 2001).

The effect of carbaryl on the neuroendocrine system was studied in rats. Spermatozoon motility was reduced progressively with the duration of the exposure of carbaryl with 70 mg/kg/day. The histochemical studies of hypophysis showed changes indicative of an increase in the activity of the cells producing a luteinizing gonadotrophy, i.e., an increase in the size of the cells, loss of granules, and hyalinization of the cytoplasm. Histological examination of the adrenal glands revealed an increase in the size of mitotic activity of cells in the zone glomerulosa. Cells with large nucleus or two nuclei were present in the fascicular zone. It is likely that the effects of carbaryl on the reproductive organs are mediated by the endocrine glands (Rybakova, 1966; Shtenberg and Rybakova, 1968; Shtenberg et al., 1970).

1.2.1.9 Effects on reproduction

The toxic effects of carbaryl related to reproductive toxicology (Schrag and Dixon, 1985; Baranski, 1993; Savitz et al., 1997; Juhler et al., 1999; Xia et al., 2005) and genetic toxicology (Grover et al., 1989; Renglin et al., 1999; Delescluse et al., 2001) have also been extensively investigated. With the
exception of a small number of studies (Ball and Su, 1979; Martin, 1982; Osterloh et al., 1983), all adverse reproductive and developmental effects were noted. Kazarnovskaya and Vasilos (1977) had shown that carbaryl suppressed mitosis, changed the rate of the mitotic phase, and significantly increased the number of pathological forms of mitosis in a human embryonic fibroblast culture and exerted a pronounced chromosome breaking effect, at a concentration of 100 μmol/litre, 26 hr and 50 hr after treatment. The cytogenetic effects induced included mostly metaphase and anaphase fragments and anaphase bridges are time-dependent (Wuu and Grant, 1966).

In a study with 101 non-exposed men with 49 men currently or previously employed in carbaryl production, no relationship was found between the intensity or duration of exposure and either sperm count (Whorton et al., 1979). On reexamining the sperm samples from this study and comparing them against a new control group of 34 non-exposed workers in the same plant, Wyrobek et al. (1981) reported morphological abnormalities in the sperm from the exposed workers, not related to estimated exposure levels. Another evaluation of these same sperm samples showed no differences in sperm count or morphology between exposed and control groups (Mac-Leod, 1982). Reviews of the reproductive effects of carbaryl (Weil et al., 1972; Kuhr and Dorough, 1976; Crammer, 1986) note frequent reports of reproductive injury by even small doses of carbaryl. Many of these
effects have not been verified in other laboratories. In these studies, rats were exposed to carbaryl by daily pre oral intubation at dosages ranging from 2-30 mg/kg/day and for periods ranging from 1 to 12 months; one study assessed reproductive effects over 5 generations. Effects reported at dosages as low as 2 mg/kg/day were functional and focal histological changes in the testes (including reduced motility and survival time of sperms), and increased hypophyseal secretion of gonadotropic hormones. Reproductive effects reported for dosages as low as 2 mg/kg/day were decreased fertility in both sexes, increases in still births and pup mortality, and delayed pup development (Rybakova, 1967, 1968; Vashakidze, 1967, 1975). Trifonova (1984) reported reduced ovarian function at carbaryl dosages of 40-80 mg/kg/day but not at 20 mg/kg/day. Vashakidze (1965) reported teratogenicity and decreased reproduction at sub-chronic intubated dosages of 100 mg/kg/day and higher; but not at 50 mg/kg/day.

The oral administration of carbaryl to male rats at 200 mg/kg/day on 3 days a week for 90 days showed no clinical signs, effects on fertility, or histopathological changes in the testes, liver, kidney or brain (Dikshith et al., 1976). Kitagawa et al. (1977) reported reduced sperm count with carbaryl per week orally for 1 year in rats. Carbaryl given to mice at 34 mg/kg/day for 5 days did not affect either the weight of the testes and sex glands or the ability of the prostate to assimilate and metabolize testosterone (Dieringer and Thomas, 1974; Thomas et al., 1974). In three-generation reproduction studies
with rats, carbaryl in the diet at 10,000 ppm (about 500 mg/kg/day, maternally toxic dosage) reduced fertility (Baron, 1991).

