EFFECTS OF CARBARYL ON THE PROTEIN METABOLISM OF THE FINAL INSTAR LARVA OF *BOMBYX MORI*

Thesis submitted to the University of Calicut for the
Degree of Doctor of Philosophy under the
Faculty of Science

By

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CERTIFICATE

This is to certify that this thesis entitled "Effects of carbaryl on the protein metabolism of the final instar larva of *Bombyx mori*." is an authentic record of research work carried out by Mr. Sebastian, C. D. from September 1999 to December 2002 under my supervision and guidance, in partial fulfilment of the requirements of the Degree of Doctor of Philosophy under the Faculty of Science, University of Calicut. No part of this thesis has been presented before for any other degree.

Dr. K. V. Lazar

DECLARATION

I, Sebastian, C. D. do hereby declare that this thesis entitled "Effects of carbaryl on the protein metabolism of the final instar larva of *Bombyx mori*" submitted by me to the University of Calicut for the award of the degree of Doctor of Philosophy under the Faculty of Science, is the bonafide record of research work carried out by me in the Laboratory of Insect Physiology and Biochemistry, Department of Zoology, University of Calicut, under the guidance of Dr. K. V. Lazar. I further declare that no part of this thesis has been submitted previously for any other degree.

Calicut University,

December 20, 2002.

Sebastian, C. D.

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INTRODUCTION

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Man has been in constant strife with insect pests right from the prehistoric days, competing for many of his fundamental needs. In early days man used inorganic materials to combat insects. The Sumerians apparently used sulphur compounds for insect control well before 2500 BC and the Chinese used plant derived fumigants and mercury and arsenic compounds in 1200 BC. The history of pesticides encompasses the last 200 years, since the first use of nicotine in the form of a tobacco tea for the destruction of aphids in 1763.

Development of insecticides and their large-scale use occurred during the late 19th century. The significant milestones include the development of Paris Green in 1865 for the control of Colorado potato beetle; the use of Bordeaux mixture for downy mildew control in 1882; the marketing of potassium dinitro-o-cresylate, the first synthetic organic pesticide, in 1892 and the development of DDT in 1939. It was followed by the development of organophosphates and carbamates in Germany and Switzerland respectively. New

pesticides such as soil fumigants, seed treatments, pre-emergent herbicides and animal and plant systemic insecticides have revolutionized farm practices and dramatically increased crop yields.

In bringing this revolution in the techniques of food production, the increasing use of pesticides has also resulted in new problems. Resistant races of pests have appeared in response to intensive natural selection. The widespread distribution of successful insecticides throughout the environment resulted in biological magnification in food chain organisms. The application of insecticides are at a critical state in view of its far reaching biological effects in human and on the environmental pollution and in the development of resistance to these chemicals by the insect itself. A better understanding on the action of these pesticides on organisms may enable us to optimize its use and thereby minimize its adverse effects on the environment. With this view, the effects of carbaryl, a carbamate insecticide, on the development of the larvae of the silkworm with special emphasis on its protein metabolism was investigated in the present study.

REVIEW OF LITERATURE

Sebastian. C.D. “Effects of carbaryl on the protein metabolism of the final instar larva of bombyx mori ” Thesis. Department of Zoology, University of Calicut, 2002

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Introduction

Carbamates are anticholinesterase insecticides, a synthetic derivative of physostigmine (commonly called eserine). It is an alkaloid extracted from the plant *Physostigma venenosum* (Calabar beans). The wine is found along the Calabar River in West Africa. Physostigmine is known to be an inhibitor of choline-esterase and Stedman and Eason (1975) developed synthetic analogues of this such as prostigmine.

Although these and similar compounds are effective inhibitors of insect cholinesterase, they are unsuitable as insecticides, for, they are water-soluble quaternary salts or amino hydrochlorides and hence too polar to penetrate the insect cuticle. Their ineffectiveness as insecticides was attributed to their low lipid solubility (Kolbezen *et al.*, 1975). Hence the modern carbamate insecticides have been modified by eliminating the polar moiety of physostigmine so that they can easily penetrate the insect cuticle as well as the nerve sheath of lipoid soluble derivatives.

The general structure of carbamate is

$R - O - C(O) - N(CH_3) - R_1$

R_1 is hydrogen, methyl, ethyl, propyl or other short chain alkyls and R is alcohol, phenol, oxime, naphthalene or other cyclic hydrocarbon rings.

A list of commonly used carbamates is given in Table A.

Table A: List of commonly used carbamates

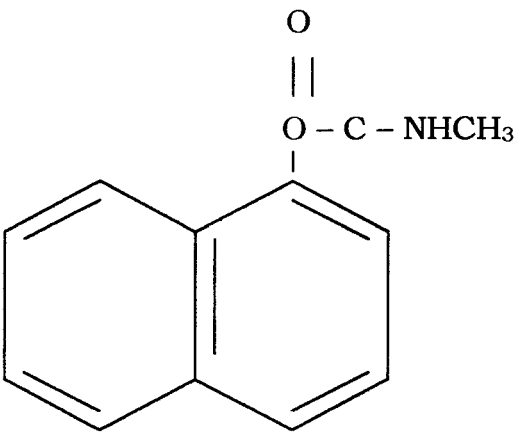
Sl. No.	Common Name	Chemical Name	Trade Name
1	Aldicarb	2- methyl- 2 (methylthio) propionaldehyde -O-(methyl carbamoyl) oxime	Temik®
2	Bendiocarb	2, 2-dimethyl-1, 3-benzodiox- ol-4-yl N-Methyl carbamate	Dycarb®, Garvox®, Niomil®
3	Carbaryl	1 - naphthyl – N - methyl carbamate	Sevin®, Carbacide®,
4	Carbofuran	2, 3 –dihydro - 2, 2 – dimethyl -1-7-benzofuranil-N- methyl carbamate	Furadan®, Brifur®
5	Carbosulfan	2, 3-dihydro-2,2-dimethyl1-7-benzofuranil[(dibutyl-amino) thio] N-methyl carbamate	Advantage®, Marshal®

6	Dioxycarb	2-(1,3-dioxolan-2-yl) phenyl-N-methyl carbamate	Electron®, Famid®
7	Formetanate HCl	3-dimethylamino methylene - amino phenyl N-methyl carbamate	Carzol®, Dicarzol®
8	Mecarbam	S-(N-ethoxycarbonyl-N-methylcarbamoyl methyl) o-o-dithylphosphorodithioate	Afox®, Murfotox®
9	Methiocarb	3,5-demethyl-4-(methylthio)-phenyl N-methylcarbamate	Draza®, Mesurol®
10	Methomyl	S-methyl-N-[(methyl carbamoyl)oxy] theoacetimidate	Lannate®, Lanox®
11	Mexacarbate	4-dimethylamino-3,5-xylyl-N-methyl carbamate	Zectran®
12	Oxamyl	N - N - dimethyl - 2 - methyl carbamoyl oxyimino – 2 - (methylthio) acetamide	Vydate®
13	Primicarb	2 - (dimethylamino) - 5, 6 – 2 -isopropoxyphenyl N, N - dimethyl carbamate	Abol®, Afox®
14	Propoxur	2 – isopropoxyphenyl – N - methylcarbamate	Baygon®, Propagon®
15	Thiodicarb	dimethyl - N, N - {thiobis-(methylimino) carbonyloxy} - bis (ethanimidothioate)	Larvin®, Nivral®
16	Trimethacarb	4:1mixture of the 3,4,5- and2,3,5-isomers of trimethyl phenyl N-methyl carbamate	Broot®, Landrin®

Carbaryl

Carbaryl is a broad-spectrum insecticide with systemic properties. It was first synthesized in 1953 and introduced in 1958 under the trade name Sevin®. It is the most widely used carbamate that controls about 150 species of insect pests. One of its major uses is for control of several cotton pests and forage pests such as insects that attack apples and pears. Carbaryl has low mammalian toxicity with an acute oral LD 50 of 250 - 850 mg / Kg in rats.

Chemically carbaryl is 1- Naphthyl N - Methyl Carbamate. The empirical formula is $C_{12}H_{11}NO_2$. The structure is as follows:



It is a white crystalline solid with a mild phenolic smell. Melting point is 142° C and vapour pressure is less than 0.005 mm Hg at 26° C. Carbaryl is rapidly metabolized in mammals to 1-

naphthol and other hydroxylation and conjugation products that are excreted in the urine. It has a short residual life and may be used right up to harvest.

The most striking difference between the clinical effects of the poisoning by carbamates and organophosphates are much more rapid and spontaneous recovery from poisoning by carbamates and the relatively wide separation between the smallest dosage of any carbamate that will cause mild illness and lethal dosage of the same compound. Both these differences have their pharmacological basis in the relatively rapid and spontaneous reactivation of acetylcholinesterase inhibited by a carbamate (Vandekar, 1965; Vandekar *et al.*, 1971; Vandekar and Wilford, 1969). The reversibility of acetylcholinesterase inhibition confers advantage to carbamates over organophosphates and chlorohydrocarbons. Read (1974) studied toxicity of the carbamate and the organophosphorous insecticide residues absorbed by rutabagas grown in treated soil. Carbamates were found to be mostly concentrated in the pulp. Residual toxicity studies of some of the commonly used insecticides of first instar larvae of spotted bollworm (Patil and Pokharkar, 1977) revealed that out of the insecticides tested, carbaryl was observed to be the most

persistent and effective which gave 37.93% mortality on the 15th day after treatment.

Toxicity of carbaryl

Mallipudi and Fukuto (1979) worked out toxicity of derivatives of insecticidal methyl carbamate esters to the honeybee. Experiments were conducted to obtain additional information relative to the effects of the herbicide atrazine on the toxicity, penetration and metabolism of carbofuran in *Musca domestica.* The selective toxic insecticidal properties of primicarb, carbaryl and methamidophos to the green peach aphid, *Myzus persicae* and its predators, *Coleomegilla maculata* and *Chrysopa oculata* were studied by Lecrone and Smilowitz (1980). Primicarb was less toxic than carbaryl and methamidophos to predators and more toxic to aphids. Kinoshita and Fukuto (1980) found the N - sulfonyl derivatives of carbaryl are non insecticidal when tested alone.

Tests conducted on residential lawns in Southern Florida on chinch bugs (Reinert, 1982) confirmed resistance to the organophosphate insecticides. A carbamate and two synthetic pyrethroids provided good control of the grapevine flea beetle, *Sealodonta strigicollis* that showed that both monocrotophos and

carbaryl had good initial toxicity for four days causing 90% mortality. Carbaryl showed residual toxicity for 32 days after spraying. Srivastava and Masoodi (1985) found mevinphos to be 8 times more toxic than carbaryl when tested against second instar larvae of *Lymantria obfuscata.* Based on the LC 50 values assessed in the laboratory studies (Visalakshi *et al.*, 1985) phorate was found to be the most toxic insecticide to banana rhizome weevil followed by carbofuran, aldicarb and carbaryl in the decreasing order. Hemingway *et al.* (1993) studied the possible mechanisms of organophosphorous and carbamate insecticide resistance in German cockroaches and found that out of 14 strains, 13 were resistant to organophosphorous and 12 to carbamate.

To manage aphids, maggots and leaf cutting caterpillars in the apple orchard, Bostanian *et al.* (2000) used five insecticides - carbaryl, azinphos-methyl, dimethoate, phosmet and phosalone. Among them carbaryl and dimethoate were found to be most toxic to nymphs and adults. Nymphs showed much resistance than adults. Hill and Foster (2000) in their studies of insecticide toxicity on the diamond black moth described a significantly high larval mortality with carbaryl and permethrin after 72 hours of treatment.

Studies of Ahmad *et al.* (2002) revealed that carbamates have much intrinsic toxicity against the oblique-banded leafroller, *Christoneura rosaceana* than the eighteen other insecticides used. The insect exhibited a very low level of resistance against it. In the experiments on insecticide susceptibilities of cat fleas (Bossard *et al.*, 2002) showed that of the eleven strains tested only two field strains developed tolerance against carbaryl. Studies on relative toxicity of insecticides upon soil organisms (Mostert *et al.*, 2002) showed that carbaryl was most toxic to *Pheretima* group with an LC 50 value of 77 mg / Kg.

Biological effects of carbaryl

Biological effects of some organophosphate and carbamate insecticides on the confused flour beetle, *Tribolium confusum*, revealed that these insecticides prolonged the larval period (Khatiyar and Lemonde, 1972). Lawrence *et al.* (1973) reported prolonged larval and pupal stages and emergence was lowest among *Chrysopa rufilabris* topically treated with carbaryl. In female western corn rootworm sublethal dosages of carbofuran and carbaryl stimulated oviposition and extended longevity (Ball and SU, 1979). Robertson (1980) noted that tolerance tended to increase with progressively older larval stages of western spruce budworm when

sprayed with carbaryl. Endosulfan and carbaryl were moderately effective against shoot and fruit borer and ash weevil but proved superior over synthetic pyrethroids in controlling aphid and jassid (Tewari and Krishnamoorthy, 1983). Studies were conducted by Krishnamoorthy (1983) on the susceptibility of the phyloscid mite, *Amblyseius tetranychivorous,* a native predator of spider mites of vegetable crops, against 14 pesticides, which are commonly recommended for pests and diseases of horticultural crops under laboratory conditions to select less toxic chemicals which in turn can effectively be used in integrated pest control program. It revealed that carbaryl at 0.1% were very highly toxic to the predatory mite, inflicting 100% mortality within 24 hours after spraying. The residues of these chemicals were also highly toxic even after 9 days of post treatment.

Sharma (1988) evaluated some synthetic pyrethroids and carbaryl for the control of bollworm and their effect on yield parameters of cotton and was found that carbaryl were the most effective. Effects of repeated application of carbaryl on zooplankton communities in experimental ponds with or without the predator *Chaoborus* was studied by Hanazato (1991). An increased mortality of both was found even days after the treatment. Elzen *et al.* (1992) studied resistance to carbamate insecticides in field population of

tobacco budworm and similar results were obtained. The residual effects of insecticides on colony vitality and behavior of the bumblebees, *Bombus impatiens,* were tested by Gels *et al.* (2002). When carbaryl and a group of other insecticides applied as dry, non-irrigated residues there were severe impacts on colony vitality. The foraging workers tend to avoid insecticide treated areas.

Biochemical effects of carbaryl on higher organisms

In isolated rat hepatocytes carbaryl increase cytochrome P 450 level, reduce oxygen consumption and carbon dioxide production, inhibit gluconeogenesis, reduce lactate dehydrogenase and aspartate aminotransferase activities and enhance glucose-6-phosphatase activity (Parafita and Otero, 1983; 1984). Borady *et al.* (1983) gave rats methomyl, a carbamate, at 40 mg / Kg / day for eight days *via* oral intubation to examine the effects on fat metabolism and blood enzymes. At 24 hour of postdosing, significant increases were noted in serum activities of alkaline phosphatase, aspartate aminotransferase, serum triglycerides, phospholipids, free fatty acids and cholesterol. The *in vitro* studies of Lechner and Rehman (1985) showed that carbaryl decreases rat liver microsomal beta-glucoronidase content. Kiran *et al.,* (1985) reported a single oral dose of 500 mg / Kg carbaryl or seven doses of 71 mg / Kg / day increased

the activities of acid phosphatase, aspartate aminotransferase and alanine aminotransferase in the liver and kidney. But this did not affect the activities of alkaline phosphatase, lactate dehydrogenase or succinate dehydrogenase.

Acute oral administration of carbaryl to rats at doses ranging from 50 to about 500 mg / Kg affected blood and brain levels of a variety of enzymes, amino acids, neurotransmitters and other substances (Baron, 1991). Effects reported included decreases in serum protein levels, blood free amino acid levels and brain acetylcholinesterase concentrations. Changes in free amino acid metabolism in the liver and brain also happened. Increases were reported in the serum transaminase activities.

It has been found that the blood and liver of the fresh water cat fish, *Clarias batrachus,* which were exposed to sublethal concentration (1mg / l, 2 mg / l and 4 mg / l) of carbaryl for four days and 15 days induced perturbations in the levels of certain biochemical components including the activities of some enzymes (Sharma, 1999). Increased levels of transaminases, alkaline and acid phosphatases and lactate dehydrogenase in serum was observed. The pesticide concentration and duration of exposure determines the magnitude of the effect. This increase in activities of transaminases and acid

phosphatases indicates enhanced protein catabolism and probable hepatocellular damage in the organism. Carbaryl also causes a decrease in the levels of total protein and glucose with a prominent increase in the levels of inorganic phosphates and lactic acid in the serum. Increase in lactic acid concentration suggests enhanced rate of glycolysis due to pesticide stress.

Carbaryl treated freshwater catfish, *Clarias batrachus*, showed serious disorders of lipid metabolism in the serum (Jyothi and Narayan, 2001). The serum cholesterol levels decreased significantly throughout the exposure period with the pesticide. The studies on the effects of carbaryl on the activities of brain acetylcholinesterase and plasma butyrylcholinesterase in adult male rats revealed that they were inhibited 85% and 43% respectively (Sachana *et al.* 2001). Sogorb *et al.* (2002) reported that the rabbit serum albumin was capable of carbaryl hydrolysis. Human, bovine and chicken serum albumins also exhibited the same capacity. Their studies indicate that carbaryl residues are not likely to be accumulated in mammalian tissues.

Studies on the scorpion, *Heterometrus fulvipus*, revealed that an excessive utilization of lipids occurs under toxic impacts of insecticides like carbaryl and lindane (Rajyalakshmi and Reddy,

1988). Lipid oriented metabolic pattern was exhibited by blister beetles during pesticide exposure. After 48 hours of carbaryl and lindane treatment, the maternal tissues of *Heterometrus fulvipus* exhibit an elevation in the activity levels of ALAT and AAT that persisted for a period of one month. Lindane exerted a greater effect than carbaryl (Rajyalakshmi and Reddy, 1991).

One of the main detoxification processes of the carbamate insecticides is the hydrolysis of the carbamyl ester bond. Carboxylesterases seem to play important roles in the metabolism of carbamates in higher organisms. Sogorb *et al.* (2002) concluded the rabbit serum albumin was able to hydrolyze the carbaryl. They also suggested that hydrolysis of carbaryl and carboxyl esters occur in the same catalytic site through a similar mechanism. Human, bovine and chicken serum albumins also exhibited the same capacity.

Biochemical effects of carbaryl on insects

The effect of carbaryl on respiration and oxidation-reduction enzymes in Lepidoptera during intestinal poisoning has been studied by Berim and Bykhovets (1973). They reported an increase in oxygen consumption and a decrease in respiratory quotient at the same time. They also observed a decline in the activity

of digestive enzymes of Lepidoptera such as amylase, protease, saccharase and lipase. *Hieroglyphus nigrorepletus* treated with carbaryl and dieldrin showed increased oxygen consumption accompanied by a reduction of the amount of glycogen in the haemolymph (Joshy *et al.* 1976). Both insecticides increased the neuromuscular irritability leading to uncoordinated activities of some vital organs.

The effect of certain insecticides on the pH value of the midgut and the haemolymph of the larvae of the potato beetle *Leptinotarsa decemlineata* was studied by Blazejewska and Wyrostkiewicz (1976) and found that carbamate treatment causes an increase in the pH. Kamoshita *et al.* (1979) determined the insecticidal activity against smaller brown plant hoppers and the inhibitory activity against their acetyl cholinesterase preparation for a number of mono and poly substituted phenyl-N-methyl carbamates. A very high level of mortality and an inhibition above 30% was observed. Dabour and Sayed (1982) reported the effect of sublethal doses of some insecticides like lindane, carbaryl, nuvacrone *etc* on the black cutworm, *Agrotis ypsilon*, demonstrated that there was marked decrease in fresh larval weight but pupation, pupal weight, adult emergence and longevity were unaffected. An increase in fecundity

with carbaryl was reported. Carbaryl and endosulfan induced alterations in the intestinal amylase activity of *Pheretima postuma* (Gupta and Sundararaman, 1982). The amylase activity was inhibited to an appreciable extend. In diamond black moth, *Plutella xylostella,* carbaryl caused deformed wings but increased longevity of adults (Kumar and Chapman, 1984). It had deleterious effects on the number of larvae surviving to pupae, duration of pupal period, number of pupae surviving to adulthood and cocoon formation.

The blister beetles, *Mylabris pustulata,* when exposed to sublethal doses of carbaryl for different time showed an elevated level of proteases (Bharathi and Govindappa, 1985 a). The levels of all the four nitrogenous end products *viz.* free ammonia, glutamine, uric acid and urea were also depleted in the malpighian tubules of the treated insects (Bharati and Govindappa, 1985 b). Effect of carbaryl on the activity levels of aminotransferases and four amino acids of the haemolymph revealed elevation in the activities of ALAT and AAT in the haemolymph of exposed beetles. These changes were similar to those under stress conditions (Bharati and Govindappa, 1985 c). There was a significant depletion of total lipids during short term and prolonged exposure to sublethal dose of carbaryl (Bharathi and Govindappa 1985 d). The excretory pattern of the beetle shifted to

ammonotelism during short-term treatment. Such a derangement envisages the possibility of water loss from the body of beetles. However the beetles on prolonged exposure switched back to uricotelism. The effects of short-term exposure to the insecticide were reversible since the beetles regained normal excretory pattern after withdrawal from the treatment (Bharati and Govindappa, 1986 a). Studies on the effect of carbaryl on the digestive enzymes of blister beetle (Bharati and Govindappa, 1986 b) revealed that the activities of all digestive enzymes were inhibited in the foregut and activated in the midgut after exposure. The effects of short-term exposure of carbaryl on digestive enzymes were reversible. Carbaryl administration to the beetles adversely affected the haemolymph organic constituents and was responsible for the mortality of beetles even at sublethal doses (Bharati and Govindappa, 1987 a). The short term and long term carbaryl treatment to *Mylabris pustulata* revealed an alteration in the constituents of the gut region (Bharati and Govindappa, 1987 b).

In the experiments with final instar larvae of *Spodoptera mauritia,* Shikha (1995) observed an initial increase in midgut protease activity when treated with carbaryl. The activity declined in later stages. Similarly the levels of carbohydrates and proteins perform an initial increase in the first few days of the larval stages,

but a drastic decline was reported in late larval and pupal stages in the midgut tissue. The most affected component in the midgut tissue was lipids, which decreases from 24 hour to adult stage.

The inhibition of acetylcholinesterase activity by carbaryl was found to be at a very higher level. *In vitro* kinetic studies on the inhibition of the acetylcholinesterase activity of western corn rootworm, *Diabrotica virgifera,* showed that carbaryl and carbofuran have a four - fold higher inhibitory potency than the other insecticides tested (Gao *et al.,* 1998). Treatment of monocrotophos, dimethoate, methyl parathion, quinalphos and endosulfan on *Rhynocoris kumarii* resulted in the reduction of carbohydrates and proteins and elevation of lipids in the alimentary canal of the animal and entire body George and Ambrose (1999). Dry matter was decreased by insecticides and on contrary, water content was increased, except in the case of endosulfan.

Biochemical changes during insect development

The Major biochemical process underlying insect morphogenesis is protein synthesis. So the number and patterns of tissue specific proteins vary at different stages of development and become increasingly more complex with advances in development. The

structural or enzymatic protein is immediately responsible for the developmental stages.

At the initiation of the larval development, growth is the predominant phenomenon. In *Drosophila* the increase in total protein content parallels closely to that in both wet and dry weight during the first 72 hours of development (Church and Robertson, 1966 a, b). An important aspect of protein metabolism during larval development is the synthesis of haemolymph proteins. In general the protein concentration in haemolymph increases rapidly during the later half of larval development, falls at metamorphosis and decline to its lowest level in early adult life. An accumulation of haemolymph protein in *Calliphora* larvae was reported by Munn *et al.* (1967). Ruegg (1968) demonstrated that under *in vitro* conditions the specific rate of protein synthesis in larval fat body of *Drosophila* declines rapidly between 65 hours and pupation.

Protein metabolism during metamorphosis of holometabolous insects has been the subject of numerous studies (Chen, 1971). The majority of the earlier studies address the question to what extend the drastic morphogenetic alterations involved in the transformation of larva to adult is reflected in the pattern of protein metabolism. It is now generally accepted that nearly all proteins in the

insect haemolymph are synthesized in the fat body (Wyatt, 1980). Thus the increase in haemolymph protein concentration is accompanied by a fall in the synthetic capacity of the fat body. This is logical since following protein production the fat body changes to a storage organ for several selected haemolymph proteins for the use during adult development (Tojo, 1980; Chen, 1985; Candy, 1985).

