STUDIES ON THE EFFICACY OF SELECTED JUVENILE HORMONE ANALOGS AND THEIR EFFECTS ON THE METABOLISM OF THE EASTERN SPRUCE BUDWORM CHORISTONEURA FUMIFERANA (CLEMENS) (LEPIDOPTERA: TORTRICIDAE)

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BY

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Abstract

The efficacy of seven juvenile hormone analogs (JHAs) in disrupting metamorphosis of the eastern spruce budworm, Choristoneura fumiferana Clemens (Lepidoptera: Tortricidae), was determined by applying the JHAs topically to early sixth-instar larvae and monitoring the subsequent development. Fenoxycarb was the most effective juvenile hormone analog, with an LD_{50} of 0.26 µg per insect. Relative lethal effectiveness, based on LD_{50} values, was fenoxycarb > ZR 9892 > ZR 8487 > S-71639 > methoprene > ZR 9582 > ZR 10151. All of the JHAs, except ZR 10151, caused a wide array of morphogenetic deformities such as formation of larval-pupal intermediates, with precocious evagination of the imaginal wing disks, production of deformed pupae with hemolymph-filled blisters, supernumerary molting, mummified larval-pupal intermediates, and inhibition of molting.

At a sublethal dose, fenoxycarb and methoprene caused a general disruption in the metabolism of C. fumiferana, as shown by altered levels of carbohydrate, protein, and lipid in the hemolymph and fat bodies. Lipid levels in the hemolymph and fat bodies were severely depleted in fenoxycarb treated insects.

The predominant class of neutral lipid in the hemolymph was diacylglycerol, and in the fat body triacylglycerol.

The fatty acid complement of the hemolymph and fat body lipids was represented by several saturated and unsaturated fatty acids. These qualitative profiles were unaffected by fenoxycarb treatment.

The capacity of fat bodies from fenoxycarb treated larvae to synthesize lipids *in vitro* was impaired. The effect of fenoxycarb treatment on lipid synthesis appears to be at least partially direct, since addition of fenoxycarb to the incubation medium of fat bodies from untreated larvae resulted in reduced lipid synthesis. It was found that impairment occurred both in the pathway leading to fatty acid synthesis, and the subsequent pathway leading to complex lipid synthesis, as shown by reduced incorporation of radiolabelled precursors into lipid, as well as diminished capacity of the fat body cytosolic enzymes to synthesize fatty acids *in vitro*.

Fenoxycarb treatment also altered the types of complex lipids synthesized from labelled acetate, but not in the types of complex lipids synthesized from labelled palmitate, suggesting that the JHA affected the initial step of lipid synthesis, i.e. fatty acid biosynthesis. The ability of fat bodies from fenoxycarb treated budworms to oxidize preformed fatty acids *in vitro*, via the β-oxidation pathway, was significantly impaired in the early (24 hours post treatment) sixth instar *C. fumiferana* larvae.

These studies demonstrate that lipid metabolism was severely perturbed in C. fumiferana as a result of fenoxycarb treatment.

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INTRODUCTION

The eastern spruce budworm, Choristoneura fumiferana Clemens (Lepidoptera: Tortricidae), is a serious defoliator of balsam fir and a variety of other softwood species (i.e. spruces, larches, hemlock) in eastern North America. Outbreaks of this pest result in millions of dollars annual losses to the Canadian forest industry.

Control programs, during the last several decades, have relied upon the use of chemical insecticides (Sanders et al., 1985). The environmental acceptibility of such insecticides is now being questioned, and there is a compelling need to develop alternative control strategies. In addition to the implementation of sound forest management practices, several strategies are being explored, and some employed, including the use of biorational control agents such as pathogenic bacteria, viruses, fungi, nematodes, and parasitoid insects. The bacterial insecticide Bacillus thuringiensis Berliner was developed for use against C. fumiferana (Morris et al., 1986).