1.3 Studies on the hemolymph of insects

One of the interesting features of insect hemolymph is that it contains very large amounts of free amino acids, much more than in the body fluids of other animals (Chen, 1962). These amino acids contribute to the osmotic value of hemolymph and account for a substantial portion of the cations and anions of hemolymph (Tembhare, 1997; Nation, 2002). The subject has been reviewed by Auclair (1953); Buck (1953); Bheemeswar (1959); Wyatt (1961); Chen (1962, 1966); Chefurka (1965); Jeuniaux (1971) and Florkin and Jeuniaux (1974). About 35-65% of non-protein nitrogen of hemolymph represents the amino acids. The free amino acid content of the blood of insects is strikingly high in comparison with that of other animals. All the known amino acids have been demonstrated in hemolymph of various insect-species (Srivastava and Auclair, 1975; Febvay et al, 1995; Sasaki and Ishikawa, 1995; Sanstorm and Moran, 2001; Calatayud et al., 2002). About 35-60% of non-protein nitrogen of hemolymph represents the amino acids (Tembhare, 1997).

The various aspects of the hemolymph proteins in insects have been studied by many investigators (Price, 1973; Chen, 1978; Wyatt and Pan, 1978; Gope and Prasad, 1980). It has established that the protein
concentration of insect hemolymph is generally higher than that of the internal fluids of other invertebrates and is almost similar to that of the blood of man (Florkin and Jeuniaux, 1974). The total hemolymph proteins occur within the range of 1 to 20 mg/100 ml (Tembhare, 1997). The presence of an open circulatory system in insects means that hemolymph percolates around and through the fat body lobes and the adipocytes that are arranged for maximum exposure to the circulatory fluid. The 10 amino acids considered essential for insects in general: arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine (Dadd, 1985). Quite a number of amino acids in the hemolymph such as alanine, aspartic acid, glutamine and tyrosine play an important role in the synthesis of chitin, polyphenols and other important constituents of the cuticle (Dhillon and Sidhu, 1977).

1.4 Studies on phosphatases

Acid phosphatases (ACPH) are considered as marker enzymes of lysosomal activity and have great significance in biochemical studies. Acid phosphatases have gained importance as clinical diagnostical tools in the detection of gynaecological conditions, metastasizing prostate cancer, bone conditions including rheumatic osteoblastoma, bone cancer, osteogenesis imperfecta, liver diseases such as Goucher's disease, hyperparathyroidism and chronic renal failure (Nakasato et al., 1999; Macejewski et al., 2001).
During massive destruction of tissues, lysosomal activity is an important factor (Van Prett-Verkuil, 1978). The histolysis represents a programmed cell death, a hormonally induced and neurologically activated cytolytic mechanism. These phosphatases catalyse the hydrolysis of phosphoric acid esters. Of the phosphoric acid esters, phosphomonoesters and phosphodiesters are of importance as constituents of cells. Day (1949) studied distribution of alkaline phosphatase histochemically in different regions of digestive tract of a number of insects. Functional significance of alkaline phosphatase was studied first by Moog (1946). Its localization in plasma membrane perhaps played an important role in transport of phosphate through cellular membranes. Hardonk and Koudstaal (1976) reported that this activity is facilitated by phosphatase or phosphotransferase action. Pearse (1961) and Srivastava (1966) studied that high activity of alkaline phosphatase indicated increased phosphate transfer from one alcohol to the other. Ide and Fischman (1969) and Farquhar et al., (1974) suggested that the lysosomal enzymes undergo metabolic transformation in vivo, resulting in change of substrate specificity. Hiromu (1969) reported that this enzyme helps in metabolism and transphosphorylation. The pivotal studies of de Duve and colleagues led to the characterization of lysosomes as the membrane-limited sub-cellular organelles which contain acid hydrolases (de Duve and Wattiaux, 1966; de Duve, 1970; Bainton, 1981).
Beel and Feir (1977) studied the changes in acid phosphatase activity in the testes and hemolymph of the 5th instar male *Oncopeltus fasciatus* at various time intervals and found that the activity of ACPH was higher in the hemolymph. In *Drosophila melanogaster*, the ACPH is present in higher levels during embryogenesis (Yasbin *et al.*, 1978). Tissues having energy requirements need a readily available source of phosphate which is provided by acid phosphatase (Blum, 1970). Under physiologic conditions the enzyme acid phosphatase may be involved specifically in the dephosphorylation of naturally and physiologically occurring phosphate esters. Acid phosphatase plays an important role during stress condition. It releases inorganic phosphorus to the system and helps to maintain the metabolic activity. The lysosomes function *in situ* as scavenging organelles and help in degradation of macromolecules of cellular origin and from invading microorganisms (Bainton, 1981).