Fat body of insects is analogous to the liver and adipose tissue of mammals in their functional aspects but its functional diversity cannot be equated to any other metazoan cell type (Wyatt, 1980). The insect fat body consists of loosely aggregated or compact masses of cells enclosed in a membranous sheath that are freely suspended in the haemocoel. It is structurally organized to provide maximal exposure to the haemolymph. The fat body is well suited for both absorbing and releasing metabolites since it is the principal metabolic-storage tissue in an organism having open, diffusion type circulatory system.

The insect fat body is a major organ of multiple metabolic processes. Metabolism involves, among other things, the utilization of substances absorbed from the gut, their assimilation in to substances in the body or their oxidation to provide energy. Fat body is composed of two or three cell type. The predominant metabolic storage cells are

the adipocytes. In a well nourished insect, the cytoplasm of these cells is packed with droplets of fats, glycogen and proteins showing that the tissue serves as an important storage depot for reserve materials. The second common cell type is urocyte that sequesters uric acid for storage-excretion.

The fat body appears to be most conspicuous in the larvae of holometabolous insects. The fat body undergoes growth and development along with other insect tissue and its functions change in accordance with the developmental stage of the insect. Shigematsu (1958) was the first person who confirmed that insect haemolymph proteins are synthesized by the fat body. Protein synthesis shows variation with the age of the insect. Price (1966) observed that highest level in four-day-old larvae of *Calliphora erythrocephala* and the rate of synthesis falls as the larvae grows old. In *Diatraea grandiosella,* fat body protein increased from 1.15mg / insect larva to 7.9 mg in the newly ecdysed pupa (Chippendale, 1970). The fat body of holometabolous insect larvae synthesizes major haemolymph proteins during the feeding stage and during prepupal stage the fat body cells incorporate these proteins (Roberts and Brock, 1981; Levenbook, 1985). During pupal life the accumulated storage proteins are hydrolyzed and their amino acids are utilized for the synthesis of

adult proteins (Munn and Greville, 1969; Levenbook and Bauere, 1984). While immature stages of holometabolous species are known to store nutrients needed for adult development in the fat body, it is mainly a biosynthetic organ during larval and adult life. (Keeley, 1985).

Extensive study of blood proteins during insect development was carried out by Laufer (1960) and concluded that the fat body was the source of several proteins found in the blood. Price and Bosman (1966) found that the pattern of blood proteins in 4-7 days old larvae was fairly constant as in the pattern produced by fat body. In *Calliphora* the increase in total soluble protein which occurs during early stage of the last instar larva has been correlated with a high rate of protein synthesis by the fat body and the rapid export of this material into the haemolymph (Martin *et al.*, 1971).

In general, the rate of protein synthesis in the fat body is high in early growing larvae and declines rapidly with the advance of larval life. The increase in the haemolymph protein concentration is accompanied by a fall in the synthetic capacity of the fat body. Following protein production, the fat body changes to a storage organ for several selected haemolymph proteins for use during adult development (Tojo *et al.*, 1980). The synthetic activity of fat body

reflects the cyclical nature of growth and development. In the fat body of *Calpodes*, there are intermoult / moult cycles of activity (Locke, 1970; 1980 a, b). The first is a period of preparation for larval synthesis of fat body cells that begins shortly after ecdysis at the fourth to fifth moult. This is followed by a phase of massive synthesis of fat body cells that which ends with preparation for metamorphosis. A few hours after ecdysis, the cells are small, with little cytoplasm, in which mitochondria appear conspicuous.

Haemolymph is the circulating body fluid in insects that fills body cavity or haemocoel. The haemolymph serves as an excellent barometer in determining the biochemical status of the developing insect. The involvement of haemolymph in the transfer of metabolites is particularly evident in Endopterygota, at the beginning of metamorphosis (Wyatt, 1961). The chemical composition of haemolymph is highly variable among the diverse species examined and at different developmental stages of the same species (Florkin and Jeuniaux, 1974).

The volume of haemolymph varies widely according to age and developmental stages. Haemolymph volume is large at ecdysis, but is reduced after the moult (Reynolds, 1980). In *Schistocerca gregaria* it appears that the increase in haemolymph volume occurring

prior to ecdysis is derived from cellular and gut water, since the total percentage of dry weight remains constant (Lee, 1961). The variability of haemolymph volume is an important adaptation in insects to deal with the changes in its external environment (Florkin, 1966).

The protein concentration in insect haemolymph is similar to that of the blood of man and other vertebrates and generally higher than that of the internal fluid of other invertebrates (Florkin and Jeuniaux, 1974). The average protein content is 5 gm per 100 ml in Hymenoptera, 4 gm per 100 ml in Coleoptera, 2 gm per 100 ml in Lepidoptera and 1gm per 100 ml in Orthoptera (Florkin, 1936).

The protein components of haemolymph comprises a structurally and functionally heterogeneous array of macromolecules that include storage proteins, vitellogenins, lipophorins, immunoproteins, clotting proteins, tanning proteins, lysozymes, enzymes *etc.* (Wyatt and Pan, 1978; Miller and Silhacek, 1982; Riddiford and Law, 1983). Fat body and midgut are considered to be the two important sources of haemolymph proteins in Lepidoptera. Many investigators proved that fat body is the major source. Apart from fat body other tissues like haemocytes (Buhlmann, 1974; Geiger *et al.*, 1977), midgut (Palli and Locke, 1987 a), epidermis (Palli and

Locke, 1987 b) and pericardial cells (Fife *et al.* 1987) also contribute to haemolymph proteins pool.

Haemolymph proteins may be used directly as a source of material for the synthesis of protein by developing adult tissues (Heller, 1932). This has been confirmed in *Phormia regina* using radioactive labeled proteins (Chen and Levenbook, 1966) and in the fifth instar larvae of *Locusta migratoria* (Tobe and Loughton, 1969). This may be the reason for the decline in the haemolymph protein concentration observed during the end of the pupal or nymphal life in insects. The number and nature of different protein fractions are variable according to sex (Yadav *et al.,* 1988) and diet (Dalhman, 1969; Shahi and Krishna, 1980).

Feeding stimulates protein synthesis in the larval fat body. This has been proved in *Bombyx mori* and the synthesis could be elicited by feeding free tryptophan (Bosquet, 1983). In locusts a protein synthesis stimulating factor appears in the haemolymph within five minutes of the initiation of feeding and its release is a response to feeding behavior (Carlisle *et al.,* 1987). The role of haemolymph proteins as a storage reserve is illustrated by changes during starvation.

At the initiation of larval development growth is the predominant phenomenon. In *Drosophila,* the total protein content parallels closely that in both dry and wet weight during the first 72 hours of development (Church and Robertson, 1966 a, b). Major changes in protein content of haemolymph occur during development (Agosin, 1978; Wyatt, 1980). The haemolymph protein levels generally increased during each instar, but decline during moulting. The levels are low in early instar. Protein levels may decline prior to larval-pupal ecdysis, rise in the pupae followed by another decline during adult development (Wyatt and Pan, 1978). Since major biochemical process underlying morphogenesis is protein synthesis, the number and pattern of tissue specific proteins vary at different stages and become increasingly more complex with advances in development.

Insect transaminases

Aminotransferase activity has been demonstrated in a few insects. Enzyme activities are found in different tissues and the transamination reactions involving alanine, glutamate, aspartate and the corresponding keto acids appear most active (Chen and Bachmann, 1964). Compared to mammals there is very little information on the structure and function of aminotransferases,

which are known to play a key role in the intermediary metabolism of amino acids.

The most important physiological functions of aminotransferases, mainly alanine aminotransferase (ALAT) and aspartate aminotransferase (AAT), are the maintenance of the amino acid pool at a proper level for protein synthesis (Meister, 1965), the supply of metabolites for energy metabolism (Saktor, 1974) and the catalysis of interactions between protein and carbohydrates metabolism (Katunuma *et al.,* 1968). The ALAT activity in *Drosophila nigromelanica* increases rapidly during larval growth, declines to a minimum at the middle of the pupal development, and rises again at emergence and during the early adult life (Schneider and Chen, 1981). As both growth and differentiation are closely related to protein synthesis, the elevated activity in growing larva and young flies appears logical. The same profile has been reported for AAT activity during development of house fly, *Musca domestica* (Mc Allen and Chefurka, 1961).

Wadhwa *et al.* (1986) suggested that ALAT and AAT activities were higher in the feeding insects than the non-feeding insects. In the army worm, *Spodoptera mauritia,* an increase in the level of enzyme activity only in the feeding stages and the activity

declines with the cessation of feeding until pupation (Lazar and Mohamed, 1988). However the larva maintains a relatively higher level of enzyme activity during larval-pupal transformation. The high levels of aminotransferases observed in the feeding stages of larva are in tune with its anabolic phase of development. During the feeding period the larva exhibited higher growth rate and urea output showing higher anabolism.

The protein synthesis requires a balanced amino acid pool and transamination is one of the chief mechanisms, which functions as a regulator for this (Reddy *et al.,* 1991). Higher transaminase implies enhanced mobilization of free amino acids in to transamination activities. The ALAT activity forms a general index of amino acid break down and AAT marks towards the mobilization of amino acids in to gluconeogenesis (Adibi, 1968; Davidson and Longslow, 1975).

MATERIALS AND METHODS

Sebastian. C.D. “Effects of carbaryl on the protein metabolism of the final instar larva of bombyx mori ” Thesis. Department of Zoology, University of Calicut, 2002

MATERIALS AND METHODS

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The experimental animal

The silkworm, *Bombyx mori* L. belongs to the Phylum Arthropoda, Class Insecta and Order Lepidoptera. They exclusively feed on mulberry leaves. The newly hatched larvae, in about four week's time, undergo moulting four times, become mature silkworms and start spinning the cocoon. Fabrication of cocoon is completed in two or three days. The silkworm pupates inside the cocoon in another two or three days during which various organs are formed rapidly to metamorphose to moth. The moth emerges after about 12 days of life as a pupa. The moth inside the cocoon secretes an alkaline fluid from its mouth to soften the cocoon layer before emerging from the cocoon. The moth usually comes out of the cocoon early in the morning, copulates on that day itself and the females lay the eggs in the evening or the following morning. After oviposition the moth weakens gradually and dies after four or five days. Each female lays about 400-700 eggs. Hatching occurs only in the early morning because the light influences hatching.

The varieties that complete only one generation in a year under natural condition are called univoltine varieties. Those that complete two generations are called bivoltine and those that complete three or more generations in a year are called multivoltine. Most of the silkworms domesticated are tetra-moulters, which moult four times during the larval stage.

The body of the silkworm is slender and long consisting of 13 segments. The body length is maximum towards the end of the fifth instar. The female is larger than male in the larval, pupal and adult stages. The bivoltine silkworm hybrid, Elite-CSR 2 x 4 was used for the present study.

Silkworm rearing

The rearing of silkworm was undertaken with procuring newly hatched larvae immediately after their brushing from Serifed, Kozhikode, Kerala, and with the help of well-grown mulberry plantation. The rearing house and all the rearing appliances were disinfected in advance with chlorine

dioxide or bleaching powder to free the rearing environment and the surrounding from pathogens.

The larvae were fed with fresh tender mulberry leaves cut in to the size of half to one-centimeter squares. Clean wet foam pads were placed around the rearing bed to ensure 80-90% humidity. The worms were fed three times a day. The rearing beds were cleaned and dried daily in the morning. Expands the size of the bed corresponding to the growth of the larvae. The worms settle for the first moult on completion of three days. During moulting rearing bed was kept completely dry and undisturbed. The duration for all the worms to come out of the first moult was about 24 hours.

The larvae resumed feeding after moulting and with 6-7 feeding the larvae were ready for second moult. The process of feeding and bed cleaning was done daily. The duration of second moult was about 24 hours. The third instar had duration of 3 – 3 1/2 days and the duration of third moult was 24 hours. The forth instar took 4 – 4 1/2 days followed by the forth moult spanning about 30 hours. The duration of fifth instar was normally about six days in

which the larvae were fed with fully matured mulberry leaves. The larvae started to spin cocoons by the end of this stage.

The present work was done on the fifth instar larvae, beginning from the first hour after its forth moult, and continued till the last day of the instar, at 24 hour intervals.

Treatment of Carbaryl

Carbaryl (50% W.D.P) was obtained from Aventis Crop Science Ltd. Mumbai, under the trade name Sevin®.

After the forth moult, the silkworm larvae were segregated into two sets. One set was fed with mulberry leaves that dipped in 50 μM carbaryl solution and drained in air for half an hour. The other set of larvae was fed with leaves dipped in distilled water and drained in air for half an hour. The leaves were fed at least three times a day for both sets simultaneously.

Analyses of biological parameters

Growth rate pattern

The fresh weights of the larvae were noted at 24-hour intervals during the development immediately after fourth moult, in the case of both normal and treated larvae. The weight of food consumed was calculated using a reference value (of 1.5 for moths) derived by Mathavan and Pandian (1974), which is expressed as a ratio between dry weight of food consumed and dry weight of excreta voided. The rate of food consumption was expressed as mg dry leaf consumed per animal at 24 hour intervals.

Fresh excreta were collected from a definite number of synchronomous larvae at 24 hour intervals starting from the first hour of the instar, till they began spinning. The excreta were dried in an oven at 80°C for one hour and then at 60°C to a constant weight. The rate of excretion was calculated and expressed as mg dry excreta voided per unit time per animal, for both normal and treated larvae.

Haemolymph volume

The measurement of the volume of total haemolymph was made directly using a fine calibrated capillary tube. For extracting the haemolymph, the larvae were anaesthetized slowly with diethyl ether as described by Mohamed (1974) since reflex bleeding was observed in the larvae as in the case of *Mylabris pustulata.* One of its thoracic legs was amputated with a sharp scissors and the haemolymph that oozed out was immediately drawn into a calibrated capillary tube and its volume found out. To ensure complete extraction of haemolymph, the larvae were gently pressed from anterior and posterior ends simultaneously until no more haemolymph was oozing out of the wound. Haemolymph volume changes of both normal and treated larvae were observed during the developmental stages at 24 hour intervals. The volume is expressed as ml per animal.

Fat body weight

The fat body was dissected out carefully in ice-cold insect ringer. The water adhered to the fat body was wiped out with a filter paper and weighed immediately. The fat bodies of

both normal and treated larvae at 24 hour intervals during the developmental stages of fifth instar were dissected out and weighed to determine changes in total fat body weight. The weight is expressed as mg per animal.

Biochemical analyses

For various estimations pooled haemolymph samples were extracted from appropriate number of both normal and treated larvae separately. The samples were immediately processed before melanization. For the estimations of fat body samples the tissue was homogenized and diluted to appropriate volume with water for all assays except enzymes. The analyses were carried out at 24 hour intervals on all larval stages of the fifth instar. Definite number of larvae from normal and treated sets was used for analyses. The results of the biochemical analyses were expressed per unit volume / weight and per total volume / weight of the haemolymph and fat body respectively.

Estimation of total protein

Total protein was estimated by the method of Lowry *et al.* (1951) using crystalline bovine serum albumin (fraction

V, Sigma) as standard. The proteins were precipitated with trichloro acetic acid. The precipitate was then successively extracted with ethanol-chloroform, ethanol-ether and finally ether at room temperature. The final residue was extracted with 0.5 N perchloric acid at 90°C for 15 minutes. The residue left over the hot extraction was dissolved in sodium hydroxide solution and used for total protein estimation. The blue color developed was measured against a reagent blank at 540 nm in a Shimadzu UV 250 Spectrophotometer.

Estimation of total free amino acids

The estimation of total free amino acids in the tissues was done by the method of Lee and Takahashi (1966). The haemolymph or homogenized fat body tissue was precipitated with 10 % sodium tungstate solution and 2/3 N sulfuric acid and centrifuged at 2000 rpm for 20 minutes. The color developed was read at 540 nm against the reagent blank in a spectrophotometer

Estimation of urea

The urea in the haemolymph and fat body was estimated using Fearon reaction, modified by Beale and Croft

(1961). The homogenates were deproteinized with 0.3 N barium hydroxide and 5% zinc sulphate and filtered. The filtrate was treated with diacetyl monoxime - phenyl anthranilic acid and then with activated phosphoric acid reagent and heated for 11 minutes. The color developed was read after cooling, against a reagent blank in a spectrophotometer at 535 nm.

Estimation of glucose

Glucose was estimated according to Morgan (1975). Haemolymph or homogenate of the fat body was deproteinized using 0.3N barium hydroxide and 5% zinc sulphate solution. The resultant filtrate was used for the estimation and the color developed was read at 540 nm against a reagent blank in a spectrophotometer.

Estimation of aspartate amino transferase (AAT) and alanine amino transferase (ALAT) activities

The activities of aspartate amino transferase (AAT) and alanine amino transferase (ALAT) were estimated following the method of Reitman and Frankel (1957) using sodium pyruvate as standard. The haemolymph or fat body

homogenate in 0.1 M phosphate buffer, pH 7.4, were used for the enzyme assay. The substrate mixture of alpha oxoglutaric acid and L-aspartic acid for AAT and the substrate mixture of alpha oxoglutaric acid and L-alanine for ALAT were incubated with the enzyme source for one hour. The color developed due to the formation of keto acid was read after 10 minutes at 520 nm against a reagent blank in a spectrophotometer. One unit enzyme activity corresponds to the formation of one mole of keto acid per minute at 37° C under the experimental conditions. The specific activity of AAT and ALAT enzymes was expressed as unit activity per mg of the protein.

The optimum pH for the activity of fat body AAT and ALAT were determined by using a series of phosphate buffers with pH 6.0, 6.5, 7.0, 7.5, and 8.0. The Km value for AAT and ALAT were determined using alanine and aspartate as substrates by the method of Lineweaver-Burk double reciprocal plot. The nature of inhibition of carbamate on the activities of AAT and ALAT were also evaluated.

RESULTS

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RESULTS

RESULTS

Growth rate pattern of the silkworm

The fifth instar larval period of silkworm was found to last for six days. The larval stages were identified by their size and feeding characteristic. The larva began feeding after 12 hours of its fourth moult. The early stages of larval life were marked by active feeding and on the fourth day the larva attain its full-grown body size. On the fifth day the silk glands were well developed and larva showed a decline in food intake. On the sixth day the larva had completely stopped feeding and was ready to start spinning the cocoon. The fabrication of the cocoon was completed in two to three days.

The larvae treated with carbaryl showed a reduction in growth and body size. There was a yellowish pigmentation on the cuticle within hours of treatment. A slightly yellowish fluid oozing from the body was also observed that lasts till the onset of spinning. The reduction in body size was very prominent when compared to the normal larvae. The result is presented in Plate 1

Plate 1

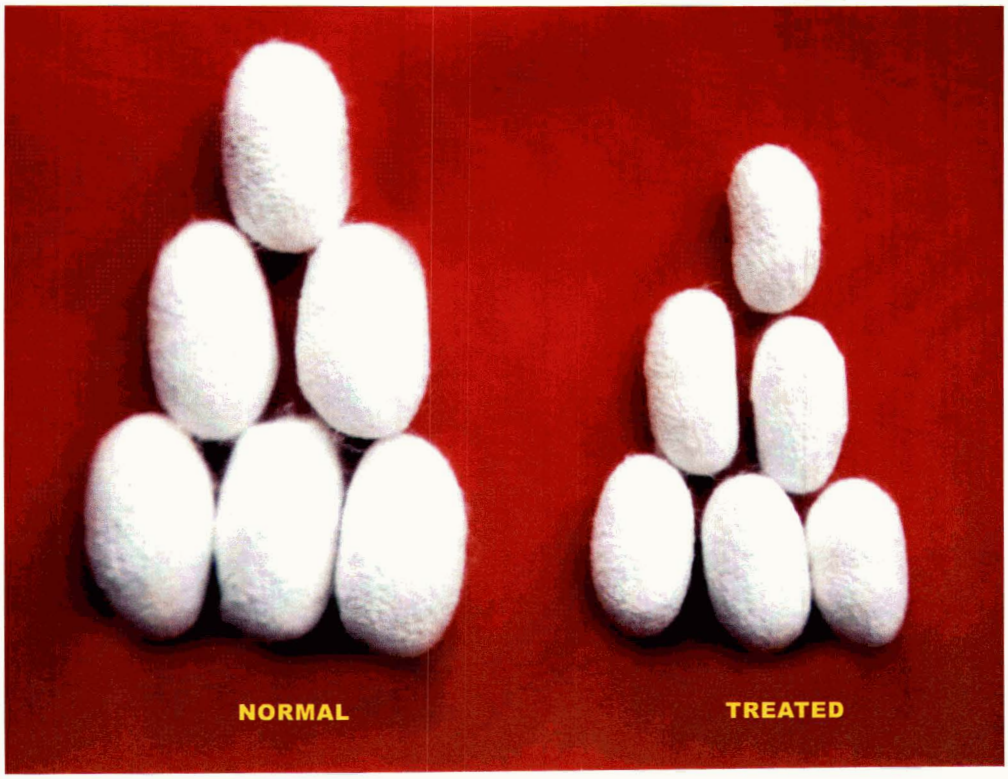

Plate 1: Normal and carbaryl treated 72-hour larvae and the cocoon of silkworm

The fresh and dry weights of both normal and carbaryl treated larvae during the fifth instar are recorded in Table 1 and represented in Figures 1a and 1b.

Table 1

Figure 1a and 1b

The fresh weight of the normal larvae was minimum at the beginning of the instar. Then it rose sharply attaining the maximum on the fourth day but declined thereafter. In the case of treated larvae a reduction in the body weight was observed after 24 hours of treatment, then increased to a maximum on the fourth day and later declined. The maximum weights of the treated larvae were found to be only 50% of that of the normal. The dry weight of the body in both sets exhibited a similar pattern.

The percentage of the total dry content in the body of both normal and treated larvae during fifth instar is represented in Table 2 and Figure 2.