A new category of insecticides which has considerable potential for spruce budworm control are the Insect Growth Regulators (IGRs). These insecticides were developed as a result of rational leads from basic research in entomology on metabolic disruptors, molt inhibitors, and behavior modifiers

of insects. Insect growth regulators constitute an assemblage of compounds that adversely interfere with the normal growth and development of insects. Such compounds are generally of low toxicity to vertebrates, low persistence under field conditions, and are effective against a variety of insect pests (Retnakaran et al., 1985). There are five basic categories of IGRs:

1) Chitin synthesis inhibitors: compounds that act directly on the epidermal cells and disrupt cuticle synthesis. e.g. diflubenzuron (Dimilin®) (Wright and Retnakaran, 1987);

2) Juvenile hormone analogs: compounds that functionally resemble natural juvenile hormone and inhibit molting and/or metamorphosis. e.g. methoprene (Altosid®) (Retnakaran et al., 1985);

3) Anti-Juvenile hormones: compounds that induce premature metamorphosis by preventing the insect from secreting juvenile hormone. e.g. precocenes (Bowers, 1985);

4) Ecdysone analogs: compounds that induce premature molting and disrupt metamorphosis in insects. e.g. ponasterone A (Wilson, 1987); and

5) Metabolic inhibitors: compounds, both naturally occurring and synthetic, that adversely interfere with metabolic processes, and disrupt normal growth and development in insects. e.g. azasterols (Svoboda et al., 1972).

Juvenile hormone analogs (JHAs) offer promising control potential against a variety of insect pests (Staal, 1975; Retnakaran et al., 1985). Preliminary studies in the laboratory and the field suggested that methoprene and several other JHAs that were among the first to be developed, were relatively ineffective in suppressing the development of C. fumiferana (Retnakaran, 1970, 1973; Retnakaran and Grisdale, 1970; Retnakaran et al., 1977, 1978; Outram, 1975). However, these studies employed a limited range of JHA concentrations on the egg, larval, and/or pupal stages of C. fumiferana. The efficacy of JHAs against the spruce budworm needs to be determined by more extensive laboratory screening of the JHAs that are commercially available in addition to more recent ones being synthesized.

Juvenile hormone analogs functionally parallel insect juvenile hormone (JH) and may or may not be similar in structure. We know in a general way that application of JHAs causes an artificial elevation in the endogenous JH titre in the insect, and disrupts the molting physiology. However, the precise effects of JHAs at the physiological and biochemical level are incompletely known, especially in C. fumiferana. Considering that eventually, these compounds may be widely used against field populations of the spruce budworm, such information is crucial. A detailed examination of the effects of JHAs on the target insect's metabolic pathways, in addition

to the associated neuroendocrine activity, is required to obtain a more complete understanding of the mode of action of this category of IGRs. Physiological studies are also required to provide important information concerning the basic physiology of this target insect.

The present study was undertaken to evaluate several new JHAs, in addition to one of the original compounds (methoprene), against sixth instar larvae of C. fumiferana and to elucidate the physiological effects of 2 JHAs, selected from those that were screened, on the insect. Finally, detailed studies were performed to determine the effects of the most potent JHA (fenoxycarb) that was evaluated on one particular aspect of metabolism (i.e. lipid metabolism) that was found to be most affected by the JHA.

1.0 Literature Review

1.1 Choristoneura fumiferana: economic impact, life history and control methods

The eastern spruce budworm, C. fumiferana, is one of the most widely distributed and the most destructive forest insects in North America. Its range includes the eastern United States from Virginia to Minnesota, and all of the forested regions of Canada from Newfoundland to Alberta,

northeastern British Columbia, the southern part of the Yukon Territory, and the southern half of the MacKenzie River basin of the Northwest Territories (Freeman, 1967; Prebble and Carolin, 1967).

It is a native species and a principle pest of conifer species such as balsam fir, Abies balsamea (L.) Mill.; white spruce, Picea glauca (Moench) Voss.; red spruce, P. rubens Sarg.; and black spruce, P. mariana (Mill.) B.S.P. (Greenbank, 1963). The spruce budworm also attacks eastern larch, Larix laricina (Du Roi) Koch, and eastern hemlock, Tsuga canadensis (L.) Carr. Periodic outbreaks of C. fumiferana in eastern Canada are known to have occurred since the early 1700's (Blais, 1965). Widespread outbreaks usually have resulted in tree mortality over extensive areas, with timber losses exceeding 50 % of the volume of the infested fir-spruce forests (Blais, 1973).