### 1.5 Histomorphology of the male reproductive system of insects

The reproductive system of the male insect has been studied in varying degrees of detail in many orders (Phillips, 1970; Roosen-Runge, 1977; Muse, 2002; Alves, 2006) including heteropteran (Ambika, 1973; Dorn *et al.*, 1992; Lemos *et al.*, 2005). The reproductive system of the male insect consists of testes, vas deferens, accessory glands and an ejaculatory duct (Tembhare, 1997; Klowden, 2002). All parts of the system may produce secretions that
aid the transfer of sperm to the female (Happ, 1992). Each testis generally consists of a number of tubes or follicles in which spermatozoa are matured (Nation, 2002). The follicles bound together by a peritoneal coat. Each tubule opens via a vas efferens into the mesodermal sperm duct or vas deferens, and the paired vasa deferentia unite to connect with the ectodermally derived ejaculatory duct which terminates in the gonopore. The vas deferens is usually expanded over part of its length to form a seminal vesicle or sperm storage organ. The testis consists of series of parallel tubes or follicles that empty into a vas deferens (Roosen-Runge, 1977). The zones of maturation stages of sperm exist along the length of a typical follicle. In the ‘Growth Zone’ (Zone I), the spermatogonia divide by mitosis into many diploid spermatocytes within a sac or cyst which generally arise from the same spermatogonial cell and their development is synchronized. The spermatocytes may undergo more mitotic divisions; there are five to eight divisions in Acrididae and seven in Melanoplus; but eventually in the ‘Zone of Maturation’ (Zone II), meiosis and haploid spermatids are produced (Klowden, 2002). Normally, four sperm are produced from each spermatocyte. In Zone III, the ‘Region of Transformation’, the mature sperm develop. Usually, the mature sperm remain bundled together in Zone III (Jamieson et al., 1999; Nation, 2002). The testis follicles of sexually mature males are full of cysts, the spermatocysts, within which spermatogenesis proceeds. A cyst is a group of germ cells surrounded by an epithelium. The proximally situated cysts contain
spermatogonia or spermatozoa. The late gonial and meiotic divisions are incomplete and the daughter cells remain connected by cytoplasmic bridges. The cells of a cyst hence form a functional syncytium. They divide in synchrony and develop as clones; the number of spermatids per cyst will thus generally be equal to an integral power of 2, for instance $2^5$, $2^6$, $2^7$, or $2^8$, depending on the species (Jamieson et al., 1999). Rasmussen (1973) found some exception to such a synchrony. The interconnected spermatids within a cyst remain aligned side by side throughout spermatogenesis, so that a transverse section through the cyst cuts all cells at the same level (Phillips, 1974). Apical cells are known to be larval structures, gradually disappearing in older animals (Carson, 1945; Menon, 1969). Numerous spermatogonia were already well packed in the terminal portion and they grew and differentiated into spermatocytes and spermatids as early as the late 4th instar. The number of all these members increases with the growth of the animal. In most insects spermatogonia and spermatocytes were developed in the pupal and nymphal stages, and the testes of the imago contain only spermatids and spermatozoa (Roosen-Runge, 1977). In some water beetles, the two testes are long and coiled single tubes (Jamieson et al., 1999). In Diptera, the testes consist of a simple, elongated and undivided sac (French and Hoopingarner, 1965). Apical cell complex has been observed in the heteropteran Oncopeltus (Bonhag and Wick, 1953; Economopoulos and Gordon, 1971). A remarkable, perhaps unique, feature of insect spermatogenesis is the association of
primary spermatogonia with a large cell or cellular complex at the apex of the testis or of each follicle, the so-called apical cell (Roosen-Runge, 1977). It is presumably derived from the primordial germ cell, but does not give rise to spermatogonia. The early spermatogonia have cytoplasmic extensions connecting them to the apical cell, as if receiving some kind of signal substance from that cell. The apical cell of this type is not found in Protura (Berlese, 1910), which is of interest as this animal group may not be closely related to the true insects (Roosen-Runge, 1977). The processes of spermatogenesis and spermiogenesis provide typical examples of the profound morphological changes that occur during terminal differentiation of specialized cells (Phillips, 1974). Deb et al. (1983) recorded the follicle of testes contains a succession of zones, in Chrysocris stollii (Pentatomidae). Bhalerao et al. (1991) showed that first zone of testis contained spermatogonia which have distinct nuclei. The second zone has spermatocytes. In the last zone spermatozoa are present in bundles. Sperms of 6.64 mm long (Yanders and Perras, 1960) and more than 10 mm long (Joly and Bressac, 1994) have been reported for Drosophila hydei.