Table 2

Figure 2

Table 1

Changes in the body weights of normal and carbaryl treated larvae during development

Larval age hours	Fresh body weight g / larva		Dry body weight g / larva	
	Normal	Treated	Normal	Treated
0	1.732 ± 0.156	1.685 ± 0.236	0.209 ± 0.021	0.202 ± 0.019
24	2.486 ± 0.219	1.317 ± 0.154	0.315 ± 0.030	0.163 ± 0.021
48	3.765 ± 0.227	1.778 ± 0.168	0.549 ± 0.041	0.226 ± 0.023
72	4.413 ± 0.425	2.036 ± 0.127	0.701 ± 0.055	0.269 ± 0.024
96	4.028 ± 0.177	1.732 ± 0.195	0.687 ± 0.051	0.242 ± 0.018
120	3.801 ± 0.213	1.647 ± 0.119	0.684 ± 0.046	0.239 ± 0.016
144	3.069 ± 0.364	1.489 ± 0.134	0.658 ± 0.049	0.236 ± 0.022

Values are the means of five determinations with standard deviation

Table 2

Percentage of dry content of the total fresh body weight during larval development

Larval age hours	Normal	Treated
0	12.07	11.99
24	12.67	12.38
48	14.58	12.71
72	15.88	13.21
96	17.06	13.97
120	17.99	14.51
144	21.44	15.85

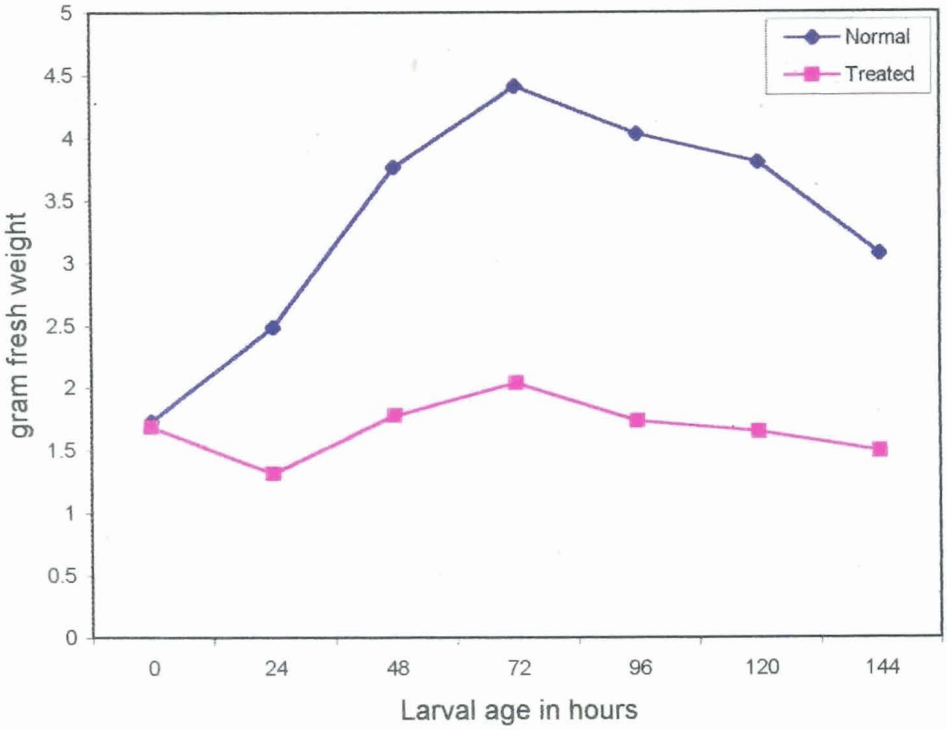

Figure 1 a : Changes in the fresh body weight of the larva during development

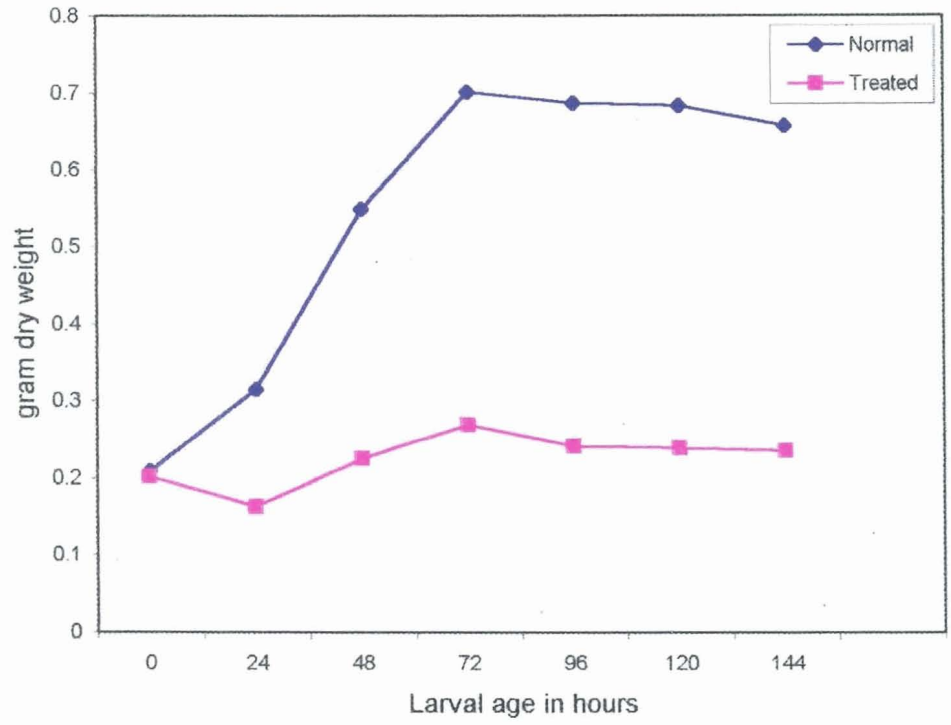

Figure 1 b : Changes in the dry body weight of the larva during development

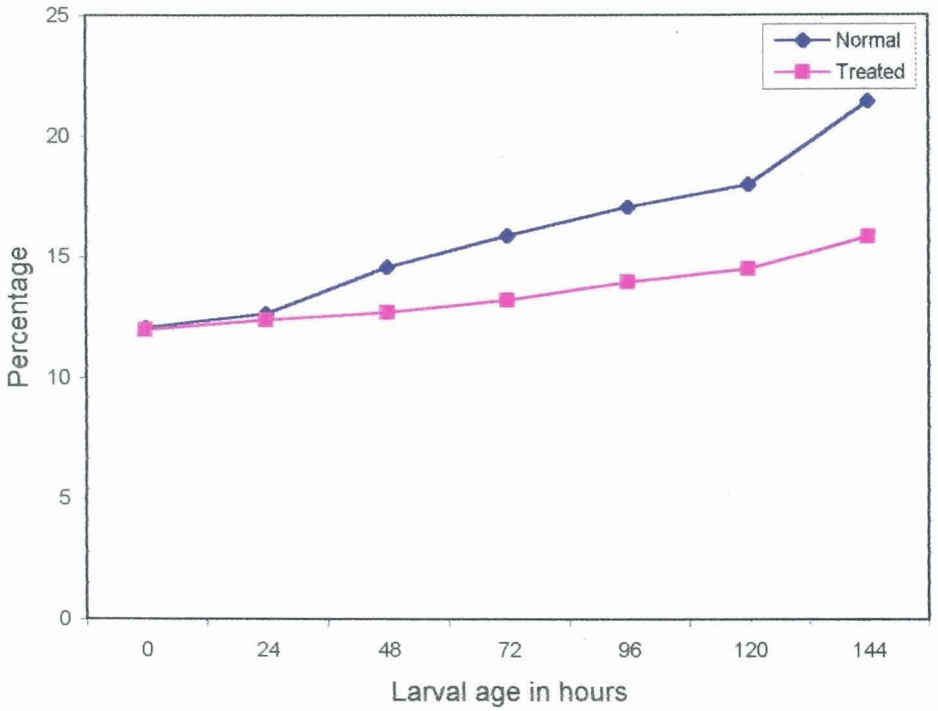

Figure 2 : Percentage of dry content in the larval body during development

In both normal and treated larvae the percentage of dry content increases steadily till the pupal formation. But in the case of treated larvae the percentage of total dry content was much lower than that of the normal.

The rates of excretion were calculated for 24 h intervals and are recorded in Table 3 and Figure 3.

Table 3

Figure 3

The rate of excretion showed an increase from the first day to the fourth day and then declined. Both normal and treated larvae showed similar pattern of excretion but in a higher scale in the former.

The rates of consumption of food are presented on Table 4 and Figure 4.

Table 4

Figure 4

The food intake was low on the first day of the instar, then rapidly shoots to a maximum on the fourth day

Table 3

Changes in the rate of excretion during larval development

Larval age in days	Normal mg dry excreta / larva / day	Treated mg dry excreta / larva / day
1	88.67 ± 1.43	37.92 ± 1.19
2	144.47 ± 1.78	121.64 ± 1.89
3	189.63 ± 2.06	176.17 ± 1.64
4	221.98 ± 2.71	178.49 ± 2.17
5	161.44 ± 1.90	140.86 ± 2.04
6	106.15 ± 1.06	97.82 ± 1.71

Values are the means of five dterminations with standard deviation

Table 4

Changes in the rate of food consumption during larval development

Larval age days	Normal mg dry weight / larva / day	Treated mg dry weight / larva / day
1	133.01 ± 1.52	56.88 ± 1.21
2	216.71 ± 1.63	182.46 ± 1.86
3	284.45 ± 2.01	264.26 ± 1.67
4	332.97 ± 2.37	267.74 ± 2.09
5	242.16 ± 1.85	211.29 ± 2.01
6	Nil	Nil

Values are the means of five determinations with standard deviation

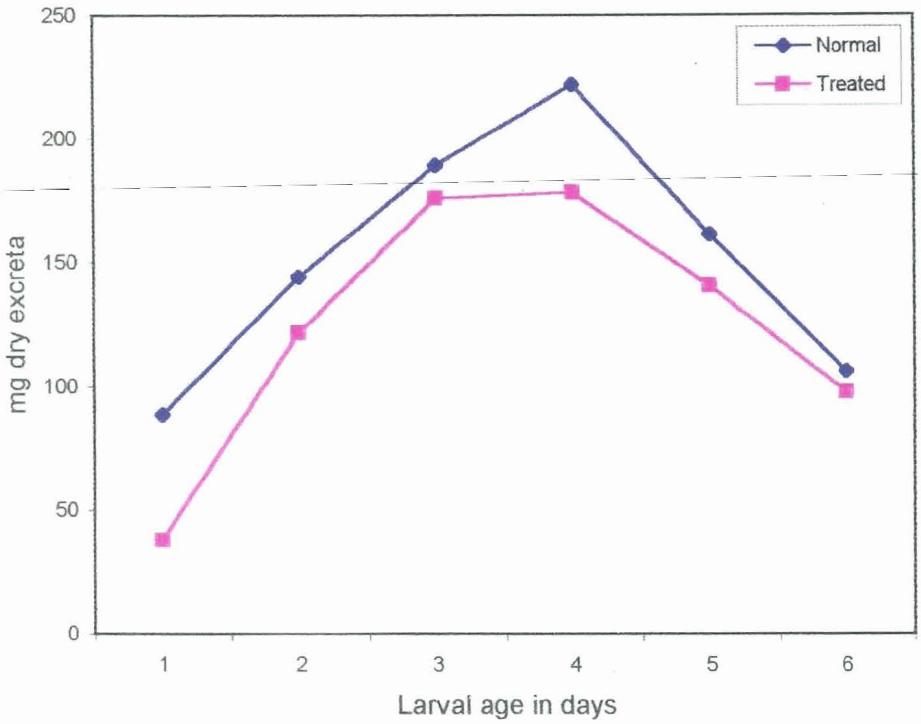

Figure 3 : Changes in the rate of excretion during larval development

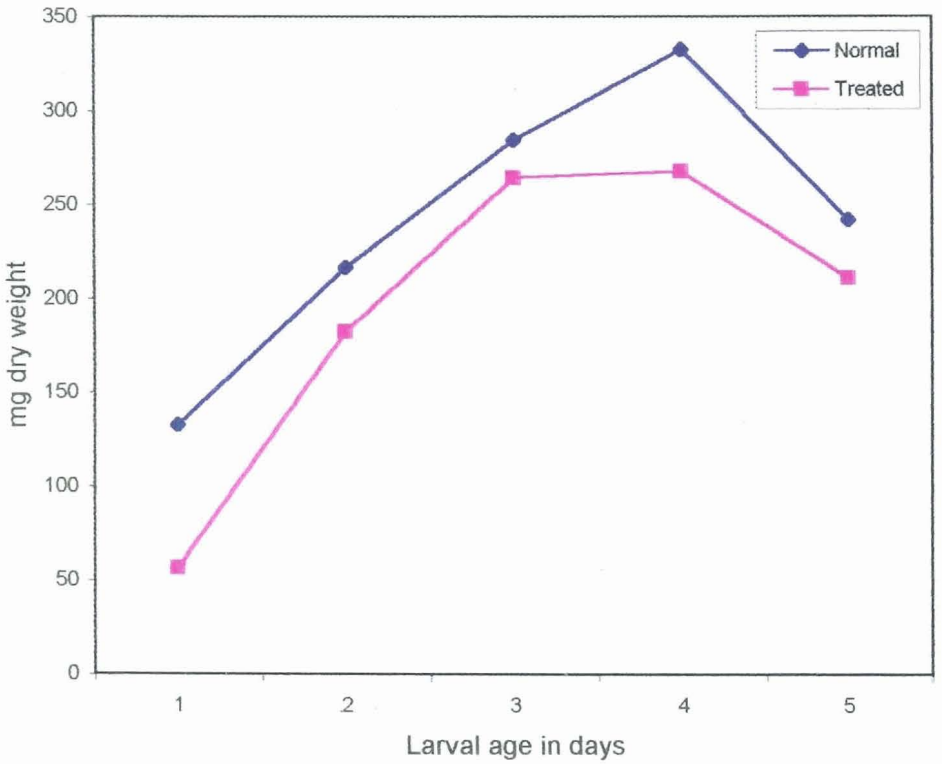

Figure 4 : Changes in the rate of food consumption during development

and then declined. There was no food consumption on the final day of the instar. The treated larvae showed reduced food consumption. There was a 40% reduction in the food intake by the treated insects on the first day compared to that of the normal.

The changes in volume of haemolymph in the normal and treated larvae during fifth instar are given in Table 5 and Figure 5.

Table 5

Figure 5

The haemolymph gradually became denser with the development of the larva. But in carbaryl treated larvae the haemolymph was less dense, compared to normal. The volume of haemolymph was low on the first day, steadily rose to a peak on the fourth day and then declined. The treated larvae also showed similar pattern of changes but at the peak it was only 37% of that of the normal.

The changes in the development of fat body were expressed as its fresh weight and are presented in Table 6 and Figures 6a and 6b.

Table 6

Figure 6a and 6b

The fresh weight of the fat body increased sharply to a maximum on the fourth day, which was more than six fold to that of the first day of the normal larvae, whereas in treated larvae the increment was just about two fold. Considering the dry weight it was gradually increased till the final stage of the fifth instar with a slight alteration in treated larvae. But at the termination of larval life the dry weight of the fat body of the normal was four times higher than that of the treated larvae. The fat body appeared yellowish in the carbaryl treated larvae whereas it was creamy white in normal ones.

Biochemical Analyses

Total protein

The concentration of protein in the haemolymph and fat body of both normal and treated larvae during its development are recorded in Tables 7a and 7b and Figures 7a(i), 7a(ii), 7b(i) and 7b(ii).

Table 5

Changes in the volume of haemolymph during larval development

Larval age hours	Normal ml / larva	Treated ml / larva
0	0.125 ± 0.013	0.128 ± 0.018
24	0.264 ± 0.017	0.124 ± 0.015
48	0.419 ± 0.021	0.201 ± 0.013
72	0.568 ± 0.026	0.246 ± 0.018
96	0.673 ± 0.019	0.254 ± 0.021
120	0.526 ± 0.023	0.217 ± 0.020
144	0.437 ± 0.028	0.194 ± 0.017

Values are the means of five determinations with standard deviation

Table 6

Changes in the weights of the fat body during larval development

Larval age	Fresh weight mg / larva		Dry weight mg / larva	
hours	Normal	Treated	Normal	Treated
0	34.43 ± 1.02	35.64 ± 1.01	8.67 ± 0.74	8.79 ± 0.42
24	52.01 ± 1.21	41.73 ± 1.93	13.03 ± 0.63	9.35 ± 0.61
48	116.32 ± 0.51	63.46 ± 2.07	24.44 ± 1.04	10.48 ± 0.54
72	163.78 ± 1.16	82.31 ± 3.04	36.76 ± 1.39	9.73 ± 0.21
96	221.14 ± 1.02	81.43 ± 2.41	42.68 ± 1.43	9.49 ± 0.29
120	214.83 ± 0.76	78.62 ± 1.30	46.27 ± 1.72	10.47 ± 0.75
144	211.64 ± 1.21	75.64 ± 1.71	49.79 ± 1.06	11.74 ± 1.06

Values are the means of five determinations with standard deviation

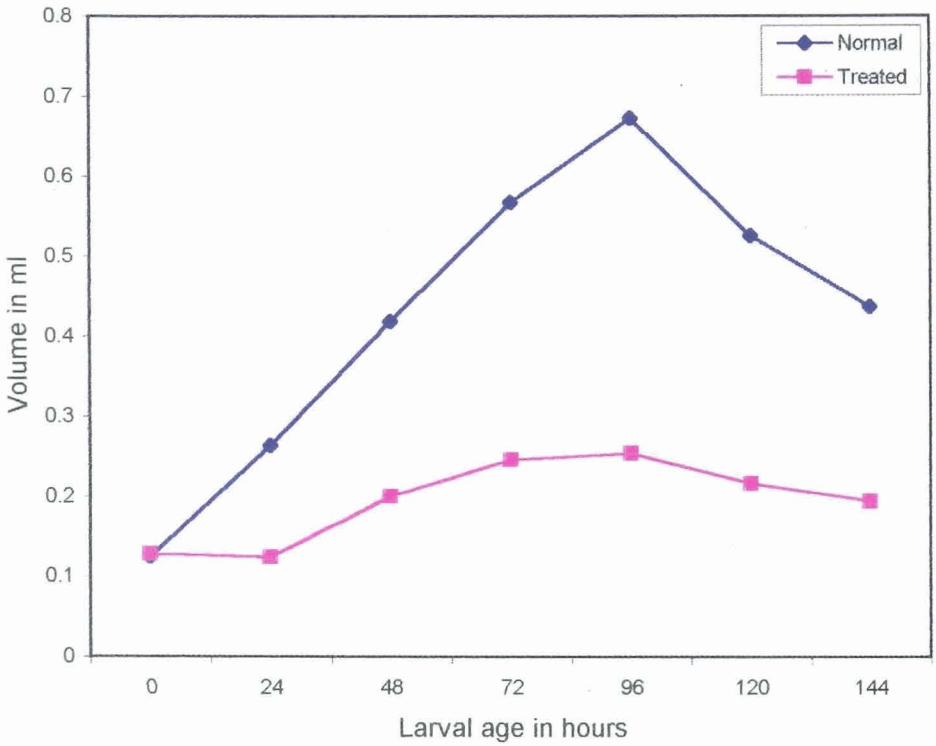

Figure 5 : Changes in the volume of haemolymph during development

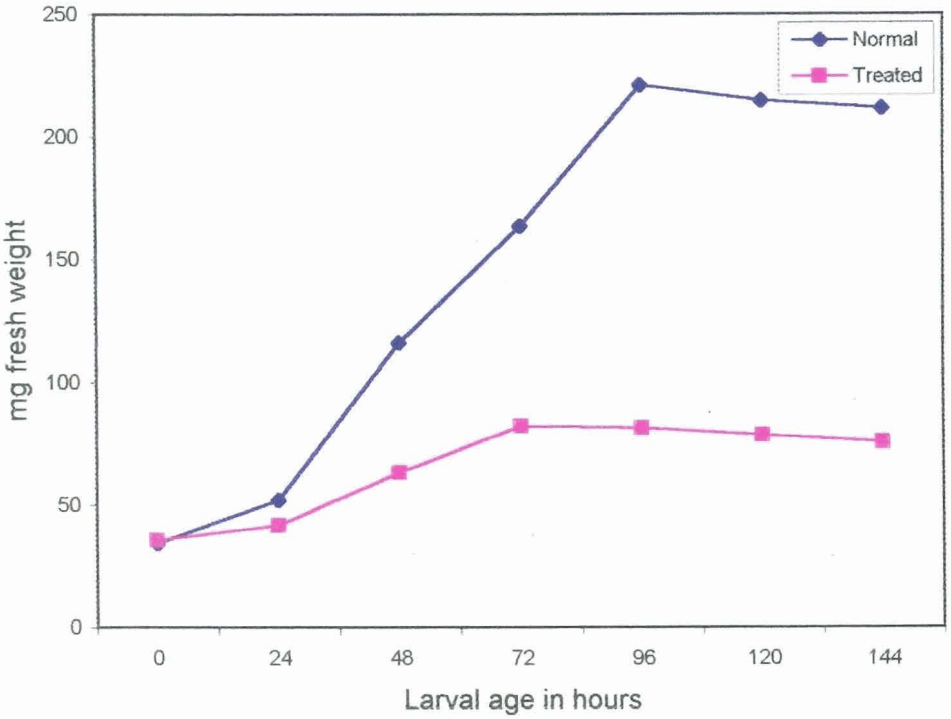

Figure 6 a : Changes in the fresh weight of the fat body

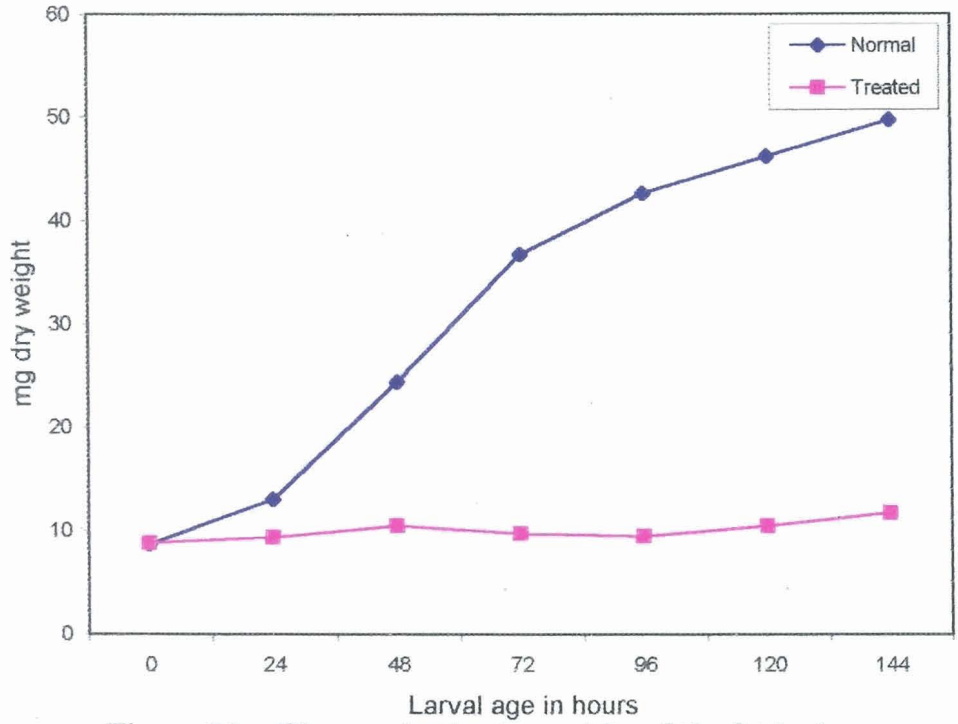

Figure 6 b : Changes in the dry weight of the fat body

Table 7a and 7b

Figure 7a(i) and 7a(ii)

Figure 7b(i) and 7b(ii)

The total haemolymph protein increased with larval age in the normal insect. But in the treated larvae its peak concentration was observed on the fourth day followed by a decline. On the basis of the total tissue the titre of protein showed a sharp rise upto 96 h followed by a dip thereafter. The treated larvae also showed a similar variation on the basis of total tissue but with a low magnitude.