Outbreaks of the spruce budworm are a natural phenomenon associated with the biota of the boreal forest; by killing extensive stands of mature and overmature spruce and fir, they prevent the perpetuation of decadent forests and bring about their rejuvenation (Blais et al., 1981). Two main conditions required for the development of budworm outbreaks are favorable weather characterized by warm-dry conditions in late spring and early summer, and extensive stands of mature balsam fir (Otvos and Moody, 1978; Blais et al., 1981). In recent

times, the forest has become an important source of raw material for the needs of modern humankind, and any situation seriously affecting the supply of wood and fibre can have far-reaching socio-economic consequences.

The damage caused by the spruce budworm is characterized by severe defoliation of the host trees, which results in reduced growth and eventually death (Raske, 1981). Radial growth of the tree is reduced first and to the greatest extent, in the immediate vicinity of the bole where defoliation occurs (McLintock, 1955). The radial growth loss during an outbreak generally ranges between 35 % and 90 % (Batzer, 1973). Reduction of height growth, as well as loss of height through top killing, are also a common form of budworm damage (Raske, 1981). Budworm outbreaks usually leave a large proportion of trees with dead tops, often more than 50 % (Kulman, 1971; Miller, 1977). During a five year outbreak in Minnesota, height growth of damaged trees averaged 48 cm compared to 130 cm in undamaged trees (Batzer, 1973). In pulpwood stands damaged by C. fumiferana, volume loss is caused by reduced tree size, tree deformities, and increased wood decay (Miller, 1977). Stem deformities, such as forked tops, multiple tops, and curved tops; and rootlet mortality are also common in budworm damaged stands (Raske, 1981). Defoliation of the new growth causes a decline of vigour, predisposing the trees to diseases (Wargo and Houston, 1974)

or to other destructive agents that normally do not seriously damage a tree, such as bark beetles (Thomas, 1958). Mortality of the host trees may also occur after five years of continuous defoliation of the new growth (Prebble and Carolin, 1967). Additionally, the spruce budworm infestation exerts a negative impact on aesthetic quality of the forest, by changing vegetational color, texture or form, e.g. to the red and brownish-black color of defoliated and dead trees (Case, 1981).

Generalized accounts of the life history, habits, ecology, population dynamics, and biology of C. fumiferana have been described by several authors, including McGugan (1954), Bean and Waters (1961), Miller (1963), Morris (1963), Prebble (1975), Hudak and Raske (1981), Schmitt et al. (1984), and Sanders et al. (1985). The following is a generalized account of the life cycle of spruce budworm in eastern North America. The life cycle may vary somewhat, depending on the geographic locality. The life cycle of the budworm spans two calendar years; the eggs are laid in July and early August of one year and larvae feed in the following year (Prebble, 1975). Female moths deposit light-green egg masses, overlapping like shingles, on the needles of the host trees (McKnight, 1968). Each female lays about 200 eggs (Miller, 1963). The larvae hatch in about 10 days (McGugan, 1954) and spin silken hibernation shelters (hibernacula) in crevices of

bark, under bud scales or lichens, and in the cups of old staminate flowers (Grisdale, 1984).

The spruce budworm has six larval instars (McGugan, 1954). First-instar larvae are pale, yellowish-green with brown heads, and are approximately 2 mm long (Crummey, 1976). First larval molt occurs in late August in the hibernacula; the second-instar larvae remain there without feeding (i.e. overwinter) until the next spring (McGugan, 1954; Grisdale, 1984). The spruce budworm has obligate diapause (Harvey, 1957). Overwintering larvae emerge in late April or early May, and commence mining needles, tunnel into the unopened buds, or feed on early opening staminate flowers when these are available (Grisdale, 1984). The two major periods of dispersal for the larvae are during these stages; first in the fall by first-instar larvae, and the other in the spring by second-instar larvae (Shaw and Little, 1973). After about 7-10 days, larvae move to opening vegetative buds and feed on the needles under a protective silken shelter (Grisdale, 1984). These larvae are yellowish-orange with a blackish-brown head, and a pale-brown prothoracic shield (Crummey, 1976).