In many cases, accessory glands are formed as diverticula from the vas deferens (Tembhare, 1997). In other insects the mesodermally derived ducts are themselves glandular (Jamieson et al., 1999). The male accessory genital glands of insects may be ectodermal or mesodermal in origin, known as ectadenia and mesadenia, respectively (Tembhare, 1997). The number and
arrangement of the accessory glands varies considerably between the different groups of insects (Nation, 2002). Each accessory gland consists of a single layer of epithelial cells, the fine structure of which depends on their stage of development and the nature of the secretion produced (Chapman, 1998).

The morphological and histochemical studies on the spermatogenesis in the bug, *Halys parvus* (pentatomidae: Homoptera) were carried out in detail (Sareen and Kaur, 1987). Histomorphological observations on the spermatogenesis in the normal and the transplanted testis of the *Dysdercus cingulatus* (Heteroptera) were also studied (Ambika, 1973). Srivastava et al. (1985) studied the histopathological effects of X-irradiation on the testes of the *Dysdercus koenigii*. The testicular degeneration in *D. Koenigii* after microwave exposure has been studied (Bhalerao et al., 1991). Rajendra et al. (2001) reported the male sterility associated with the over expression of noncoding of *hsro* gene in cyst cells of testes of *D. melanogaster*.

1.5.1 Ultrastructure of testes

The wall of the testes of insect has three layers. The outermost is the peritoneal coat, followed by a middle muscle fibre supporting the inner most coat of epithelial cells. A number of mitochondria, endoplasmic reticulum and some ribosomes are seen in the peritoneal cells (Tembhare, 1997; Klowden, 2002). The muscle fibers are usually single cell in thickness but at some
places even double cells are seen. There are a few mitochondrial bodies in them.

1.5.2 Ultrastructure of sperm

Insect spermatozoa have been described at ultrastructural level (Baccetti, 1972). Diversity among insect spermatozoa is seemingly endless. The length of the spermatozoon can vary from 1.7 μm as in the termite *Reticulitermes lucifugus* (Baccetti *et al.*, 1981), to 58,000 μm as in *Drosophila bifurcata* (approximately 20 times the length of the male producing it) (Pitnick *et al.*, 1995). A great diversity of shapes exists in insect sperms and any of the four main constituents (nucleus, acrosome, mitochondria and flagellum) may be lacking or be the dominant one (Jamieson *et al.*, 1999).

**Heteropteran sperm**

The investigation of spermatozoal ultrastructure in eight families in four of the seven infra orders of the Heteroptera (classification of Stys and Kerzhner, 1975), Dallai and Afzelius (1982) considered the four characters for heteropteran sperm such as the presence of two or three crystals within each of the two mitochondrial derivatives, (rather than a single one), presence of bridges between the two mitochondrial derivatives and axonemal microtubules 1 and 5, the absence of longitudinal accessory bodies and a
prominent Zipper-line along the sperm tail to be unique synapomorphies (autapomorphies) for the Heteroptera, within a framework of many variations, and have confirmed the taxon-specific nature of sperm morphology. The crystalline material in heteropteran mitochondrial derivatives has a "fish bone" pattern in longitudinal sections; the patterns have been elucidated by Rosati et al. (1976) and Baccetti et al. (1977).