The total protein concentration in fat body increased steadily in both normal and treated larvae throughout the fifth instar when total tissue was considered. The values per unit weight did not show sharp variation but when the whole tissue was considered the total protein content increased more than nine fold from 0 h to 144 h of development in normal ones. But in the treated larvae the fat body proteins per unit weight showed a decline upto 96 h followed by increase upto pupation. In treated larvae the total protein content at the final stage was just more than double

Table 7a

Changes in the levels of total haemolymph proteins during larval development

Larval age hours	Normal mg / ml	Treated mg / ml	Normal mg / larva	Treated mg / larva
0	13.981 ± 1.340	13.621 ± 0.523	1.678 ± 0.112	1.635 ± 0.125
24	22.673 ± 0.713	15.437 ± 0.196	5.895 ± 0.110	1.852 ± 0.173
48	31.432 ± 0.932	16.214 ± 1.057	12.887 ± 0.712	3.248 ± 0.281
72	47.604 ± 1.167	18.473 ± 0.972	26.658 ± 0.823	4.434 ± 0.226
96	60.313 ± 1.714	18.363 ± 1.231	40.410 ± 1.167	4.591 ± 0.137
120	66.185 ± 1.036	16.292 ± 1.183	34.416 ± 1.031	3.421 ± 0.126
144	67.843 ± 1.281	16.173 ± 1.356	29.172 ± 1.002	3.073 ± 0.217

Values are the means of five determinations with standard deviation

Table 7b

Changes in the levels of total fat body proteins during larval development

Larval age hours	Normal mg / mg tissue	Treated mg / mg tissue	Normal mg / larva	Treated mg / larva
0	0.117 ± 0.012	0.121 ± 0.002	4.028 ± 0.341	4.312 ± 0.071
24	0.123 ± 0.003	0.103 ± 0.007	6.397 ± 0.261	4.298 ± 0.292
48	0.126 ± 0.008	0.094 ± 0.007	14.656 ± 0.382	5.965 ± 0.571
72	0.141 ± 0.005	0.073 ± 0.003	23.093 ± 0.921	6.009 ± 0.247
96	0.147 ± 0.002	0.091 ± 0.006	32.508 ± 0.625	7.410 ± 0.489
120	0.160 ± 0.007	0.101 ± 0.005	34.373 ± 0.321	7.941 ± 0.393
144	67.843 ± 1.281	16.173 ± 1.356	29.172 ± 1.002	3.013 ± 0.217
144	0.182 ± 0.008	0.126 ± 0.007	38.518 ± 0.675	9.531 ± 0.529

Values are the means of five determinations with standard deviation

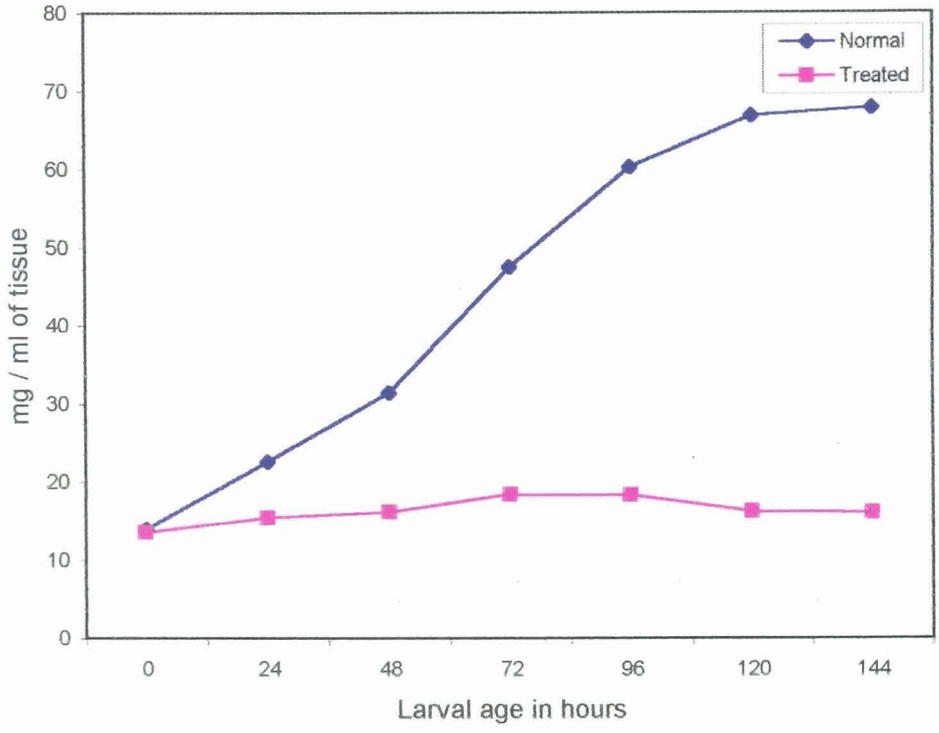

Figure 7 a(i) : Changes in the levels of protein in the haemolymph

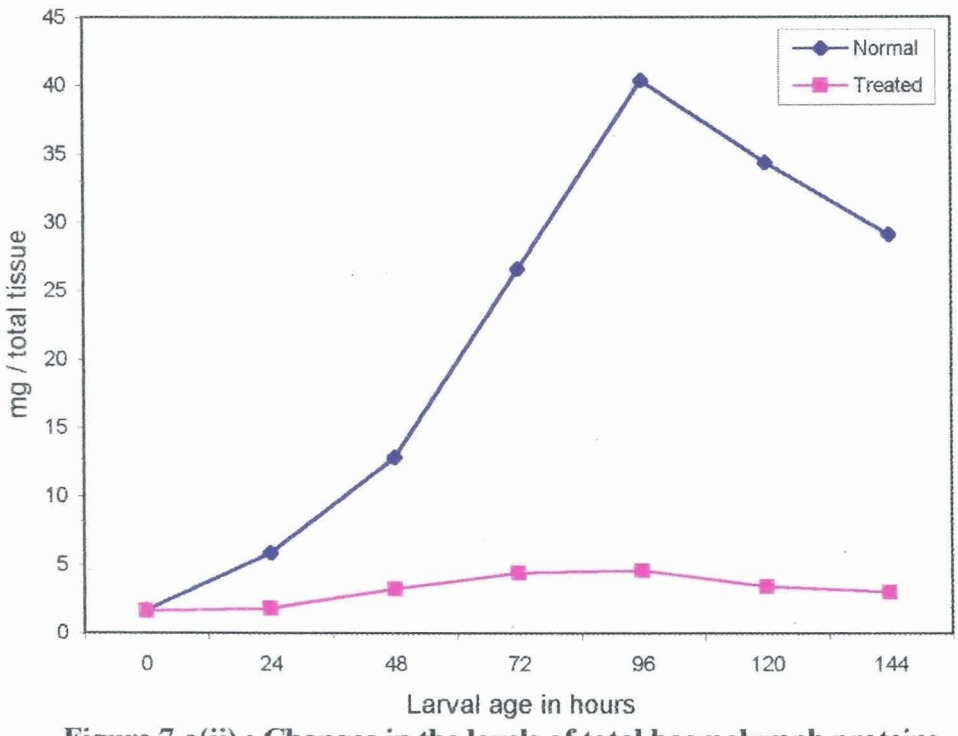

Figure 7 a(ii) : Changes in the levels of total haemolymph proteins

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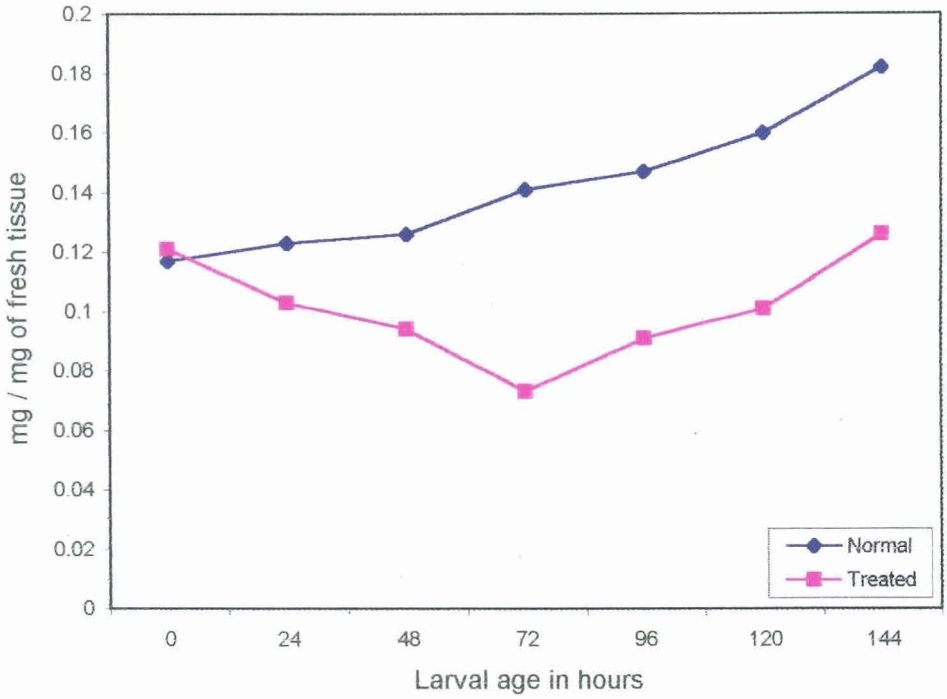

Figure 7 b(i) : Changes in the levels of protein in the fat body

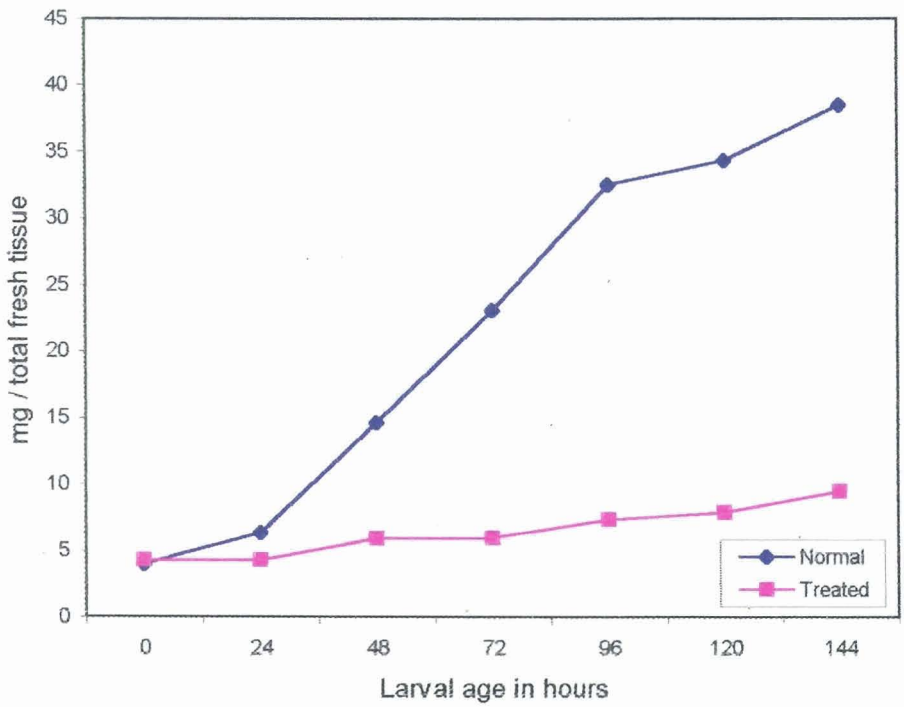

Figure 7 b(ii) : Changes in the levels of protein in the total fat body

to that of the initial stage. On the final day of the larval life the treated larvae contain only 25% of total fat body protein of the normal.

Total free amino acids

The total free amino acid concentration in the haemolymph and fat body of the normal and treated larvae during final instar are given in Tables 8a and 8b and Figures 8a(i), 8a(ii), 8b(i) and 8b(ii).

Table 8a and 8b

Figure 8a(i) and 8a(ii)

Figure 8b(i) and 8b(ii)

The total free amino acids in the haemolymph of the normal larvae sharply increased from 0 h to 72 h and then declined when the values per unit volume are considered. There was a 3 - fold increase in the amount of free amino acids in the haemolymph during the larval development. In the case of treated larvae the increase was more or less gradual and reached the peak at 120 h of development.

Table 8a

Changes in the levels of total haemolymph free amino acids during larval development

Larval age hours	Normal mg / ml	Treated mg / ml	Normal mg / larva	Treated mg / larva
0	5.172 ± 0.191	5.314 ± 0.141	0.621 ± 0.023	0.638 ± 0.017
24	9.622 ± 0.780	6.013 ± 0.233	2.502 ± 0.203	0.722 ± 0.028
48	11.794 ± 0.125	5.647 ± 0.314	4.836 ± 0.003	1.134 ± 0.063
72	14.983 ± 0.903	6.231 ± 0.274	8.390 ± 0.506	1.495 ± 0.066
96	12.842 ± 0.981	6.817 ± 0.461	8.604 ± 0.510	1.704 ± 0.115
120	7.886 ± 0.034	7.335 ± 0.525	4.101 ± 0.018	1.540 ± 0.110
144	7.047 ± 0.061	7.012 ± 0.067	3.030 ± 0.026	1.332 ± 0.013

Values are the means of five determinations with standard deviation

Table 8b

Changes in the levels of total fat body free amino acids during larval development

Larval age hours	Normal mg / mg tissue	Treated mg / mg tissue	Normal mg / larva	Treated mg / larva
0	5.813 ± 0.191	5.634 ± 0.216	199.677 ± 16.57	200.796 ± 7.698
24	6.134 ± 0.363	5.013 ± 0.175	319.029 ± 18.88	209.192 ± 7.175
48	6.394 ± 0.987	5.099 ± 0.321	743.750 ± 14.80	323.583 ± 20.37
72	6.816 ± 0.013	5.416 ± 0.412	1116.32 ± 21.11	445.790 ± 26.14
96	6.800 ± 0.436	5.621 ± 0.478	1503.75 ± 26.35	458.207 ± 18.86
120	5.194 ± 0.215	5.781 ± 0.392	1115.82 ± 16.18	454.502 ± 30.81
144	5.016 ± 0.273	5.613 ± 0.261	1061.58 ± 27.60	424.567 ± 20.65

Values are the means of five determinations with standard deviation

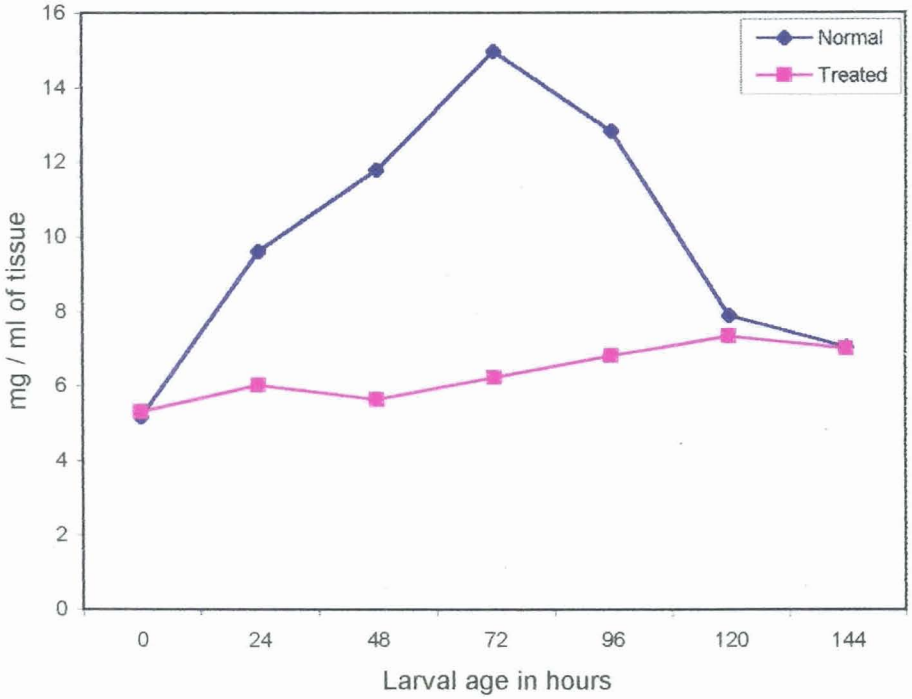

Figure 8 a(i) : Changes in the levels of total free amino acids in the haemolymph

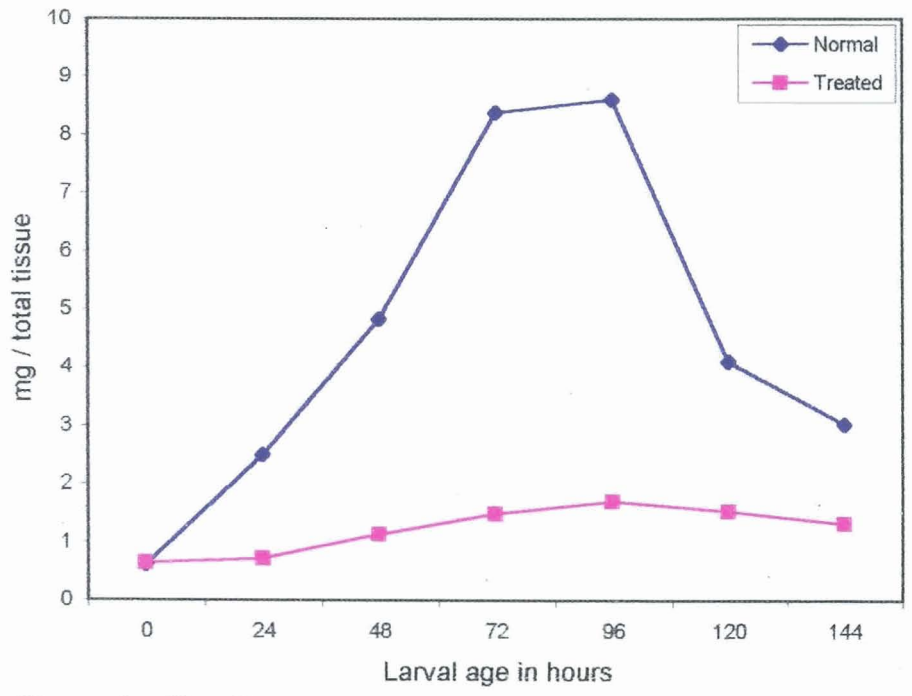

Figure 8 a(ii) : Changes in the levels of total free amino acids in the total haemolymph

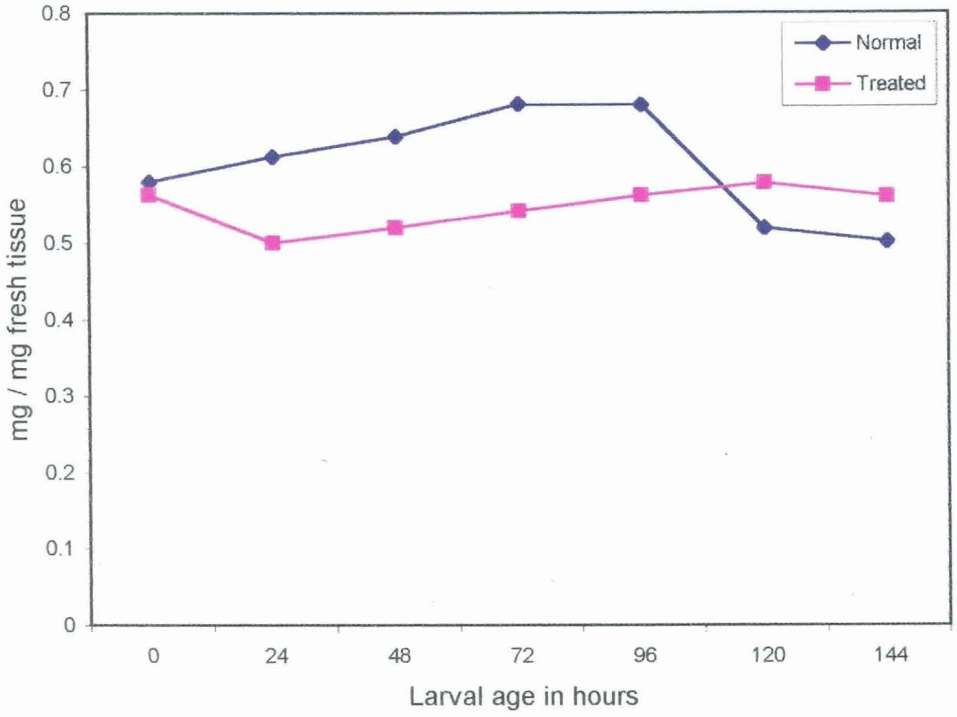

Figure 8 b(i) : Changes in the levels of total free amino acids in the fat body

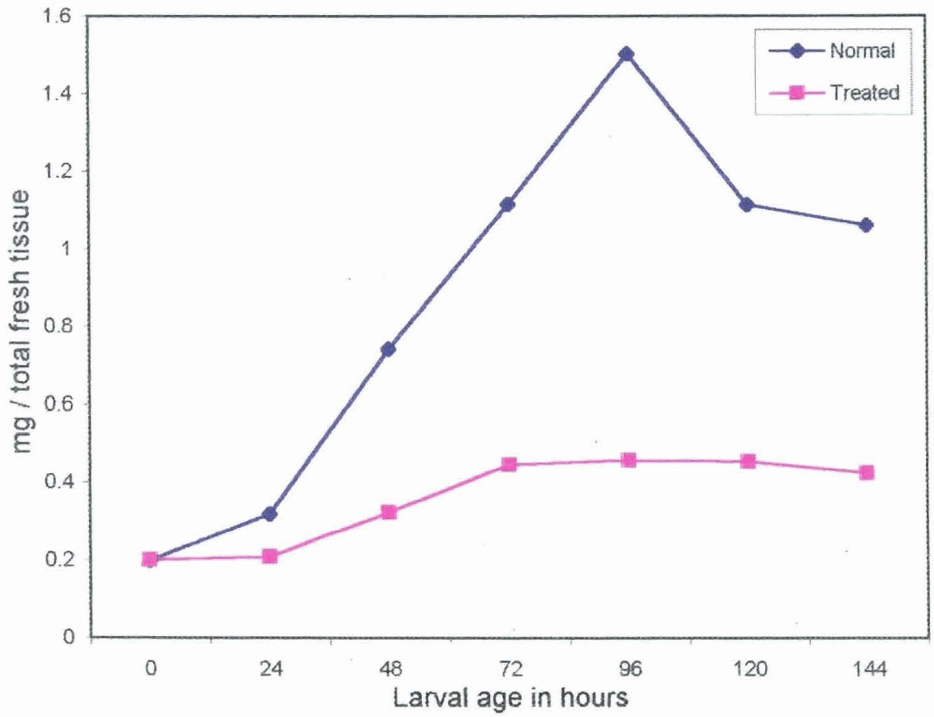

Figure 8 b(ii) : Changes in the levels of free amino acids in the total fat body

On the basis of total free amino acids in the total haemolymph both normal and treated larvae showed the peak value at 96 h of development. The rise from 0 h to 96 h was 14 - fold in normal but only about 3 - fold in the case of carbaryl treated larvae. The later contains only 20% of the total free amino acids in the haemolymph of a normal when peak values are considered.

The total free amino acids in the fat body of normal larvae steadily increased from 0 h to 96 h of development and then declined. Treated larvae also showed a similar pattern. When concentration in the total fat body tissue was estimated, it was found that at the peak concentration carbaryl treated larvae contain only 30% that of free amino acids observed in the normal.

Urea

The concentration of urea in the haemolymph and fat body of normal and treated larvae during the development of the final instar is shown in Tables 9a and 9b and Figures 9a(i), 9a(ii), 9b(i) and 9b(ii).

Table 9a and 9b

Figure 9a(i) and 9a(ii)

Figure 9b(i) and 9b(ii)

The urea levels in the haemolymph declined conspicuously in both normal and treated larvae during development. The changes were sharper in the case of treated larvae. There was a 10 to 15 - fold variation in the titre of the material during larval development.

In the fat body the urea level dropped sharply in both normal and treated larvae when values per unit weight were considered. However on the basis of total fat body, urea content increased to a peak at 72 h in normal larvae. In the case of carbaryl treated larvae no such increase was noted but the level gradually declined up to 144 h larval stage.