The spermiogenesis in most insect means an elongation of the entire cell and not least of the nucleus. This elongation is accompanied by, perhaps caused by, the appearance of microtubules encircling the acrosome and the nucleus and collectively named the manchette (Kessel, 1966, 1970). It is found not only in insects but in most animal spermatozoa. Fawcett et al. (1971) are of the opinion that the shape of the sperm head is probably not the consequence of external modelling by pressures applied to the condensing spermatid nucleus by microtubules in the perikaryal cytoplasm but may be largely determined from within by a specific genetically controlled pattern of aggregation of the molecular subunits of DNA and protein during condensation of the chromatin.

The sperm tails are usually free and beat in a coordinated fashion. Large and small spermatozoa are also seen in pentatomid bugs, such as Murgantia histrionica and Rhaphigaster sp. (Bowen, 1920). In the sperm of the fire-bug, Pyrrhocoris apterus (Pyrrhocoridae), the derivative-axonemal
bridges have usually large end-feet which are curved and solid (Jamieson, et al., 1999). In *Lygaeus equestris* (Lygaeidae) the derivatives are irregular in outline and contact each other at one point only. Rosati et al. (1976) have described the mitochondrial derivatives of *G. lineatum italicum* and *Nezara viridula smarogdula*. They have remarkably uniform derivatives, regarded as partially crystallized. Their position is, however, different in *G. lineatum* where the derivatives arise close together in the centriole region whilst in *N. smarogdula* they arise one behind the other from a small cavity in the nucleus. The inner region is filled with structural material visible in and between the coils. It appears like a series of parallel lines, evidently corresponding with 30 Å thick longitudinally arranged globular filaments that in some regions become elements of a honeycomb network (Jamieson et al., 1999). The classical form of the insectan axoneme was established by Phillips (1966), chiefly for the scavenger fly *Sepsis*. Baccetti (1972) found that all ptergote insects have 9+9+2 axoneme except for Ephemeroptera. The paracrystalline material often has a herringbone pattern in longitudinal section as in the Diptera, Homoptera, Odonata, Dermaptera, Psocoptera, Hemiptera, Neuroptera, Coleoptera and Hymenoptera (Phillips, 1970). In the typical insectan sperm tail the axoneme is flanked not only by two mitochondrial derivatives but also by two elongate bodies, or less commonly one body, which may show a paracrystalline structure (Jamieson et al., 1999).
a) **Acrosome formation**

The early spermatid contains a prominent Golgi body that will give rise to the acrosome. It is sometimes called the acroblast. It produces a secretion vesicle on its concave side (sometimes called maturing or secretory side), which migrate to the nucleus. In some insects the acrosomal vesicle is the only component of the acrosome region (Jamieson *et al.*, 1999). In some insects, such as the heteropteran *Gerris, Notonecta* and *Hydrometra*, the acrosome has an intricate geometry of tightly packed tubules (Tandler and Moriber, 1966; Dallai and Afzelius, 1980) or a regular meshwork with a honeycomb pattern (Afzelius *et al.*, 1976; Werner *et al.*, 1988). The acrosome is lacking in many species, but is more than 2.5 mm long in the heteropteran, water strider, *Gerris* (Tandler and Moriber, 1966). The acrosome of most insect species has a conical or rod like shape, although in some group more complicated types are seen such as arrow heads in Tettigoniidae (Baccetti *et al.*, 1970 b; Guerra *et al.*, 1990), a wine-glass shape in several Neuroptera (Afzelius and Dallai, 1979) and a large flattened disc in some saldid bugs (Afzelius *et al.*, 1985).

The acrosome of insect sperm is formed from the Golgi apparatus (Clayton *et al.*, 1958; Cruz-Landim and Ferreira, 1971; Cruz-Landim, 1979 a; Kaye, 1962; Shay and Bieseke, 1968) which is usually termed the acroblast and which lies distal to the nucleus. The outer laminated part of the acroblast,
the externum, is horse-shoe shaped whereas an inner area, the internum, is relatively structureless. The acrosome typically forms in the internum within the concavity of the laminae of *Acheta domestica* (Clayton *et al.*, 1958; Kaye, 1962), *Centhophilus secretus* (Shay and Biesege, 1968), *Myogryllus* sp. (Cruz-Landim, 1979a).

b) **Mitochondrial transformation**

Mitochondria in insect spermiogenesis generally undergo a remarkable series of transformations, first to form what is called a 'Nebenkern' (a German term meaning alongside the nucleus) and later to form the so-called mitochondrial derivatives. Pratt (1968) has described several steps in Nebenkern formation in the hemipteran *M. histrionica*. She found that many filamentous mitochondria aggregate in late telophase of the second meiotic division and occupy the space between the cleavage furrow and the chromosome plate. Gradually the mitochondria fuse to form longer units and approach each other closely. They also anastomose with their neighbours. A network is then formed that consists of two unconnected and interlocked network of rings. In a cross section the Nebenkern will look like a jigsaw puzzle with the many mitochondrial profiles that form two halves, each consisting of several concentric layers (Tokuyasu, 1975). Finally two halves of the Nebenkern extend to become the two elongated mitochondria which extend in parallel along the flagellum. The two mitochondrial derivatives are
derived from a spherical mass (Nebenkern) formed by the fusion of all the mitochondria of spermatid. With the growth of flagellum of the spermatid, these mitochondria become realigned to give the two longitudinal mitochondrial derivatives of the mature spermatozoon (Payne, 1966; Meyer, 1968; Pratt, 1968; Szöllösi, 1975; Cruz-Landim, 1979a).

Pratt (1968) assumed that the process of Nebenkern formation may be one that will divide the mitochondrial material equally in two parts to maintain symmetry and that it is one that will organize the mitochondrial material for its specific role in the mature spermatozoon. The two mitochondrial derivatives in many species have unequal diameters. It is a process where the mitochondrial DNA complements from all mitochondria of a single spermatid are given the opportunity to meet, perhaps for a proof-reading and correction of the genetic information (Tokuyasu, 1975).

Evidence for origin of the mitochondria of the spermatid in association with the nuclear envelope and from, or at least under the control of nuage material emanating from the nucleus is presented by Cruz-Landim (1979c) for Myogryllus sp. The two mitochondria formed during spermiogenesis extend along the flagellum in most insect species and differ from their equivalents in somatic cells in three respects: (1) their length and size is relatively enormous and in some species they occupy the largest part of the spermatozoon with a length of several millimetres (Afzelius et al., 1976;
Mazzini, 1976; Pitnick et al., 1995), (2) the mitochondrial cristae tend to be regularly spaced and oriented perpendicularly to the longitudinal axis (Phillips, 1970, 1974) and (3) the mitochondrial matrix contains a conspicuous crystalline material, which may occupy most of the mitochondrial space or part of it; in later case it is close to the mitochondrial membrane at the side bordering of flagellar axoneme. The mitochondrial crystalline protein, crystallomitin, present in the sperm of most insects (largely from a study of the heteropteran Notonecta) has been defined by Baccetti et al. (1977).

c) Flagellar growth

There are insect spermatozoa with no flagellum (Dallai et al., 1975; Dallai and Afzelius, 1994) and those with a hundred flagella (Baccetti and Dallai, 1978). The flagellum has a central core of nine microtubular doublets surrounding two central singlet microtubules, the well-known 9+1 organelle. The wall of the two central microtubules contains 13 protofilaments as does the dynein arm carrying A-tubule of the doublets (Jamieson et al., 1999). The wall of B-tubule contains 10 protofilaments and a smaller filament. The lumen of these various tubules appears clear or electron-dense, depending on the species (Dallai and Afzelius, 1990). The newly formed flagellum has a simple 9+2 axonemal structure, but eventually accessory tubules (also called peripheral singlets) develop. In most insect orders, the axoneme is
characterized with the short hand formula 9+9+2, i.e., nine accessory tubules, nine doublets and two central microtubules (Alves, 2006).