Glucose

The changes in the concentration of glucose in the haemolymph and fat body of both normal and treated larvae during the development stages of fifth instar are presented in

Table 9 a

Changes in the levels of total haemolymph urea during larval development

Larval age hours	Normal mg / ml	Treated mg / ml	Normal mg / larva	Treated mg / larva
0	1.201 ± 0.120	1.236 ± 0.110	0.144 ± 0.014	0.148 ± 0.013
24	1.110 ± 0.091	1.015 ± 0.093	0.289 ± 0.024	0.122 ± 0.011
48	0.832 ± 0.076	0.616 ± 0.044	0.341 ± 0.031	0.123 ± 0.009
72	0.695 ± 0.038	0.437 ± 0.016	0.389 ± 0.021	0.105 ± 0.004
96	0.437 ± 0.045	0.218 ± 0.021	0.293 ± 0.030	0.055 ± 0.005
120	0.155 ± 0.021	0.093 ± 0.007	0.081 ± 0.011	0.020 ± 0.002
144	0.143 ± 0.026	0.081 ± 0.009	0.062 ± 0.011	0.015 ± 0.001

Values are the means of five determinations with standard deviation

Table 9 b

Changes in the levels of total fat body urea during larval development

Larval age hours	Normal mg / mg tissue	Treated mg / mg tissue	Normal mg / larva	Treated mg / larva
0	0.421 ± 0.040	0.392 ± 0.021	14.495 ± 1.377	13.971 ± 0.748
24	0.293 ± 0.091	0.216 ± 0.011	15.239 ± 4.733	9.014 ± 0.459
48	0.176 ± 0.011	0.148 ± 0.007	20.472 ± 1.280	9.392 ± 0.444
72	0.113 ± 0.007	0.095 ± 0.004	21.783 ± 1.146	7.819 ± 0.329
96	0.064 ± 0.005	0.046 ± 0.002	14.153 ± 1.106	3.746 ± 0.163
120	0.032 ± 0.002	0.025 ± 0.002	6.875 ± 0.430	1.966 ± 0.157
144	0.024 ± 0.002	0.017 ± 0.001	5.079 ± 0.423	1.286 ± 0.076

Values are the means of five determinations with standard deviation

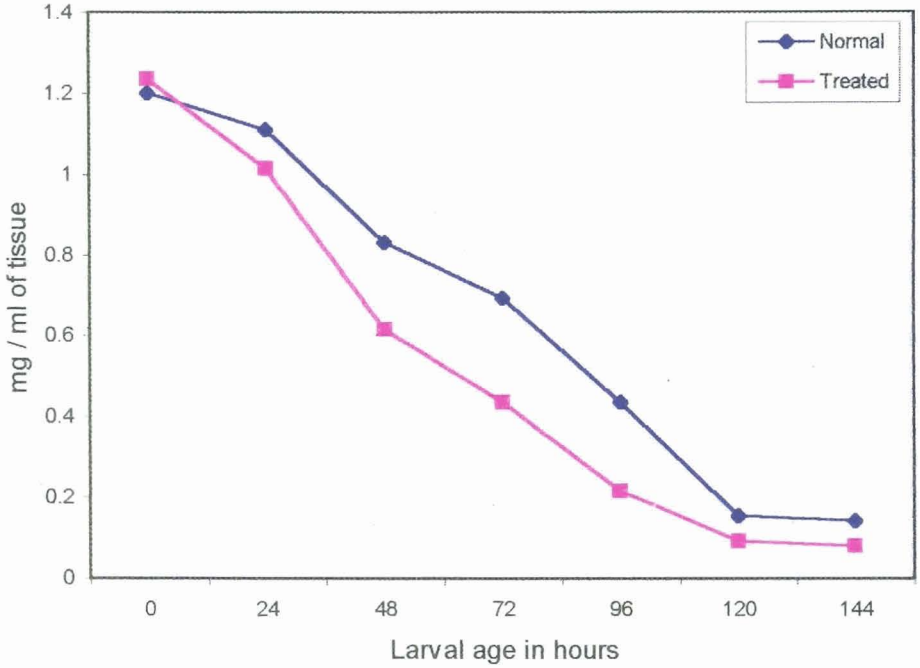

Figure 9 a(i) : Changes in the levels of urea in the haemolymph

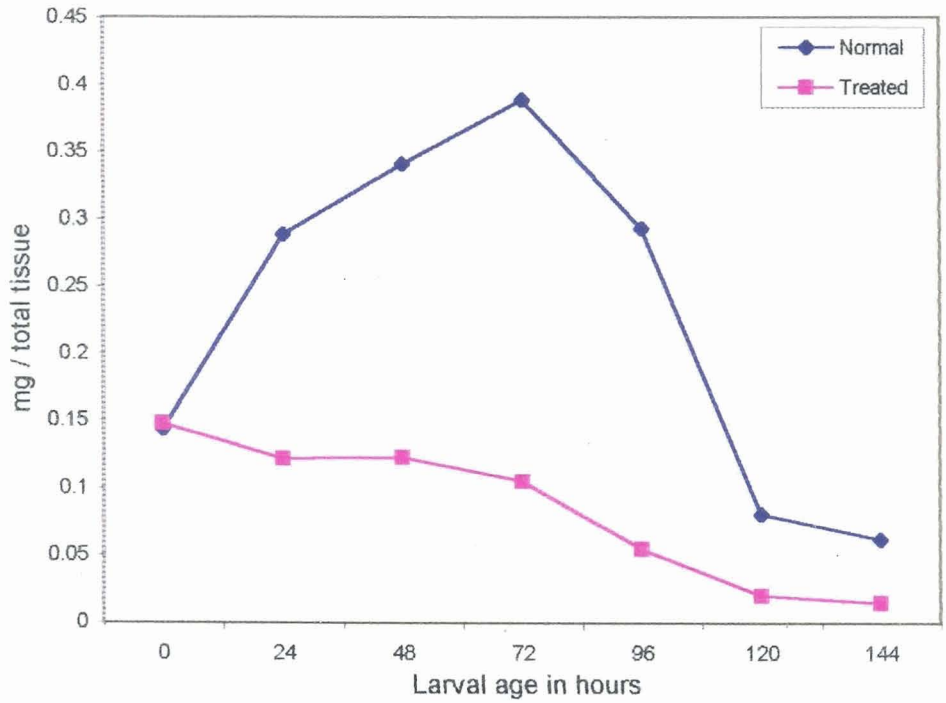

Figure 9 a(ii) : Changes in the levels of urea in the total haemolymph

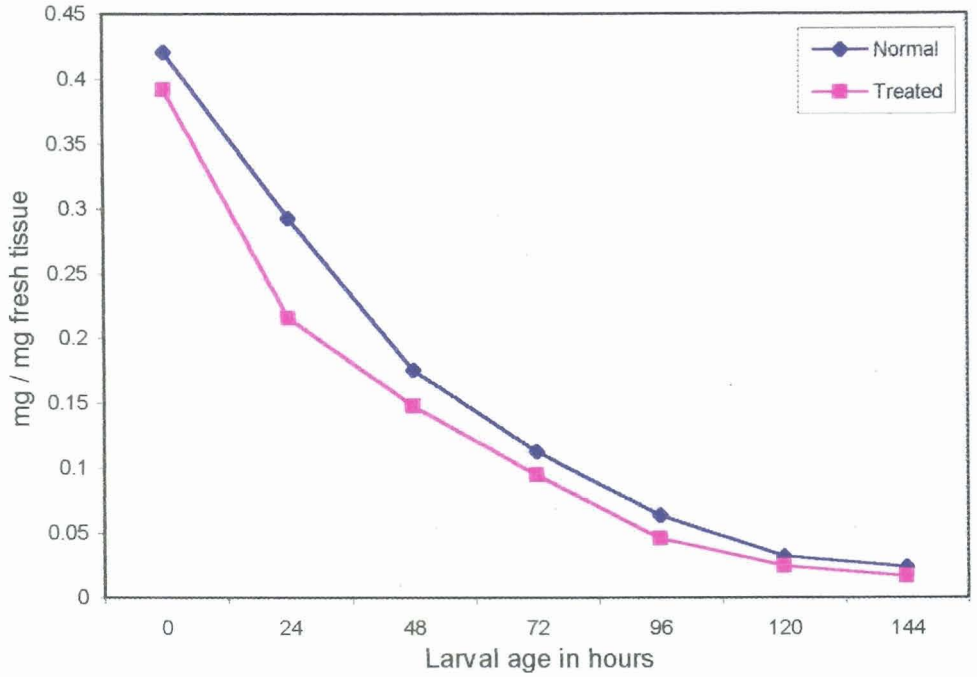

Figure 9 b(i) : Changes in the levels of urea in the fat body

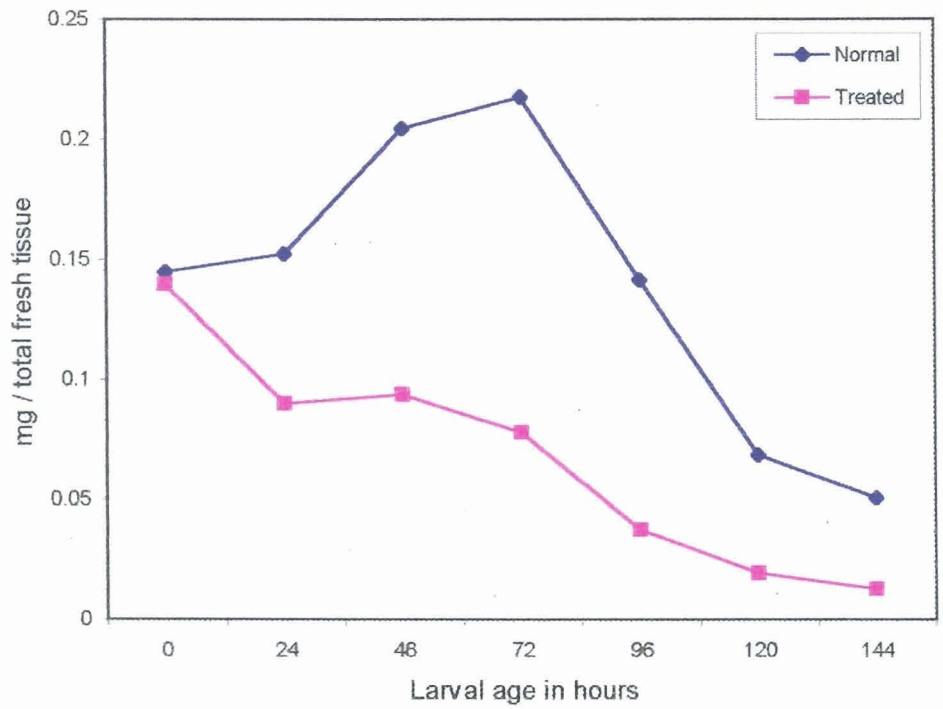

Figure 9 b(ii) : Changes in the levels of urea in the total fat body

Tables 10a and 10b and Figures 10a(i), 10a(ii), 10b(i) and 10b(ii).

Table 10a and 10b

Figure 10a(i) and 10a(ii)

Figure 10b(i) and 10b(ii)

In the haemolymph of normal larvae the glucose content steadily increased with the development and reached a peak at 72 h stage. Then it declined gradually up to the final day of larval development. When the total haemolymph glucose concentration was considered, the pattern of variation was similar, but the peak value was at 96 h stage. This peak value recorded was almost 17 times to that found at the beginning stage of the larva. In the case of carbaryl treated larvae the concentration of the glucose was initially low followed by gradual increase, which was similar to that of normal.

The fat body glucose also exhibited a variation in accordance with haemolymph in normal larvae with the maximum at 72 h stage. This maximum concentration was 10

Table 10 a

Changes in the levels of total haemolymph glucose during larval development

Larval age hours	Normal mg / ml	Treated mg / ml	Normal mg / larva	Treated mg / larva
0	0.813 ± 0.091	0.835 ± 0.081	0.098 ± 0.011	0.100 ± 0.010
24	1.131 ± 0.067	0.716 ± 0.035	0.294 ± 0.017	0.086 ± 0.004
48	1.256 ± 0.334	0.987 ± 0.027	0.015 ± 0.137	0.194 ± 0.005
72	2.815 ± 0.137	1.125 ± 0.036	1.576 ± 0.077	0.270 ± 0.009
96	2.536 ± 0.082	1.304 ± 0.117	1.699 ± 0.055	0.326 ± 0.029
120	2.491 ± 0.054	1.328 ± 0.089	1.295 ± 0.028	0.279 ± 0.019
144	2.163 ± 0.031	1.265 ± 0.092	0.930 ± 0.013	0.240 ± 0.017

Values are the means of five determinations with standard deviation

Table 10 b

Changes in the levels of total fat body glucose during larval development

Larval age hours	Normal mg / mg tissue	Treated mg / mg tissue	Normal mg / larva	Treated mg / larva
0	3.031 ± 0.526	3.105 ± 0.123	104.357 ± 18.11	110.622 ± 4.384
24	4.028 ± 0.077	2.416 ± 0.093	209.080 ± 4.005	100.820 ± 4.090
48	5.107 ± 0.038	1.925 ± 0.101	594.046 ± 4.420	122.161 ± 6.409
72	6.351 ± 0.757	1.736 ± 0.087	1040.167 ± 12.40	142.890 ± 7.160
96	4.432 ± 0.631	1.443 ± 0.116	1040.167 ± 11.39	117.503 ± 9.446
120	4.219 ± 0.446	1.095 ± 0.074	1040.167 ± 12.81	86.089 ± 5.818
144	4.073 ± 0.281	1.032 ± 0.086	1040.167 ± 10.40	78.060 ± 6.505

Values are the means of five determinations with standard deviation

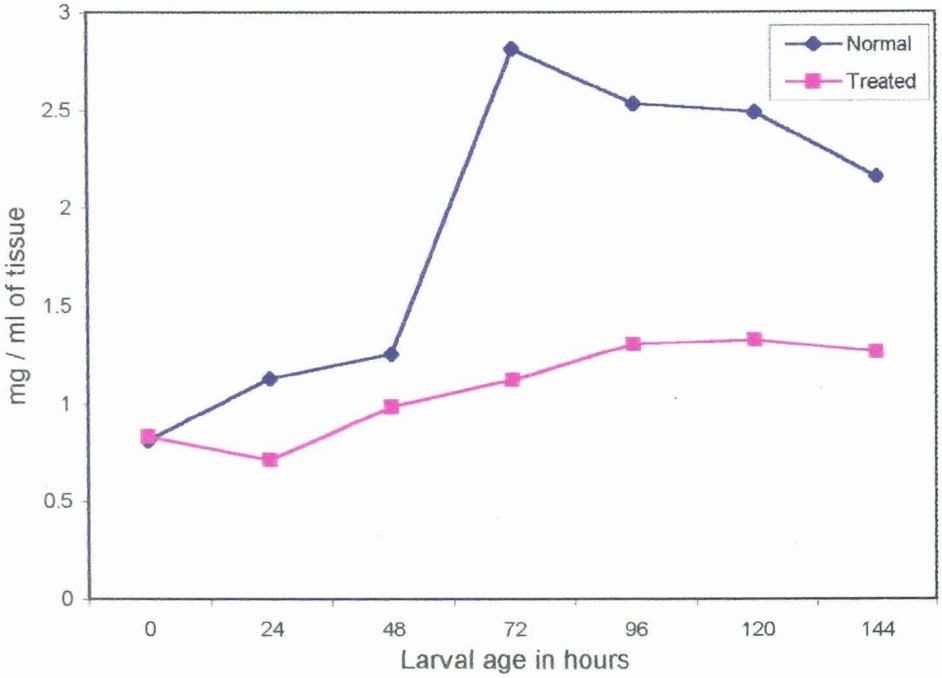

Figure 10 a(i) : Changes in the levels of glucose in the haemolymph

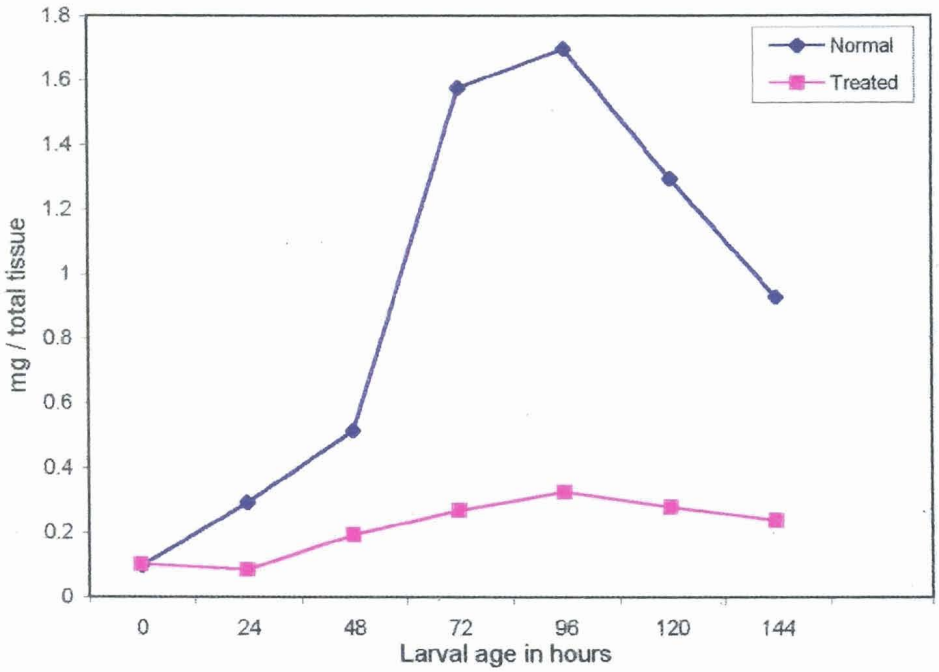

Figure 10 a(ii) : Changes in the levels of glucose in the total haemolymph

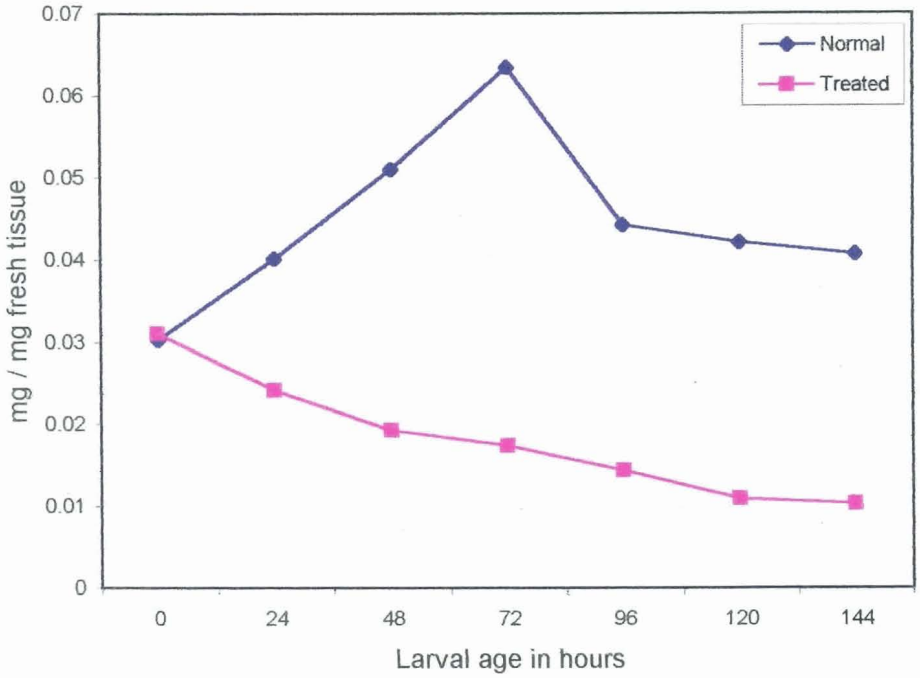

Figure 10 b(i) : Changes in the levels of glucose in the fat body

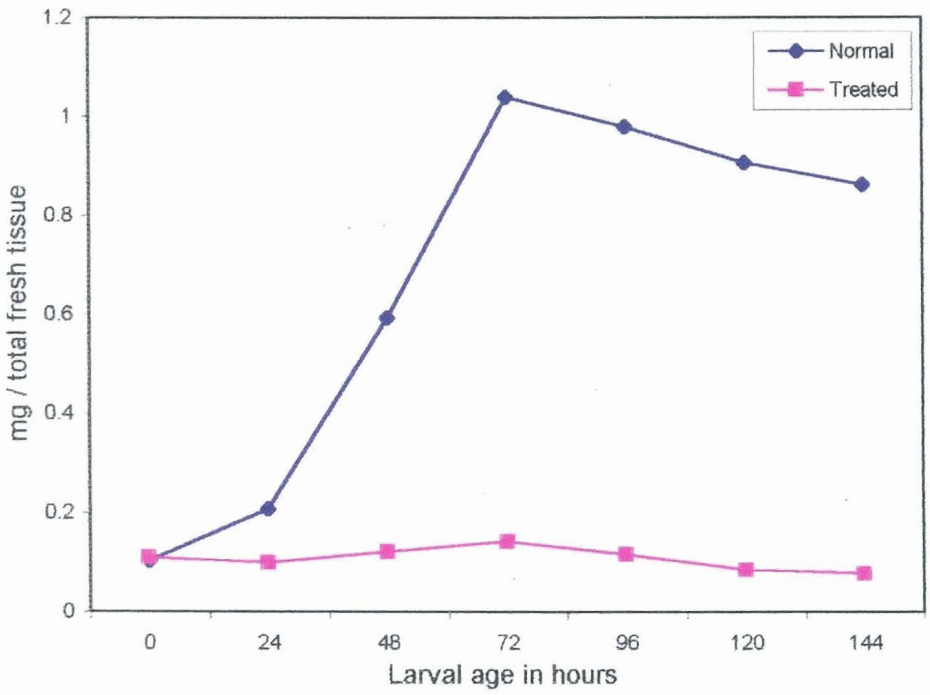

Figure 10 b(ii) : Changes in the levels of glucose in the total fat body

times higher than that at the beginning stage of fifth instar when the total content was considered.

The glucose content in the fat body of carbaryl treated larvae exhibited a constant reduction from 0 h to 144 h stage when estimated per unit weight of fat body. At the end of the larval period the glucose concentration was only 33% of that of the beginning. On the basis of the total fat body glucose, the amount was initially low but increased up to 72 h followed by a dip thereafter.

Aspartate aminotransferase (AAT) activity

The effect of change in pH on AAT activity or glutamate oxaloacetate transaminase (GOT) activity are presented in Table 11 and Figure 11

Table 11

Figure 11

The optimum pH for AAT activity was found to be at pH 7.5 during the present investigation.

The changes in the AAT activity of the fat body of 72 h larvae with different substrate and substrate-carbaryl

mixture concentrations were observed to find the Km values and the Lineweaver-Burk double reciprocal plot for the same presented in Table 12 and Figures 12a and 12b

Table 12

Figure 12a

Figure 12b

The results indicate that the inhibition of carbaryl upon larval AAT activity was a mixed type of both competitive and noncompetitive enzyme inhibitions. The Km value of AAT activity was found to be 0.083.

The AAT activity in the haemolymph and fat body of both normal and treated larvae during its development is recorded in Tables 13a and 13b and Figures 13a(i), 13a(ii), 13b(i) and 13b(ii).

Table 13a and 13b

Figure 13a(i) and 13a(ii)

Figure 13b(i) and 13b(ii)

Table 11

Change in the AAT activity of 72h larval fat body with the pH

pH	AAT activity units / mg x 10^{-3}
6	0.973 ± 0.026
6.5	1.486 ± 0.101
7	2.012 ± 0.113
7.5	2.031 ± 0.082
8	1.256 ± 0.091

Values are the means of five determinations with standard deviation

Table 12

Effect of inhibitor concentration on AAT activity of 72h larval fat body

Concentration micro moles	Substrate only units / mg x 10^{-3}	Substrate + Carbaryl units / mg x 10^{-3}
0.1	3.012 ± 0.121	2.004 ± 0.031
0.2	3.390 ± 0.106	2.717 ± 0.132
0.3	3.745 ± 0.119	3.077 ± 0.104
0.4	4.082 ± 0.103	3.301 ± 0.127
0.5	4.255 ± 0.112	3.461 ± 0.193

Values are the means of five determinations with standard deviation

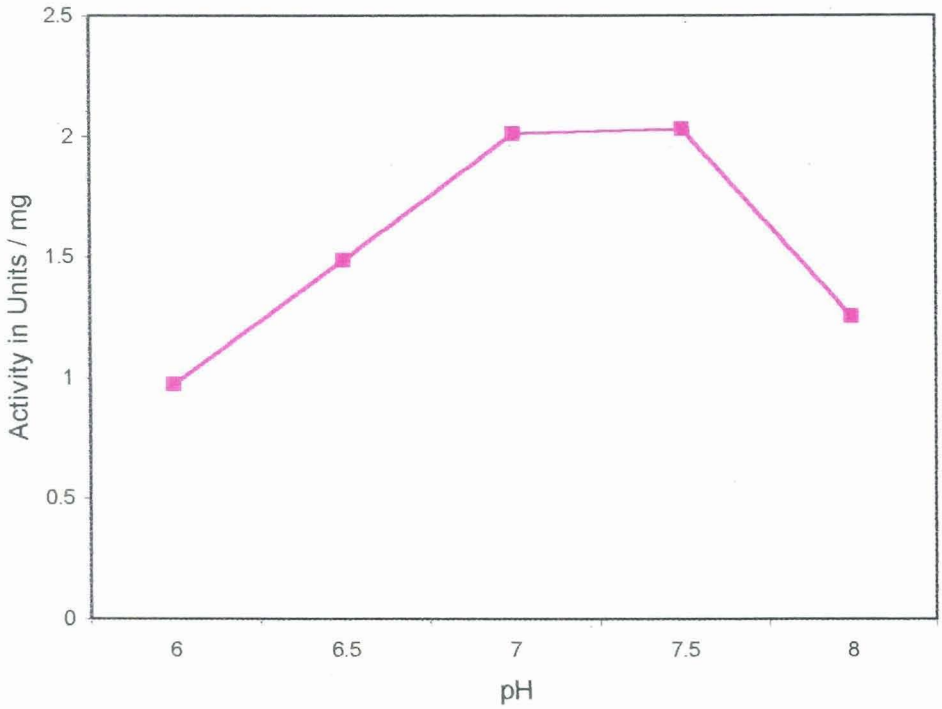

Figure 11 : Changes in the AAT activity with different pH

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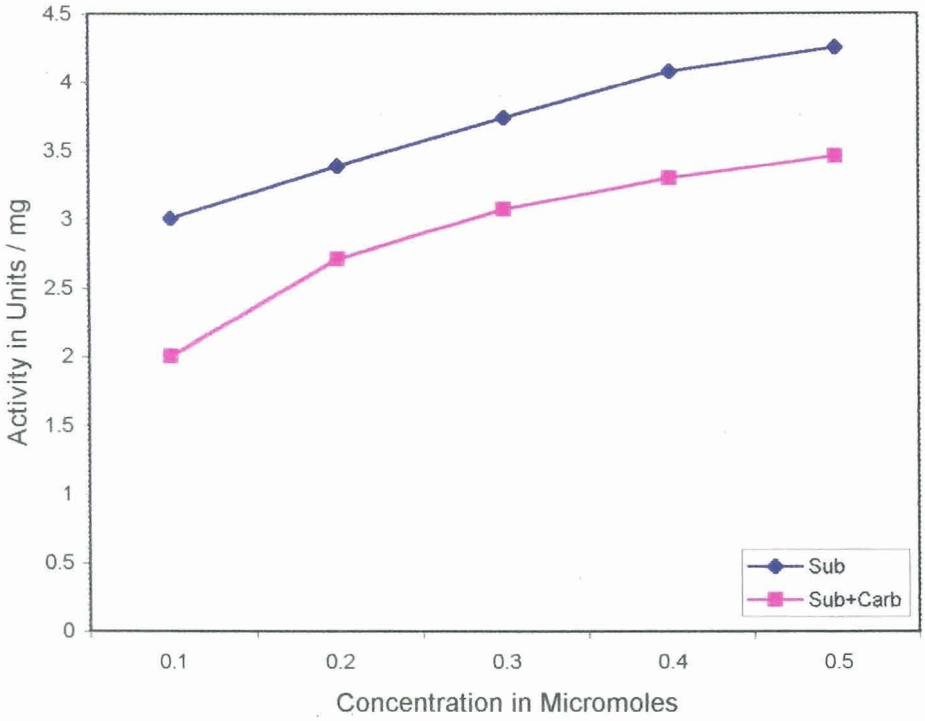

Figure 12 a : Effect of inhibitor concentration on fat body AAT activity

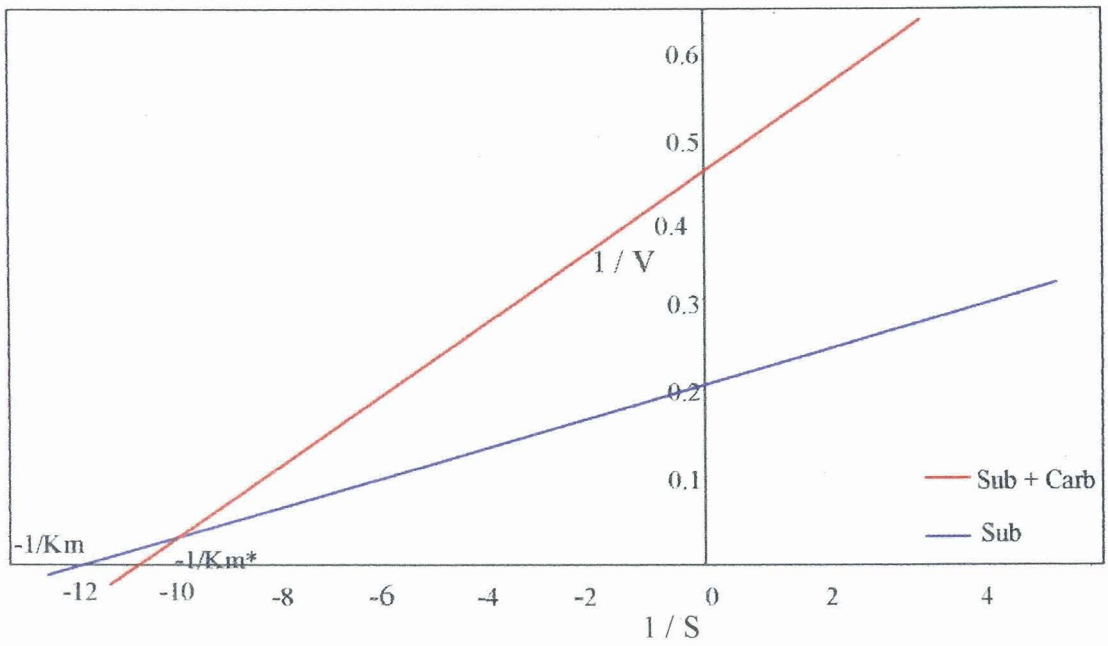

Figure 12 b : Lineweaver-Burk double reciprocal plot on AAT activity

Table 13 a

Changes in the levels of haemolymph AAT activity during larval development

Larval age hours	Normal units / ml $x10^{-3}$	Treated units / ml $x 10^{-3}$	Normal units / larva $x10^{-3}$	Treated units / larva $x10^{-3}$
0	0.634 ± 0.021	0.617 ± 0.025	0.076 ± 0.003	0.074 ± 0.003
24	0.815 ± 0.062	0.938 ± 0.076	0.212 ± 0.016	0.113 ± 0.009
48	0.983 ± 0.047	1.715 ± 0.109	0.403 ± 0.019	0.343 ± 0.022
72	1.256 ± 0.096	2.980 ± 0.114	0.703 ± 0.054	0.715 ± 0.027
96	0.968 ± 0.041	1.499 ± 0.092	0.649 ± 0.027	0.375 ± 0.023
120	0.713 ± 0.033	0.821 ± 0.077	0.371 ± 0.017	0.172 ± 0.019
144	0.591 ± 0.041	0.732 ± 0.081	0.254 ± 0.018	0.139 ± 0.015

Values are the means of five determinations with standard deviation

Table 13 b

Changes in the levels of fat body AAT activity during larval development

Larval age hours	Normal units / mg $x10^{-3}$	Treated units / mg $x 10^{-3}$	Normal units / larva $x10^{-3}$	Treated units / larva $x10^{-3}$
0	2.201 ± 0.110	2.175 ± 0.113	75.780 ± 03.787	77.517 ± 4.027
24	2.615 ± 0.232	3.035 ± 0.157	136.006 ± 12.066	126.651 ± 6.552
48	2.726 ± 0.173	4.168 ± 0.231	317.088 ± 20.123	264.501 ± 14.659
72	2.071 ± 0.195	4.377 ± 0.215	339.188 ± 31.937	360.271 ± 17.697
96	1.823 ± 0.151	2.986 ± 0.131	403.138 ± 33.392	243.150 ± 10.667
120	1.418 ± 0.096	1.732 ± 0.088	304.629 ± 20.624	136.170 ± 6.919
144	1.093 ± 0.091	1.652 ± 0.113	231.323 ± 19.259	124.957 ± 8.547

Values are the means of five determinations with standard deviation

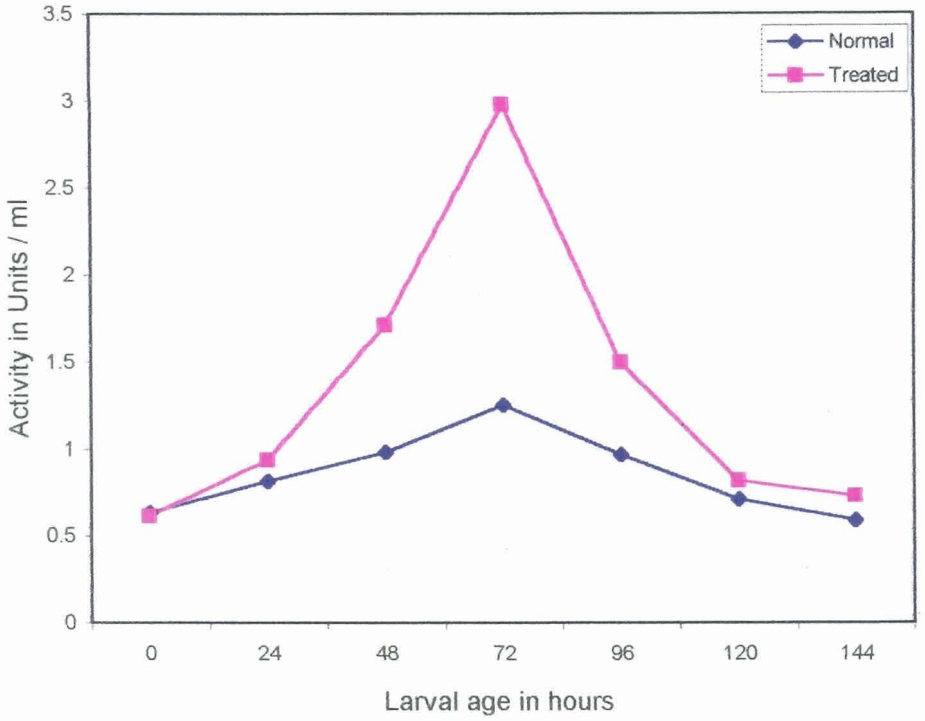

Figure 13 a(i) : Changes in the levels of haemolymph AAT activity

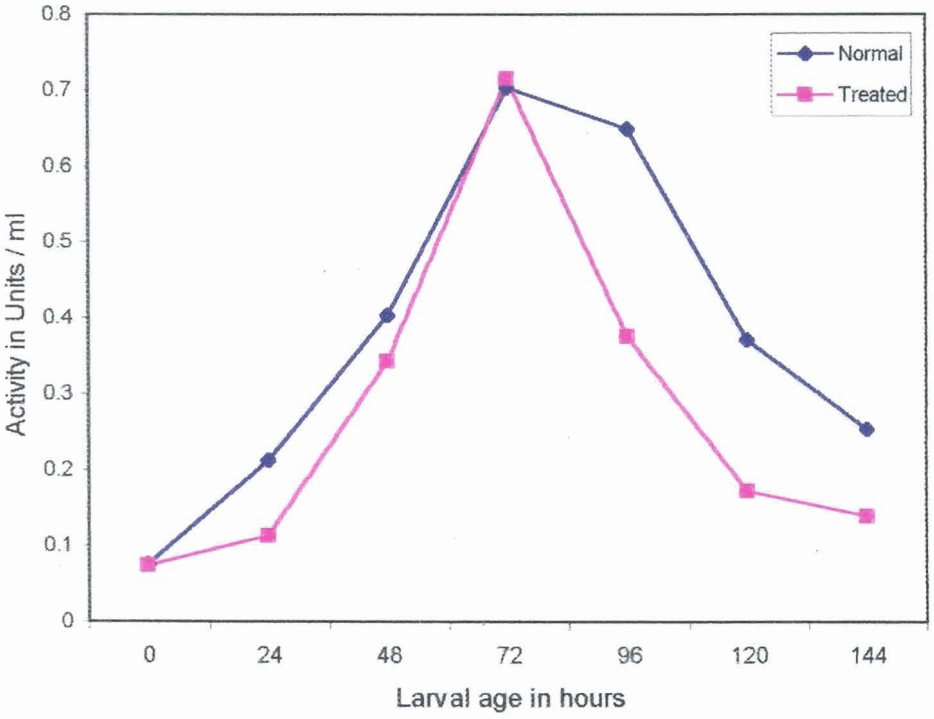

Figure 13 a(ii) : Changes in the levels of total haemolymph AAT activity

SIF

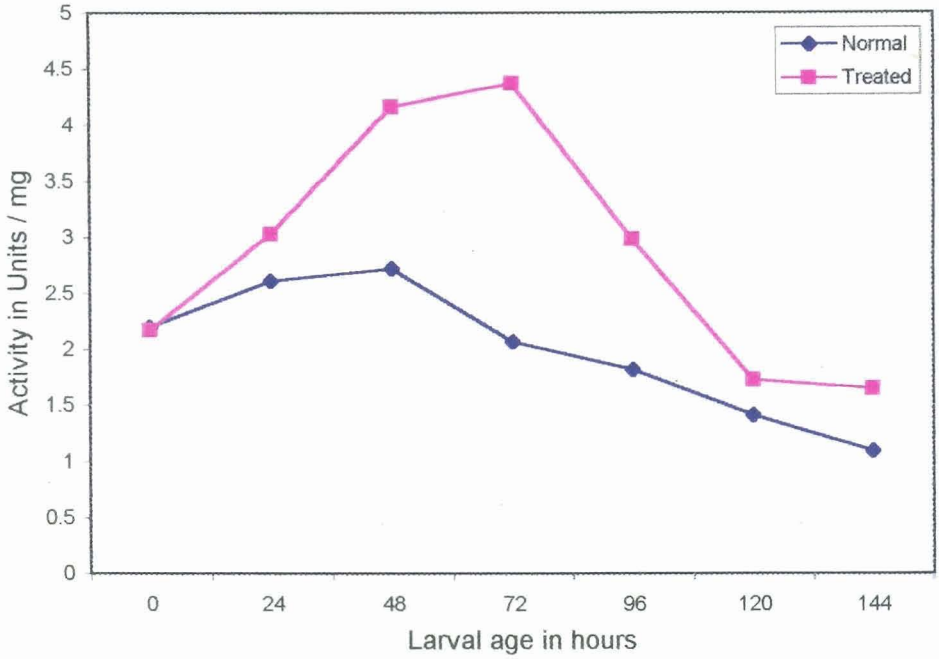

Figure 13 b(i) : Changes in the levels of fat body ALAT activity

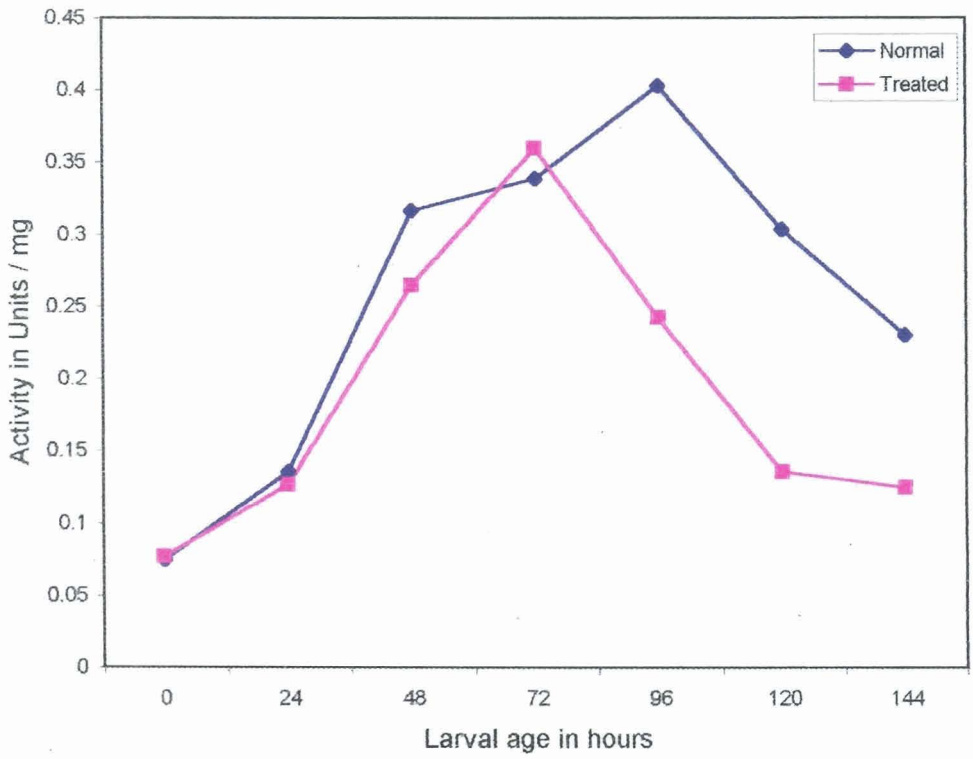

Figure 13 b(ii) : Changes in the levels of total fat body ALAT activity

In the haemolymph of the normal larvae AAT activity increased with development attaining a maximum at 72 h stage. When activity units per unit volume were considered the hike was 2 - fold whereas activity per total tissue showed a 10 - fold increment. Then the activity declined steadily as the development progressed.

The treatment of carbaryl caused an increased AAT activity in the larval haemolymph. However the pattern of variation in activity was similar to that of the normal. At the peak level the activity in normal was only 40% of that found in the treated larvae. The fat body of normal and treated larvae also showed a similar variation in its AAT activity. The activity was much higher in the case of carbaryl treated insects.

The specific activities of AAT in the haemolymph and fat body of the normal and treated larvae were recorded in Tables 14a and 14b and Figures 14a and 14b.

Table 14a and 14b

Figure 14a and 14b

Table 14 a

Changes in the levels of specific activity of AAT in the haemolymph during larval development

Larval age hours	Normal units / mg of protein x 10^{-3}	Treated units / mg of protein x 10^{-3}
0	0.045 ± 0.004	0.045 ± 0.003
24	0.036 ± 0.003	0.061 ± 0.004
48	0.031 ± 0.002	0.106 ± 0.007
72	0.026 ± 0.002	0.161 ± 0.004
96	0.016 ± 0.001	0.082 ± 0.005
120	0.011 ± 0.001	0.051 ± 0.002
144	0.009 ± 0.001	0.045 ± 0.001

Values are the means of five determinations with standard deviation

Table 14 b

Changes in the levels of specific activity of AAT in the fat body during larval development

Larval age hours	Normal units / mg of protein x 10^{-3}	Treated units / mg of protein x 10^{-3}
0	18.812 ± 0.604	17.975 ± 0.903
24	21.261 ± 1.003	29.466 ± 1.004
48	21.635 ± 0.762	44.341 ± 2.017
72	14.689 ± 1.002	59.959 ± 2.034
96	12.401 ± 0.341	32.813 ± 1.128
120	8.863 ± 0.746	17.149 ± 0.901
144	6.005 ± 0.231	13.111 ± 0.784

Values are the means of five determinations with standard deviation

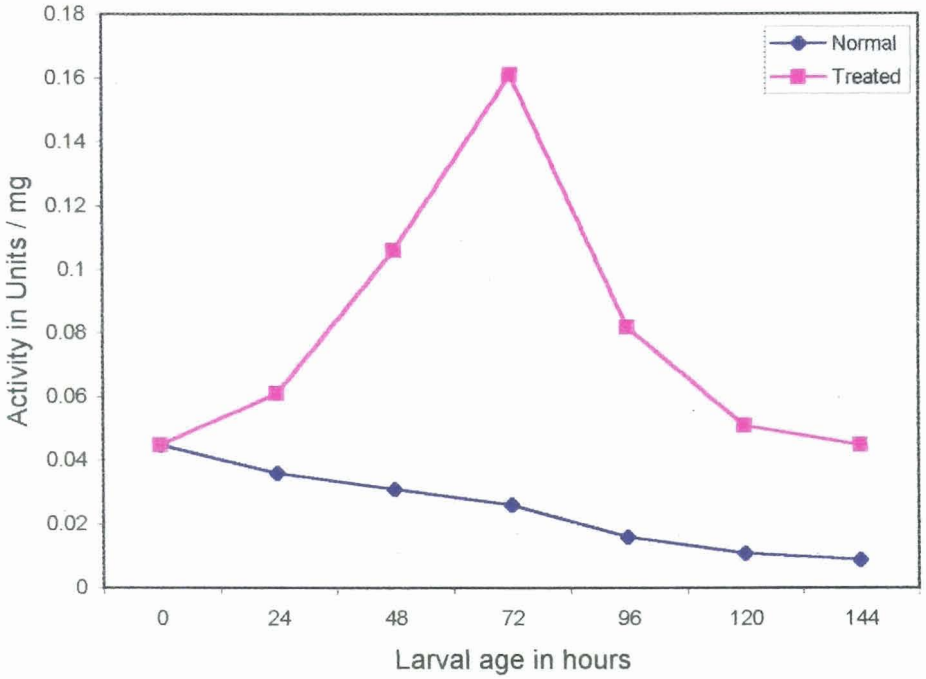

Figure 14 a : Changes in levels of specific activity of haemolymph AAT

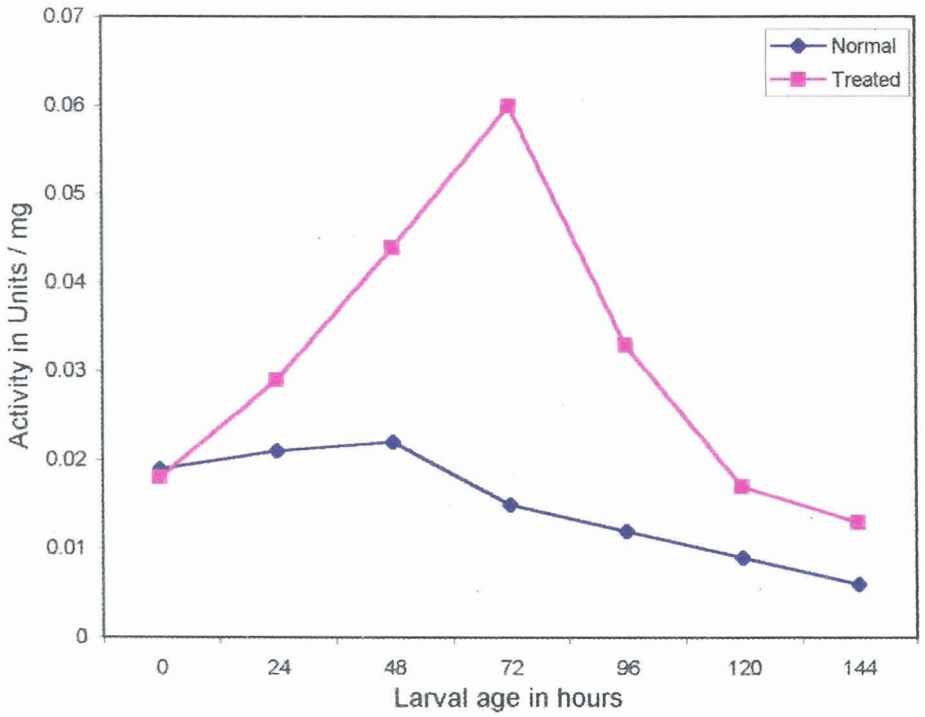

Figure 14 b : Changes in the levels of specific activity of fat body AAT

The specific activity shows a gradual decline from 0 h to 144 h in the case of normal larvae. On the final day of the larval instar the value reduced to 20% of that of the first day. But in treated larvae it was in tune with that of the tissue activity levels with the peak on 72 h. The activity levels in the fat body were in accordance with that of the tissue AAT in both normal and carbaryl treated larvae.

Alanine aminotransferase (ALAT) activity

The change in the ALAT activity or glutamate pyruvate transaminase (GPT) activity of the 72 h larvae with different pH were observed and the data are given in Table 15 and Figure 15.

Table 15

Figure 15

The optimum pH for ALAT activity was found to be at pH 7.1.

The changes in the activity of ALAT of 72 h larvae with different substrate and substrate-inhibitor

concentrations are presented in Table 16 and Figures 16a and 16b.

Table 16

Figure 16a

Figure 16b

The Km value of ALAT was found to be 0.125. The Lineweaver-Burk double reciprocal plot of the substrate and substrate with carbaryl against reaction velocity of ALAT showed that its mode of inhibition was of a mixed type. Similar to the observations of AAT activity, the mode of inhibition was found to be of a mixed type.

The ALAT activities or glutamate pyruvate transaminase (GPT) activities in the haemolymph and fat body of both normal and treated larvae during its development in fifth instar is presented in Tables 17a and 17b and Figures 17a(i), 17a(ii), 17b(i) and 17b(ii).