The early insectan spermatid contains a single centriole (Friedländer and Wahrman, 1966, 1971), whereas other animals generally have a diplosome. In most animals, the centrioles replicate after the first meiotic division, which gives the first spermatocyte with four centrioles; no such replication occurs in insects according to Friedländer and Wahrman (1971). In this connection it is of interest to note that the primary spermatocytes of lepidopteran insect *Spodoptera littoralis* (Godula, 1985) and dipteran insect, *D. melanogster* (Rasmussen, 1973) are reported to have four flagella.

**d) Sperm surface**

Freeze-fracture replicas of the heteropteran spermatozoa have shown that the sperm tail has a triple row of intramembranous particles running along the spermatid (Dallai and Afzelius, 1982). They are seen mainly in the P-face of the membrane. This structure was called the Zipper-line in analogy with the better-known Zipper in mamalian spermatozoa (Friend and Fawcett, 1974). Contrary to the mammalian zipper, the hemipteran sperm tail has an asymmetric location, as it is located between the axoneme and one of the two mitochondrial derivatives. Zipper-lines are also seen in the freeze-fracture replicas of fruit fly (Baccetti et al., 1971) and mosquito spermatozoa (Báo and de Souza, 1992) and might be common in insect spermatozoa.
e) **Centriole adjunct**

The centriole of the mature spermatozoon is surrounded by a material termed centriolar adjunct. In most insects it has a rather homogeneous or finely granular appearance (Dallai *et al*., 1996). The centriolar adjunct consists mainly of proteins, although the presence of RNA has also been demonstrated (Baccetti, *et al*., 1970 a; Cantacuzene, 1970). Species of at least 15 orders of the Insecta have been reported to contain a centriole adjunct (Lindsey and Biesele, 1974). Although the adjunct varies morphologically in the species studied, it usually surrounds, asymmetrically, the bases of the mitochondrial derivatives and the axial filament complex where all of these structures attach to the posterior end of the nucleus. In some insects, however, centriole adjunct material present early in spermiogenesis disappears before maturity. The function of the centriole adjunct has not been established but it is generally considered as a head to tail attachment structure (Gatenby and Tahmisian, 1959), and a nidus for the attachment of the upper ends of the mitochondrial derivatives, needed for cushioning the robust flagellar movement (Lindey and Bieseie, 1974).

f) **Nucleus**

The nucleus is usually an elongated, anteriorly tapered cylinder, and truncated posteriorly. It is sometimes helically coiled and may even coil around the flagellum (Jamieson *et al*., 1999).
g) Accessory bodies

In the typical insectan sperm tail, the axoneme is flanked not only by two mitochondrial derivatives but also by two elongate bodies, or less commonly one body, which may show a paracrystalline structure. These are the deltoid or accessory bodies. In heteropterans longitudinal accessory bodies are absent (Dallai and Afzelius, 1980).

h) Centriole

The studies of Phillips (1970) in 195 species of 15 orders of insects concluded that, in all sperms both centrioles disappear during spermiogenesis and absence of a centriole was directly demonstrated for Homoptera and Heteroptera.