Table 17a and 17b

Figure 17a(i) and 17a(ii)

Table 15

Change in the ALAT activity of 72h larval fat body with the pH

pH	ALAT activity units / mg x 10^{-3}
6	4.613 ± 0.131
6.5	5.824 ± 0.107
7	6.241 ± 0.091
7.5	6.228 ± 0.101
8	4.731 ± 0.085

Values are the means of five determinations with standard deviation

Table 16

Effect of inhibitor concentration on ALAT activity of 72h larval fat body

Concentration micro moles	Substrate only units / mg x 10^{-3}	Substrate + Carbaryl units / mg x 10^{-3}
0.1	5.821 ± 0.231	4.001 ± 0.121
0.2	6.013 ± 0.173	5.128 ± 0.237
0.3	7.042 ± 0.119	6.135 ± 0.249
0.4	7.692 ± 0.186	6.849 ± 0.106
0.5	8.216 ± 0.238	7.353 ± 0.308

Values are the means of five determinations with standard deviation

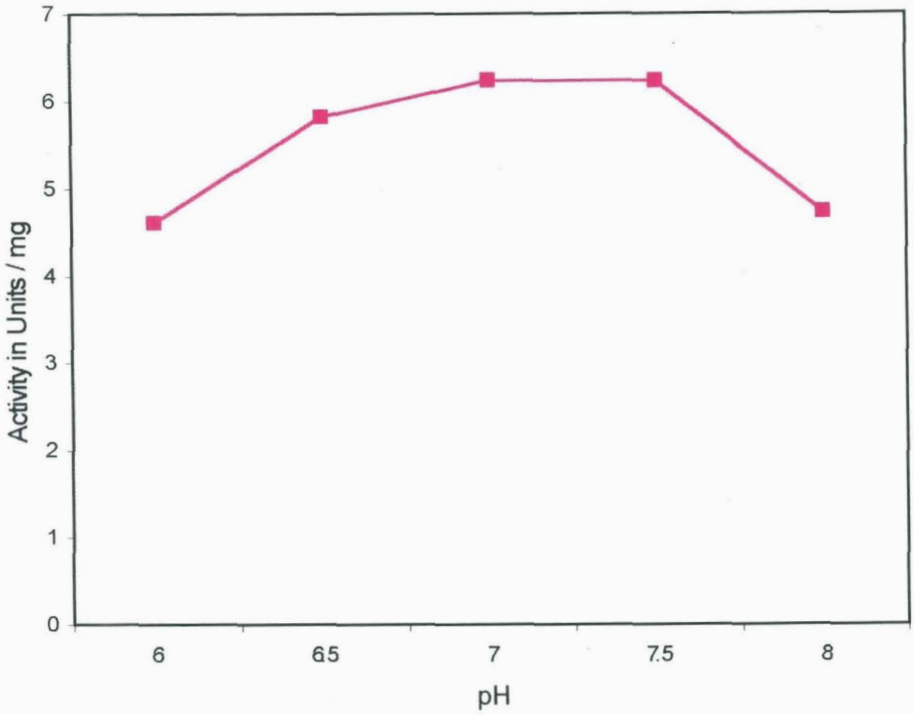

Figure 15 : Changes in the fat body ALAT activity with different pH

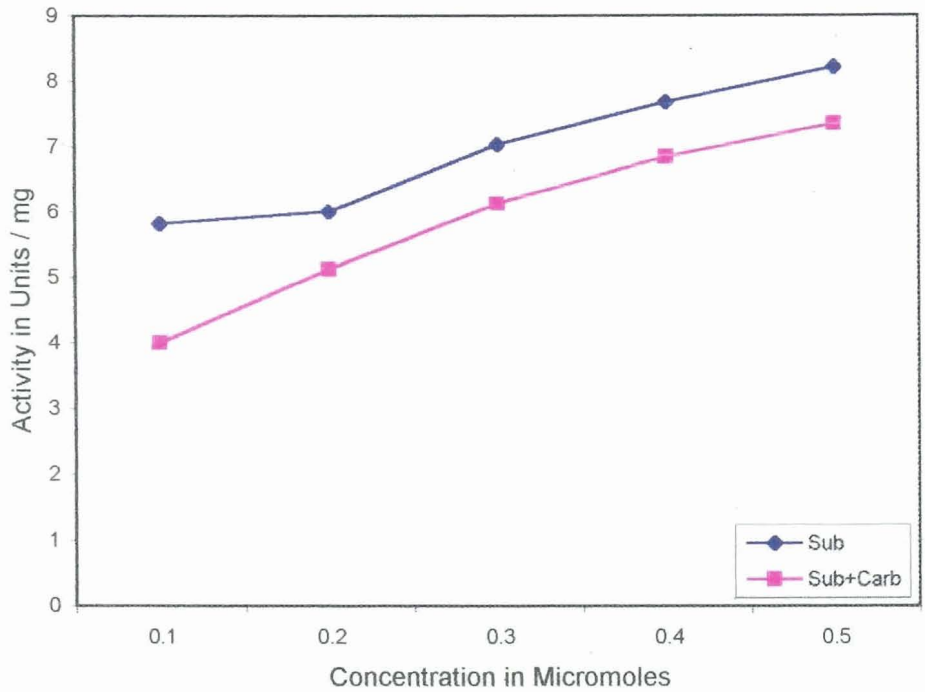

Figure 16 a : Effect of inhibitor concentration on fat body ALAT activity

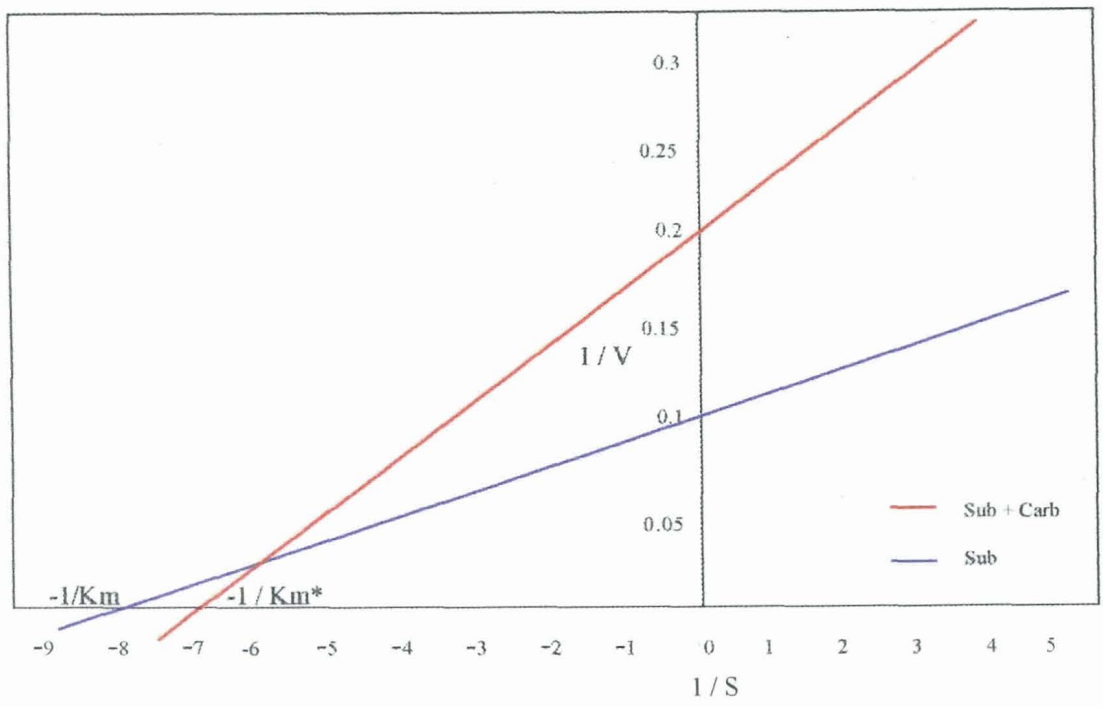

Figure 16 b : Lineweaver-Burk double reciprocal plot on ALAT activity

Table 17 a

Changes in the levels of haemolymph ALAT activity during larval development

Larval age hours	Normal units / ml $x10^{-3}$	Treated units / ml $x 10^{-3}$	Normal units / larva $x10^{-3}$	Treated units / larva $x10^{-3}$
0	1.432 ± 0.133	1.415 ± 0.073	0.172 ± 0.016	0.170 ± 0.009
24	1.725 ± 0.081	2.016 ± 0.103	0.449 ± 0.021	0.242 ± 0.012
48	2.338 ± 0.056	3.348 ± 0.039	0.959 ± 0.023	0.670 ± 0.008
72	2.416 ± 0.079	4.617 ± 0.198	1.353 ± 0.044	1.108 ± 0.048
96	2.232 ± 0.065	3.035 ± 0.167	1.495 ± 0.043	0.759 ± 0.042
120	1.527 ± 0.113	1.762 ± 0.121	0.794 ± 0.059	0.370 ± 0.025
144	1.316 ± 0.121	1.582 ± 0.135	0.566 ± 0.052	0.301 ± 0.026

Values are the means of five determinations with standard deviation

Table 17 b

Changes in the levels of fat body ALAT activity during larval development

Larval age hours	Normal units / mg $x10^{-3}$	Treated units / mg $x 10^{-3}$	Normal units / larva $x10^{-3}$	Treated units / larva $x10^{-3}$
0	5.361 ± 0.125	5.124 ± 0.231	184.579 ± 04.30	182.619 ± 08.233
24	5.932 ± 0.166	6.457 ± 0.411	308.523 ± 08.63	269.451 ± 14.648
48	7.045 ± 0.213	8.738 ± 0.316	819.474 ± 24.77	554.513 ± 20.053
72	6.187 ± 0.174	9.414 ± 0.337	1013.307 ± 28.50	774.866 ± 27.738
96	5.718 ± 0.232	6.836 ± 0.259	1264.479 ± 51.30	556.655 ± 21.090
120	5.229 ± 0.193	5.147 ± 0.193	1123.346 ± 41.46	404.657 ± 15.174
144	5.015 ± 0.296	5.095 ± 0.312	1061.375 ± 32.64	385.386 ± 23.600

Values are the means of five determinations with standard deviation

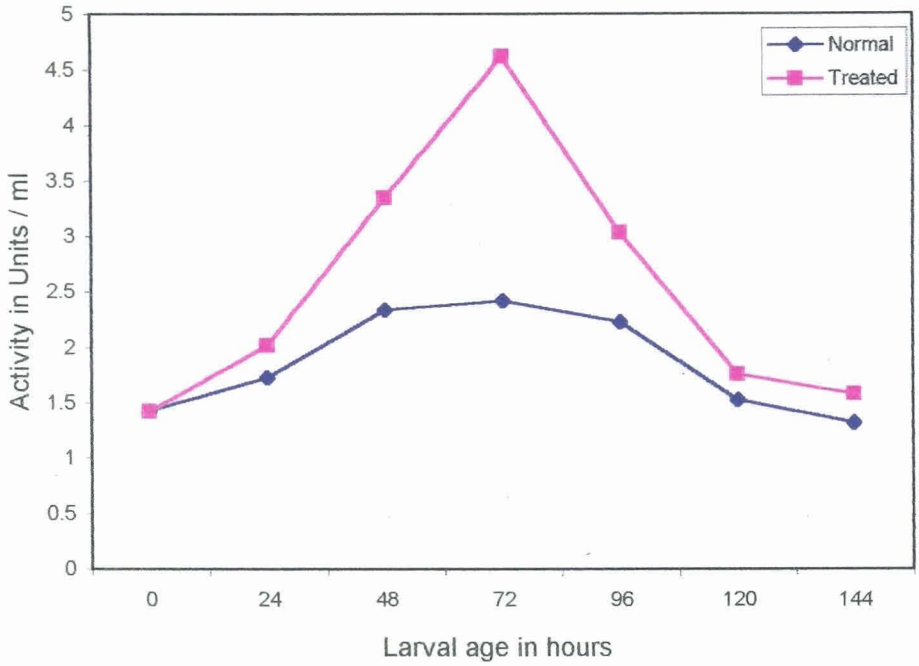

Figure 17 a(i) : Changes in the levels of haemolymph ALAT activity

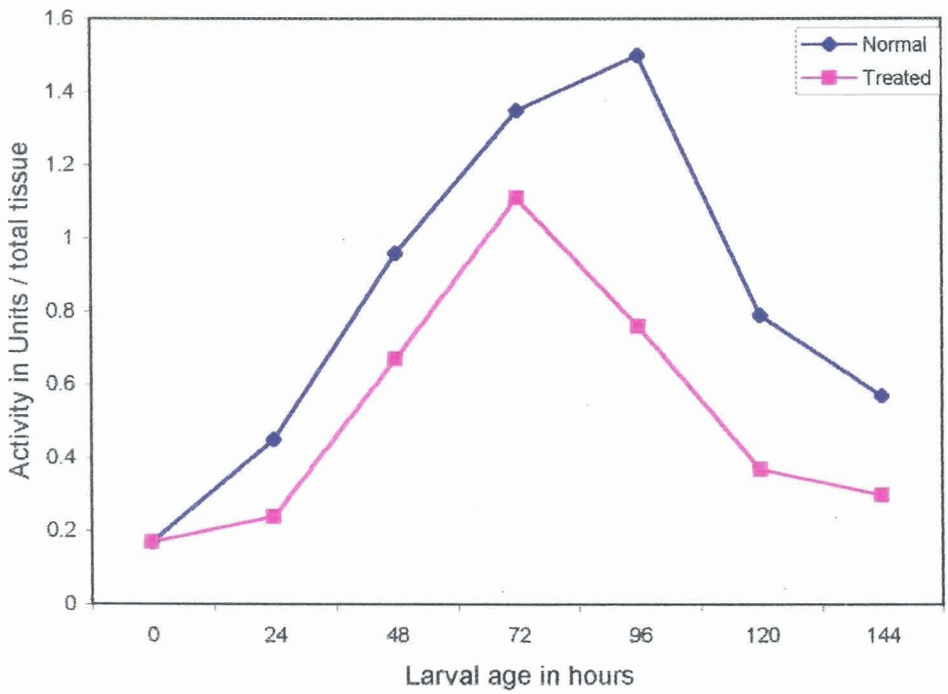

Figure 17 a(ii) : Changes in the levels of total haemolymph ALAT activity

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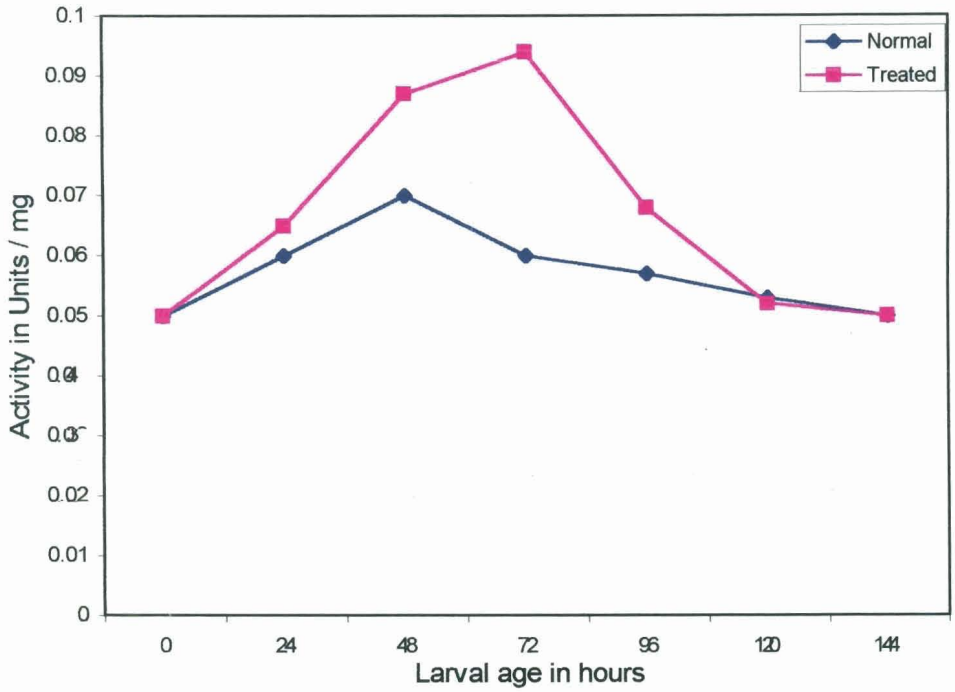

Figure 17 b(i) : Changes in the levels of fat body ALAT activity

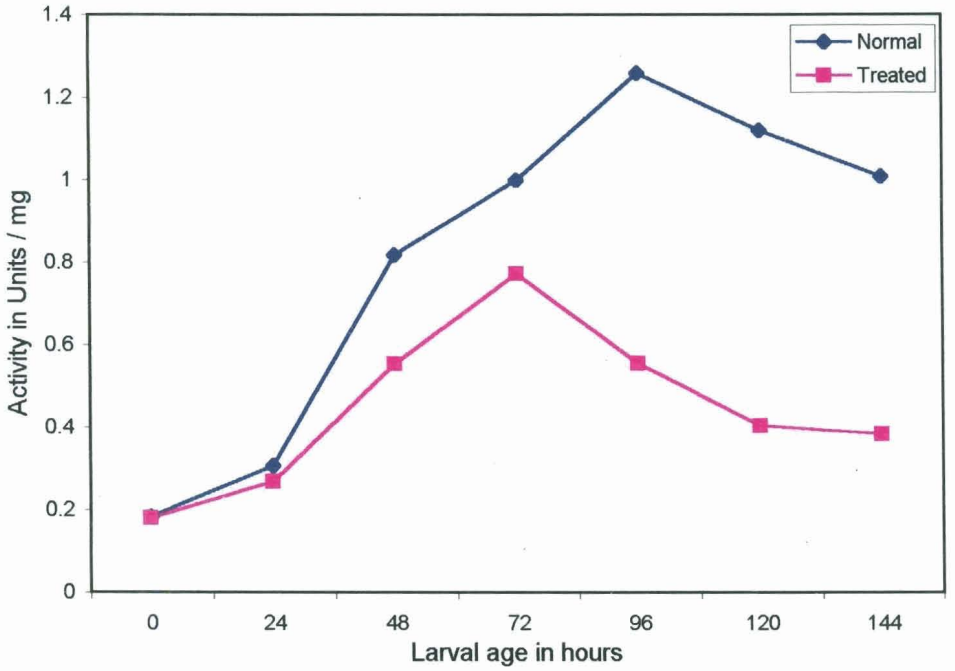

Figure 17 b(ii) : Changes in the levels of total fat body ALAT activity

Figure 17b(i) and 17b(ii)

In the haemolymph of normal larvae the ALAT activity steadily increased with larval development and registered the peak at 72 h stage followed by a decline thereafter. In treated larvae the variation of ALAT activity exhibited a similar pattern but with a high magnitude.

In the fat body the change in ALAT activity was almost similar to that of haemolymph in both normal and treated larvae. The treated larvae exhibited higher activity than the normal in every corresponding stage.

The specific activities of ALAT in the haemolymph and fat body of the normal and treated larvae were recorded in Tables 18a and 18b and Figures 18a and 18b.

Table 18a and 18b

Figure 18a and 18b

The specific activities showed a gradual decline from 0 h to 144 h in the case of normal larvae, which was similar to that of specific activity of AAT. On the final day of the larval instar the specific activity of ALAT was only 18% of

Table 18 a

Changes in the levels of specific activity of ALAT in the haemolymph during larval development

Larval age hours	Normal units / mg of protein x 10^{-3}	Treated units / mg of protein x 10^{-3}
0	0.102 ± 0.011	0.104 ± 0.013
24	0.076 ± 0.007	0.131 ± 0.009
48	0.074 ± 0.002	0.206 ± 0.027
72	0.051 ± 0.004	0.251 ± 0.014
96	0.037 ± 0.001	0.165 ± 0.009
120	0.023 ± 0.002	0.108 ± 0.006
144	0.019 ± 0.001	0.098 ± 0.004

Values are the means of five determinations with standard deviation

Table 18 b

Changes in the levels of specific activity of ALAT in the fat body during larval development

Larval age hours	Normal units / mg of protein x 10^{-3}	Treated units / mg of protein x 10^{-3}
0	45.821 ± 1.624	42.347 ± 2.563
24	48.227 ± 1.443	62.689 ± 1.059
48	55.913 ± 2.754	92.957 ± 2.027
72	43.879 ± 1.023	128.96 ± 2.095
96	38.898 ± 1.765	75.121 ± 3.145
120	32.681 ± 0.946	50.961 ± 0.929
144	27.555 ± 1.231	40.437 ± 1.737

Values are the means of five determinations with standard deviation

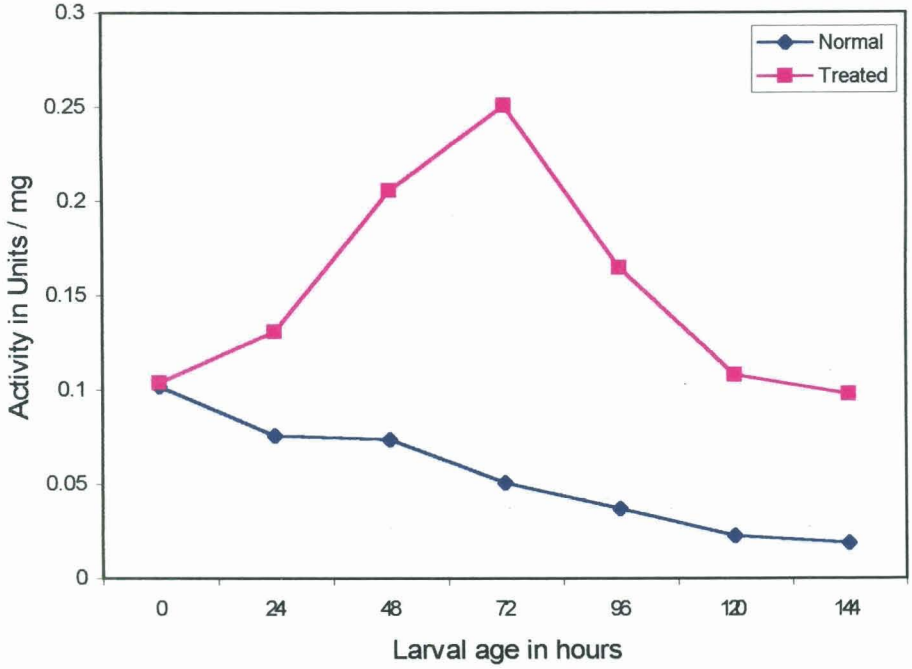

Figure 18 a : Changes in the levels of haemolymph specific ALAT activity

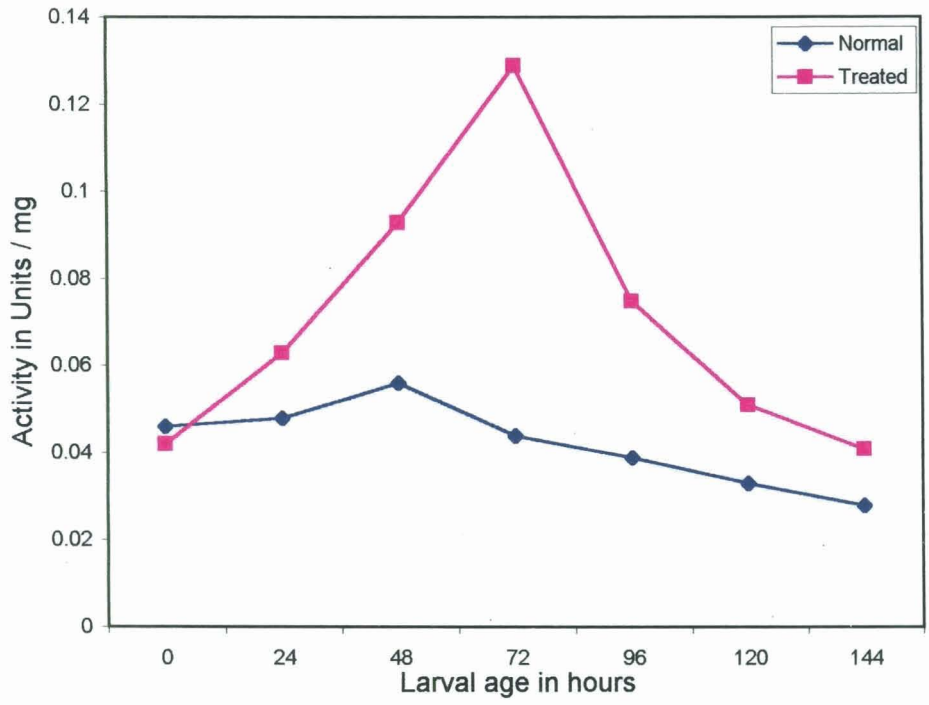

Figure 18 b : Changes in the levels of fat body specific ALAT activity

that observed in the first day. But in treated larvae it was in tune with that of the tissue activity levels with the peak on 72 h. The activity levels in the fat body were in accordance with that of the tissue AAT in both normal and carbaryl treated larvae. It was peculiar to note that the highest value in the treated larvae is about 3 -fold of that at the first day.

DISCUSSION

Sebastian. C.D. “Effects of carbaryl on the protein metabolism of the final instar larva of bombyx mori ” Thesis. Department of Zoology, University of Calicut, 2002

DISCUSSION

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DISCUSSION

Growth rate of the larva

The final instar larvae of lepidopterans are the most voraciously feeding and actively growing stage in their life cycle. Hugar and Kaliwal (1998) proved that the final instar larva of the silkworm, *Bombyx mori*, is the most actively feeding period during which the animal accumulate large quantity of biomolecular reserves in various tissues. They are endowed with unique biochemical adaptations to conserve nutritional reserves for cocoon spinning, metamorphosis and reproduction. The growth rate pattern of the final instar larvae of *Bombyx mori* observed in the present work is in tune with the above. In both normal and carbaryl treated larvae the duration of the fifth instar larval period was six days and on the final day the larvae stopped feeding completely and started to spin the cocoon. Even though the gross pattern of growth was similar, the carbaryl treated larvae started cocoon spinning almost eight to ten hours prior to that of the normal ones. Shikha (1995) made similar observations with *Spodoptera mauritia* larvae treated with carbaryl. The carbaryl treated larvae started pupation at 96 h while under normal conditions they pupated at 120 h only.