1.5.3 Ultrastructure of accessory glands

The accessory glands differ in size, shape, number, anatomical placement and embryological origin (Adiyodi and Adiyodi, 1975; Grassé, 1977). The muscular layer outside the epithelium constituted of a variable number of sheets of muscular fibers. These muscles are generally innervated (Kimura et al., 1987). Most accessory glands consist of a secretory epithelium surrounded by a muscular sheath, for most glands there are 2 or more layers of muscle cells which spiral obliquely to the right or to the left, around the generally cylindrical gland (Happ, 1992). The thickness of the muscle coat
seems to be correlated with the consistency of the product and is surrounded by two or three layers of muscle cells. Basement membranes surround the muscle layer and also coat the basal surface of the secretory cells. Tracheoles, enclosed in their cellular coats, run between the muscle cells and deep into the secretory epithelium (Happ, 1992). The materials produced by the accessory glands are transferred to the female during copulation, and they frequently have a long term effect on her reproductive behaviour and physiology (Chen, 1984; Happ, 1992; Schooneveld et al., 1997; Wolfner, 1997; Gillott, 1998). The accessory reproductive gland components exerts their effects at all phases of the reproductive biology of the mated females, from the moment sperm is deposited in the reproductive tract to egg deposition. Components with sperm-related functions seen likely to act mainly within the female reproductive tract, whereas those molecules that influence female behaviour and physiology typically appear to pass through the wall of the tract into the hemocoel to reach their site of action (Eberhard, 1996; Gillott, 1988), female insects are far from being "silent partners". Thus, it may be anticipated that females strongly influence the manner and extend to which male accessory reproductive gland (ARG) materials function. The past two decades have seen the publication of two comprehensive review of male ARG (Chen, 1984, Gillott, 1988), as well as several that deal with specific aspects, namely structure and development of ARG (Happ, 1984, 1992), endocrine regulation of ARG secretory activity (Gillott and Gaines, 1992; Gillott, 1996), and the
role of ARG secretions in egg production (Vahed, 1998; Gwyne, 2001; Gillott, 2002) and protection (Blum and Hilker, 2002; Eisner et al, 2002; Gillott, 2002). For males which mate repeatedly, the glands must go through recurrent secretory cycles to produce the charge of semen and paraseseminal material during copulation. The maturation of accessory glands is regulated by endocrine and neuroendocrine factors in almost all groups (Happ, 1992).

Biochemistry of accessory gland secretions

As like mammals, insect semen and its accompaniments are an aggregation of biochemical constituents derived from morphologically diverse and complex glands. The heterogeneous products of these glands include both the seminal fluids and paraseseminal fluid mixtures which promote sperm maturation, facilitate transfer of sperm, provide nourishment for stored sperm, contribute nutrients for investment into egg yolk and modulate the behaviour or the physiology of the female (Leopold, 1976). The mesadenia, simplex and duplex are just a few of the terms which describe the organs that produce seminal or paraseseminal secretions (Happ, 1992). Some of these glandular products are proteins. Smaller molecules including sugars and lipids are reported in the semen (Gilbert, 1982). Secreted proteins appear to be manufactured de novo within the epithelium. Delivery of sperm to the female requires a vehicle which is produced by the accessory glands. Secretions of male accessory glands contain a variety of bioactive molecules. When
transferred during mating, these molecules exert wide-ranging effects on female reproductive activity. The accessory gland secretions may affect virtually all aspects of the female's reproductive activity. Male-derived accessory gland proteins that are transferred to female during mating have profound effect on female reproductive physiology including mating inhibition, and effect on sperm utilization and storage (Gillott, 2003).

Although proteins and carbohydrates were regarded as ubiquitous components of the accessory glands of insects (Leopold, 1976) more work has been done on the analysis of protein components of the accessory glands of several species of insects (Chen, 1984). The accessory secretions may mingle with the sperm, may precede the sperm mass, may enclose it, or may follow along afterward. It is accepted that the glycogen serves as source of energy for various activities of insect (Steele, 1982). Blum et al. (1962) demonstrated presence of fructose, glucose and trehalose in the ARGs of bee, *Apis mellifera*. Inositol is supposed to be the main carbohydrate present in the ARG complex of *Periplaneta americana* (Leopold, 1976). An ovipositor stimulant in the male accessory gland extract of *S. littura* has been studied by Sridevi et al. (1987). Baumann (1974) reported the presence of glucose and xylose as the important components that stimulate egg maturation and oviposition in the female insect. A complex mixture of protein in the accessory gland of *A. domestica* was reported by Kaulenas (1976). Lange and Loughton (1984) demonstrated the presence of a variety of proteins with a
wide range of molecular weights in the accessory glands of *Locusta migratoria*. There are about 80 secreted accessory gland proteins (Wolfner et al., 1997; Swanson et al., 2001; Mueller et al., 2004) in *D. melanogaster*. The genes of male reproductive tract that encode proteins and peptides have strong effect on male and female fitness (Chapman, 2001; Wolfner, 2002). Davies and Chapman (2006) identified cysteine-rich secretory protein genes in the accessory gland of *D. melanogaster*. Several studies have shown that the proteins from the accessory reproductive glands are transported during copulation (Leopold et al., 1971; Terranova et al., 1972; Kaulenas, 1976; Chen, 1984).