It has been suggested that the insecticide treatment will cause a delay in larval-pupal transformation and prolonged juvenile state (Abdul Kader *et al.*, 1991; Saxena *et al.*, 1992). These authors suggested that the prolonged larval life of *S. mauritia* on the treatment of diflubenzuron was mainly because of its antifeedant nature and its effects in the general metabolism of the larva. The results of the present study indicate that carbaryl affects the general growth of the larva of silkworm with a reduction in its body size and showing the symptoms of early aging.

When the total fresh body weight was considered, the larvae attained their maximum body size on the fourth day and thereafter no active growth. This coincides with the feeding habit. The carbaryl treated larvae exhibited an initial decrease in body weight for the first day but later kept a uniform variation with the normal. This initial loss of weight was an indication of the reduction in feeding on the initial exposure to the insecticide. However the organism gets habituated with the insecticide very soon and came in tune with normal growth rate pattern.

The dry weight of the larva was almost constant from the fourth day onwards. It was similar for both normal and treated larvae since increased accumulation of storage materials is a usual

phenomenon during later stages of development. Though a similar pattern of growth observed in both, the dry body weight of the carbaryl treated larvae were only 50% of that of normal. The result points to the deleterious effects of carbaryl on larval growth and metabolism.

The percentage of dry content of the larva indicate a steadily increase with a sharp rise on the final day. It was established that the bulk of the fresh weight of the body of *S. mauritia* larva was due to its water content and that the larva lost most of its water content at the commencement of pupation (Lazar, 1983). The dry content in the body of carbaryl treated larvae show a similar pattern of variation but with a low magnitude. This reduced dry content is a clear indication of water accumulation in the body under toxic stress. It has been seen that the treatment of carbaryl to the blister beetle, *Mylabris pustulata,* resulted a change in excretion from uricotelism to ammonotelism pointing to the accumulation of large amount of water in the beetles to prevent ammonia toxicity (Bharathi and Govindappa, 1985a). The results of the study are in tune with the above. However it is not certain whether the compound acts as an anti-diuretic agent or causing any other mechanism for water retention.

The rate of food consumption and rate of excretion was highest on the fourth day in both normal and treated larvae since they are proportional. In normal larvae the food intake on the fourth day was 2.5 times much than that on the first day. It correlates with the fresh body weight of the larvae. The carbaryl treated larvae showed a much reduced feeding rate on the first day of insecticide exposure which could be to its antifeedant effect. From the second day onwards the rate of food consumption increased and came in tune with the normal. The reduced feeding rate on the administration of insecticide to the normal food is a general phenomenon in insects (Abdul Kader, 1990).

The changes in the volume of haemolymph and weight of the fat body during the development of the larva point to the importance of the evaluation of the tissue analysis on the basis of unit volume and total volume of haemolymph and unit weight and total weight of the fat body respectively. Since haemolymph is the immediate environment of the organs in the insect, the metabolic activity and the development are affected by the haemolymph (Nakayama *et al.*, 1990). Fat body plays a vital role in the storage of biomolecules and responds to the fluctuation of the metabolites in the haemolymph fairly quickly (Tojo *et al.*, 1980). The functional role of the

fat body in insects is somewhat analogous to the combined functions of the liver and adipose tissue in mammals (Wyatt, 1980).

The changes in the volume of haemolymph coincided with the larval body weight. The decrease in the volume of haemolymph prior to pupation is a rule in holometabolous insects (Chen, 1966, 1978). In the carbaryl treated larvae though the pattern of variation was similar, there was a slight decline in the volume of haemolymph from 0 h to 24 h. This can be explained on the light of the decreased feeding on the first day of exposure to insecticide. The highest value in the treated larvae was just twice that of the volume at 0 h. It was suggested that under toxic stress insects show a decreased haemolymph synthesis (Nakayama *et al.*, 1990).

The weight of the fat body showed an increase from 0 h to 96 h and then onwards a decline. Shikha (1995) observed the increase in weight of tissues during the first half of the final larval instar of *S. mauritia* followed by a decrease. This increase in weight may be due to the growth and development taking place when the larvae consume a good amount of food as well as its functional changes in accordance with the development of the insect. The fat body of the holometabolous insect larvae synthesizes major haemolymph proteins during the feeding stage and during prepupal

stage the fat body cells incorporate these proteins. In carbaryl treated larvae the maximum fat body weight was only 37 % of that of normal. This reduction coincides with other biological parameters of normal and treated larvae. Considering the dry weights of fat body, the normal larvae have more than 20 % dry content during all developmental stages in final instar whereas in the treated ones it was only 10 to 15%. This points to the water accumulation in tissues under toxic stress.

Total proteins

Insects maintain a high level of protein concentration in their blood and the proteins are the chief organic constituents in the body (Florkin and Jeuniaux, 1974). They are concerned with the regulation of all biochemical events in the organism (Harper *et al.*, 1993). Accumulation of protein in the haemolymph and fat body during final larval instar of insect development is a common phenomenon in insects. It has been established that the proteins are synthesized in the fat body and released into the haemolymph, which are subsequently sequestered into the fat body and stored there depending upon the physiological condition of the animal (Chen, 1985).

Many investigators have studied the various aspects of insect haemolymph proteins (Chen, 1966, 1978; Wyatt and Pan, 1978). The protein concentration in insect haemolymph 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 protein concentration in the haemolymph of the normal silkworm larvae was in good agreement with this general view. The concentrations of proteins per unit volume of haemolymph increased gradually with the age in the final instar of normal larvae. An increase in protein concentration in insect haemolymph during larval stages is well established (Engle and Wood, 1960; Wyatt, 1961; Jeuniaux, 1971; Chen, 1985). When the total haemolymph proteins in the larva were considered, the amount declined at the end of the larval life prior to pupation. The sequestration of the proteins into the fat body from haemolymph during late larval life (Chen, 1985) may be attributed to the reason for such a protein declination in silkworm haemolymph just before spinning the cocoon.

The amount of protein in the haemolymph of carbaryl treated larvae was only 25% of that of normal larvae. When the total haemolymph protein levels were taken, it was just 10% of that of

normal. This drastic decrease due to insecticide treatment might be a cumulative factor for causing lethality to the insects. Insects are known to their capacity to regulate the osmotic pressure of haemolymph in spite of the variation in their blood volume (Florkin, 1966). During dehydration the haemolymph volume decreases, but the osmotic pressure remains constant due to a concomitant lowering of the concentration of solutes. The various ingredients of the haemolymph may contribute to the osmotic pressure. Florkin (1966) suggested that the proteins of insect haemolymph lack the oncosmotic component of the mammalian plasma, but they are mainly enzymes. It is probable that the haemolymph proteins beyond their capacity to act as enzymes may also take part in the maintenance of haemolymph osmotic pressure (Lazar and Mohamed, 1988). The carbaryl treatment resulted in the decreased haemolymph volume in silkworm larvae and hence a reduction in haemolymph protein concentration to maintain osmotic balance.

Proteins are the most studied nitrogenous compounds in the insect fat body. The variation in the fat body proteins during the developmental stages showed that there was a slow increase from 0 h to 144 h in normal larvae when calculated per unit weight. When the total fat body proteins were considered the increase in concentration

was striking with about 10-fold increment from 0 h to 144 h in the normal larvae. A high accumulation of proteins in the larval fat body prior to pupation has been well established in insects (Kilby, 1963; Price, 1966; Wyatt, 1980; Chen, 1978).

On the treatment of carbaryl the total fat body protein of the larva increases gradually during development. When compared to the drastic increase in the normal larvae, the treated larvae exhibit only a 2 - fold rise. The reduced protein concentration on the treatment of carbaryl indicates a reduction in protein synthesis. Asif and Ali (1988) reported a decrease in protein synthesis in *Drosicha stebbingi* on treatment with malathion. The reduction in protein synthesis on insecticidal treatment, especially carbamates was reported by many investigators (Saleem and Shakoori, 1985; Abdul Kader, 1990; Rajyalakshmi and Reddy, 1991; Reddy *et al.*, 1991; Mandal and Chaudhuri, 1992; Shikha, 1995). Therefore, the reduced fat body protein levels on carbaryl treatment in *B. mori* can be attributed to the decline in protein synthesis under insecticidal stress.

Total free amino acids

A higher amino acid concentration in the final instar larvae of *B. mori* is very significant as it reflects the synthesis and

degradation of proteins in the animal. The final larval instar is a very actively feeding stage acquiring materials for its future development and cocoon spinning during pupation. The amino acids absorbed through food were retained almost completely in the body as such after satisfying the amino acid requirement for its protein synthesis during the growing larval stage. The occurrence of high titre of amino acids in the haemolymph is a universal phenomenon in insects (Corrigan, 1970; Chen, 1971; Jeuniaux, 1971; Florkin and Jeuniaux, 1974).

There was a 14-fold variation in the titre of total free amino acids during the development of the silkworm. A change in the amino acid pool will directly influence the protein turn over, that is, the synthesis and degradation of proteins, and thus obviously reflect the physiological stage of the organism (Chen, 1971). The initial increase in total free amino acid level was proportional to the growth of the larvae and feeding rate. But their reduction in the later developmental stages of final larval instar indicates that during this period there is a positive balance in protein storage. In the silkworm, *B. mori* most of the amino acids resulting from the digestion are transported directly to the silk gland *via* haemolymph (Prudhomme *et al.,* 1985). Towards the end of the larval life, the silk glands synthesize

silk at the cost of other tissues since the feeding gradually declined to zero (Noguchi *et al.*, 1974). Hence an initial increment and later decline in the haemolymph free amino acid concentration of final instar *B. mori* larvae under normal conditions can be established.

The carbaryl treated larvae also showed a similar pattern of total free amino acid levels in haemolymph during its final instar development but with a low magnitude compared to the normal. There was a 50 – 70 % reduction in the level of total free amino acids which could indicate for the higher protein turn over in the haemolymph of treated larvae. This was also in tune with the changes observed in the total protein content in the treated larvae. It is obvious that any alteration in the concentration of the dissolved components of blood may upset the osmotic concentration.

The interest in the investigation of free amino acids in the fat body is due to fact that the tissue is an active site of intermediary metabolism of amino acids (Kilby, 1963). On the basis of the unit weight of the tissue, the total free amino acid content in the fat body of normal larvae showed an increase up to 96 hr, but declined thereafter. The changes in the total content of free amino acids in the total tissue (haemolymph and fat body) of the larvae of *S. mauritia* showed a similar pattern of variation during the development (Lazar and

Mohamed, 1988). This decline corresponds with the protein synthesis in the larvae. Compared to the haemolymph, the amino acid content of the fat body was fairly low. It is established that there is a marked mobilization of free amino acids from haemolymph to silk gland bypassing the fat body during silk synthesis (Nair, 2001). These free amino acids might be mainly from the dietary source. By the end of the larval stage silkworm stopped feeding and further availability of amino acids for silk synthesis might be at the cost of other tissues including the fat body reserve.

In the treated larvae the total free amino acid content in the fat body showed an increase up to 96 h but declined thereafter. This variation is in tune with the levels of haemolymph free amino acids and total proteins in both haemolymph and fat body.

Urea

The occurrence of urea has been demonstrated in the haemolymph insects (Lazar and Mohamed, 1994). Both in normal and carbaryl treated silkworm larvae, the urea levels drop progressively in the haemolymph during final larval instar. In the fat body also the urea levels show a similar pattern. But the urea levels in the

haemolymph and fat body of the treated larvae were reduced by 50 – 70 % to that of the normal.

The changes in the levels of urea in the haemolymph and fat body indicate that the larva maintains high levels of the material in the feeding stages and low levels in the non-feeding stages. The reduction in the urea levels was drastic when the larva undergoes pupation. It has been reported that the urea concentrations tend to decline toward larval-pupal transformation in the haemolymph of *B. mori* (Sumida *et al.*, 1995) and in the final instar larva of *S. mauritia* (Lazar and Mohamed, 1989). The present study suggests that the urea synthesis is almost confined to the feeding stages of the larva. In the carbaryl treated larva there was a reduced feeding, a situation similar to starvation. The low urea levels can be explained on the light of their reduced synthesis during non-feeding stages.

Glucose

The carbohydrates form an important constituent of the intermediary metabolism of insects. They are predominant carbon source of chitin, a participant in energy metabolism and the substrate for protein and lipid synthesis (Pant, 1984). The results of the present study indicate a similar variation in the glucose level of both

haemolymph and fat body with the peak concentration at the middle of the final larval instar. The variation is in tune with the feeding and the increase in carbohydrate level may be due to the large amount of starchy food intake (mulberry leaves) during the final larval instar since reduced feeding result in glucose declination.

The carbohydrate content of the haemolymph and fat body of *Schistocerca gregaria* increased during the period of intensive feeding (Walker *et al.*, 1970). Similar observations were made by Bade and Wyatt (1962), Nettles *et al.* (1971) and Rockstein (1978). Tate and Wimer (1971) explained the carbohydrate might be utilized for protein synthesis during pupation and adult growth. In *B. mori*, the high amount of glycogen accumulated in all tissues during the period before spinning is associated with the maximum food intake during that period (Simek and Kodrik, 1986). Glucose plays an important role in muscle contraction (Saktor, 1975), which would be very prominent during cocoon spinning in silkworm. Nair (2001) suggested that the decrease in carbohydrate level just before spinning indicate an increased mobilization of carbohydrate reserves both from haemolymph and fat body towards the cocoon spinning process.

Though the carbaryl treated larvae exhibited a similar variation of glucose levels in both haemolymph and fat body, the quantity was significantly less. Exposure to DDT caused a decline in carbohydrate level in *Poppilla japonica* (Ludwig and Bartolotta, 1953) and in glucose level in *Galleria mellonella* (Beard, 1957, 1958). Pandey (1980) also reported similar results. Application of lindane, fenthion and deltamethrin on *Locusta migratoria* larvae resulted in depleted levels of haemolymphatic carbohydrate and trehalose (Moreteau and Chaminade, 1983). A decline in the organic constituents of haemolymph and the gut region occurs in the blister beetle on exposure to sublethal doses of carbaryl (Bharathi and Govindappa, 1987 a, b).

It appears that insecticide interferes in the enzyme system needed for fat utilization, which necessitates an increased use of carbohydrate as an energy source. Depletion of carbohydrate from fat body during insecticidal stress may be due to the supply of energy demanded by insect to serve the tiding over of the critical phase caused by toxic stress (Hudson, 1958). Since carbohydrates form the key substrates for energy metabolism, the decrease in glucose and glycogen suggest the availability of insufficient substrate during toxic

stress (Peter, 1973). The decreased feeding during insecticide treatment will also result in the reduction of carbohydrate level.

Aminotransferases

Aminotransferases are of fundamental importance in the metabolism of proteins and amino acids. The final instar larvae of holometabolous insects undergo physiological modifications during metamorphosis. They have a feeding phase characterized by active feeding and somatic growth and a non-feeding phase characterized by major reorganization of the tissues during the larval period.

In the present study the pH of the medium reveals the optimum pH for their activity. It was clear that the optimum pH for AAT activity was pH 7.5 while that of ALAT was pH 7.1. Bergmeyer (1965) proposed that the optimum pH for AAT is in a range of 7.4 to 7.6 and that for ALAT is 7.0 to 7.8. The Km value of AAT activity was 0.083 and that of ALAT was 0.125.

The levels of AAT and ALAT activity in the silkworm showed striking variation during the development. In the larva of *Musca domestica* (Mc Allen and Chefurka, 1961) an increase in aminotransferase has been observed during the developmental stages. Similar variation was reported in *Drosophila nigromelanica* (Schneider

and Chen, 1981). In *S. mauritia*, Abdul Kader (1990) observed an increase in the level of aminotransferase activity during feeding stages and the activity declines with the reduction in feeding until pupation. However the larva maintained a relatively higher level of enzyme activity throughout the final instar larval period. This can be interpreted in terms of histolysis and histogenesis occurring in the larva prior to pupation. As both growth and differentiation are closely related to protein synthesis, the elevated activity of transaminase in the growing larva appears normal. Aminotransferase activity was higher in the feeding insects than the non-feeding insects as suggested by Wadhwa *et al.* (1986). The high level of aminotransferases observed during feeding stages was in tune with the anabolic phase of the larva. During the feeding period the larva exhibits higher growth rate and nitrogen balance showing higher anabolism (Lazar and Mohamed, 1988).

It has been found that the activity of AAT and ALAT increased in accordance with the advancement of silk production (Klunova *et al.*, 1976). The most important physiological functions of aminotransferases are the maintenance of the amino acid pool at a proper level for protein synthesis (Meister, 1965), the supply of metabolites for energy metabolism (Saktor, 1974) and the catalysis of

interactions between protein and carbohydrate metabolism (Katunuma *et al.*, 1968). ALAT and AAT activities in the final instar larvae of *S. mauritia* showed an increase with the larval growth and development (Lazar and Mohamed, 1998). The observations of AAT and ALAT activities in the haemolymph and fat body of normal silkworm larvae in the present study are in tune with the above.

The carbaryl treated larvae showed a similar pattern of changes in the activities of AAT and ALAT of the fat body and haemolymph but with a higher magnitude compared to the normal. This can be attributed to the reduced food intake of the animal since feeding reflects aminotransferase activity levels. The larvae were found to feed a lesser quantity of treated leaves that bring the animal a condition similar to partial starvation. Starvation can induce a high aminotransferase activity in the mammalian liver (Katunuma *et al.*, 1968). Similarly the young mammals feeding on milk with low carbohydrate content and rich protein have a higher titre of aminotransferases (Dymza *et al.*, 1964). These two conditions are linked to gluconeogenesis for substituting the carbohydrates. It has been reported that there is an elevation of AAT and ALAT in the haemolymph of blister beetles, *Mylabris pustulata* (Bharathi and Govindappa, 1985 c)

The increase in enzyme activity and subsequent decline from 72 h onwards in the carbaryl treated silkworm larvae can also be related to the toxicity at the cellular level. Aminotransferases are found associated with mitochondria in *Schistocerca gregaria* (Mane and Mehrotra, 1976; 1977) and mammals (Katunuma *et al.*, 1968). The rupture of mitochondria that contain the enzymes may be a possible reason for higher aminotransferase activity in the carbaryl treated silkworm larvae since carbaryl can affect the cellular organelles as reported by Shikha (1995) in final instar larva of *S. mauritia.*

Enzyme inhibition

The enzyme inhibition is the reduction in the activity of enzymes in the presence of an inhibitor. According to Segel (1975) enzyme inhibition have been classified into three:

a) Competitive inhibition- if the inhibitor affects only the slope of the reciprocal plot,

b) Non competitive inhibition- if the inhibitor affects only the 1/V axis intercept of the reciprocal plot and

c) Mixed type- if both the slope and 1/V axis intercept are affected.

Based on the above classification the nature of the aminotransferase inhibition due to carbaryl in the larva of *B. mori* appear to be of mixed type as there is lowering of activity observed and the slope and the 1/V axis intercept are affected. Mixed inhibition system may be considered as a mixture of partial competitive inhibition and pure non-competitive inhibition. The inhibitor has a lower affinity for substrate than enzyme and the ESI complex is non-productive. It was established that many so-called inhibitors are not truly enzyme inhibitors but alter the pattern of the overall reaction to divert the course away from some product (Webb, 1963). Substances, which will not specifically inhibit an enzyme, may be called a metabolic inhibitor. In the case of acetylcholinesterase carbaryl is an enzyme inhibitor. But in the case of aminotransferases it may be referred more appropriately as a metabolic inhibitor.

SUMMARY

Sebastian. C.D. “Effects of carbaryl on the protein metabolism of the final instar larva of bombyx mori ” Thesis. Department of Zoology, University of Calicut, 2002

SUMMARY

SUMMARY

§ The final instar larvae of the silkworm, *Bombyx mori*, is the most voraciously feeding and actively growing stage in their life cycle. During this period the animal accumulates large quantity of biomolecular reserves in various tissues.

§ The duration of the fifth instar larval period was six days and on the final day the larvae stopped feeding completely and started to spin the cocoon. The treatment of the carbaryl causes an early cocoon spinning and pupation, which were almost eight to ten hours prior to that of the normal ones.

§ The larvae attained their maximum body size on the fourth day of the final instar and thereafter no active growth. The carbaryl treatment results a much reduction in growth rate, but the pattern was similar.

§ The dry weight of the larva was almost constant from the fourth day onwards in both normal and treated larvae since increased accumulation of storage materials is a usual phenomenon during

later stages of development. The carbaryl has deleterious effects on larval growth and metabolism and causes water accumulation in tissues. That means carbaryl has antidiuretic nature.

§ The rate of food consumption and rate of excretion were maximum on the fourth day in both normal and treated larvae. In the normal larvae the food intake on the fourth day was 2.5 times higher than that on the first day. The carbaryl treatment causes much reduction in the feeding rate, hence it can be considered as an antifeedant.

§ The change in the volume of haemolymph and weight of the fat body was in tune with the larval body weight. Under toxic stress insect show a decreased haemolymph synthesis and fat body weight. This reduction coincides with other biological parameters of normal and treated larvae.

§ The concentrations of proteins per unit volume of haemolymph were increased gradually with the age of the normal larvae but declined at the end of the larval life prior to pupation due to sequestration of the proteins into the fat body from haemolymph during late larval life. The decrease of haemolymph protein on

insecticide treatment is a cumulative factor for causing lethality to the insects since they have the capacity to regulate the osmotic pressure of haemolymph in spite of the variation in their blood volume. There is a high accumulation of proteins in the larval fat body of silkworm prior to pupation. The reduced protein concentration on insecticide treatment indicates a reduction in protein synthesis.

§ The high free amino acid concentration in the haemolymph of the final instar larvae is very significant that reflects the synthesis and degradation of proteins in the animal. The initial increase was proportional to the growth of the larvae and feeding rate and reduction in the later developmental stages of final larval instar points a positive balance in protein storage. In silkworms most of the amino acids resulting from the digestion are transported directly to the silk gland *via* haemolymph and towards the end of the larval life, the silk glands synthesize silk at the cost of other tissues since the feeding gradually declined to zero. The reductions in the level of total free amino acids indicate the higher protein turn over in the haemolymph on insecticide treatment.

§ Compared to the haemolymph, the free amino acid content of the fat body was fairly low. It appears that there was a marked mobilization of free amino acids (mainly of the dietary source) from haemolymph to silk gland bypassing the fat body during silk synthesis. By the end of the larval stage silkworm stopped feeding and further availability of amino acids for silk synthesis is at the cost of other tissues including the fat body reserve. Carbaryl treatment did not alter the pattern of the above changes.

§ Both in normal and carbaryl treated silkworm larvae, the urea levels drop progressively in the haemolymph and fat body during final larval instar. The larva maintains high levels of the material in the feeding stages and low levels in the non-feeding stages. The urea concentrations tend to decline toward larval-pupal transformation in the haemolymph and fat body. In the carbaryl treated larva there was a reduced feeding and hence the low urea levels due to their reduced synthesis during non-feeding stages.

§ The variation in the concentration of haemolymph and fat body glucose levels is in tune with the feeding and the increase may be due to the large amount of starchy food intake (mulberry leaves) during final larval instar since reduced feeding results in glucose

declination. The decrease in carbohydrate level just before spinning indicates an increased mobilization of carbohydrate reserves both from haemolymph and fat body towards the cocoon spinning process. The insecticide interferes in the enzyme system needed for fat utilization, which necessitates an increased use of carbohydrate as an energy source. Depletion of carbohydrate from fat body during insecticidal stress may be due to the supply of energy demanded by insect to serve the tiding over of the critical phase caused by toxic stress.

§ The optimum pH for AAT and ALAT activities in the tissues of silkworm was pH 7.5 and pH 7.1 respectively.

§ The Km value of AAT and ALAT activity in the tissues of silkworm was 0.083 and 0.125 respectively.

§ The levels of AAT and ALAT activity in the silkworm increased during feeding stages and the activity declines with the reduction in feeding until pupation. The larva maintained a relatively higher level of enzyme activity throughout the final instar larval period. This is due to the histolysis and histogenesis occurring in the larva prior to pupation. The carbaryl treatment will induce a high

aminotransferase activity in the silkworm body tissues. The rupture of mitochondria that contain the enzymes may be a possible reason for higher aminotransferase activity in the carbaryl treated silkworm larvae since carbaryl can affect the cellular organelles.

§ The nature of the aminotransferase inhibition due to carbaryl in the larva of *B. mori* appears to be of a mixed type as there is lowering of activity and the slope and the 1/V axis intercept are affected. In the case of acetylcholinesterase carbaryl is an enzyme inhibitor. But in the case of aminotransferases it may be referred more appropriately as a metabolic inhibitor.

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