Full-grown larvae are olive-brown with a black head, and a brownish prothoracic shield, and have yellowish-white tubercles (Crummey, 1976). Mature larvae pupate in the feeding webs (Miller, 1963) in late June or early July (Grisdale, 1984). Newly formed pupae are green or yellowish-green; mature

pupae are dark grey or dark brown, with no color differences between the sexes (Campbell, 1953). The moths emerge from the pupal cases about 10-14 days later, completing the annual one-generation cycle (Grisdale, 1984). The sex ratio of C. fumiferana is usually 1:1 (Miller, 1963). Adult males emerge a few days earlier than females, and the flight activity of the moths spans about 3 weeks (McKnight, 1968).

In the laboratory, several generations of C. fumiferana can be reared per year on artificial diet, both with and without diapause (Robertson, 1984). Stehr (1954) described a method for rearing spruce budworms on shoots of balsam fir that had been preserved by freezing. Subsequently, several methods have been described for mass-rearing the insect (Grisdale, 1970, 1972, 1984; Mulye and Gordon, 1990) involving the use of a wheat-germ based synthetic diet (McMorran, 1965).

Control programs, during the last several decades, have relied upon the use of chemical insecticides (Prebble, 1975). In the 1920's and 1930's, aerial insecticide dusting was used to control spruce budworm in small areas. Aerial spraying with chemical insecticides began in 1944, and has been widely used for the protection of large areas of forests from budworm attack (Prebble, 1975). In Canada, there are six chemical insecticides and one biological insecticide registered for use against the spruce budworm (Helson, 1985): acephate (Orthene®), aminocarb (Matacil®), carbaryl (Sevin®),

fenitrothion (Sumithion®), phosphamidon (Dimecron®), trichlorfon (Diptrex®), and Bacillus thuringiensis (B.t.) (Thuricide®, Dipel®). Several new chemical insecticides are also being developed for spruce budworm control: chlorpyrifos-methyl, Bolstar®, and permethrin (Armstrong, 1985); and new strains and formulations of B.t. are under study (Dimond, 1985). Other potential control methods for C. fumiferana include viruses, a nuclear polyhedrosis virus (NPV), and a cytoplasmic polyhedrosis virus (CPV) (Cunningham and Howse, 1980); sex pheromones (Sanders, 1980); protozoa (microsporidia), Nosema fumiferanae (Wilson, 1981); fungi, Entomophthora egressa, and Zoophthora radicans (Otvos and Moody, 1978); parasitoids, Trichogramma spp. (Houseweart et al., 1984); nematodes, Heterorhabditis heliothidis (Finney et al., 1982), and Steinernema spp. (Finney-Crawley, personal communication); antifeedants (Strunz and Fogal, 1981); genetic control (Retnakaran, 1971); and insect growth regulators (Retnakaran et al., 1985).

1.2 Insect Growth Regulators: diversity and economic applications

1) Chitin synthesis inhibitors: These compounds, upon ingestion, selectively inhibit the synthesis of chitin in larvae and prevent normal molting (Van Eck, 1979).

Benzoylphenyl ureas were the first chitin synthesis inhibitors synthesized by the Philips-Duphar Company (the Netherlands) in the 1970's. One of the analogs of benzoylphenyl urea, DU-19111, was shown to be effective against several insect species (Retnakaran et al., 1985). This led to the development and commercialization of diflubenzuron (PH 60-40, Dimilin®). Benzoylphenyl ureas generally consist of two substituted ring structures connected by a urea bridge (Marks et al., 1982; Retnakaran et al., 1985). Substitutions are typically halogens (chlorine and fluorine), or methyl, methoxy, trifluoromethoxy, or pentafluoroethoxy groups (Marks et al., 1982; Retnakaran et al., 1985).

Benzoylphenyl ureas have been shown to disrupt molting in several Lepidoptera, and species of other insect Orders. Mulder and Gijswijt (1973) reported that Pieris brassicae larvae, fed with a lethal dose of diflubenzuron, move within the intact exuviae, lose body fluids, imperceptibly blacken, and die while still attached to the substrate. If treatment occurred during the last larval instar, pupation was prevented, or the metamorphic molt was initiated but not concluded (Mulder and Gijswijt, 1973). Similar results have been obtained in Spodoptera littoralis, Malacosoma neustra, and other Lepidoptera (Zabel and Ostojic, 1973; Salama et al., 1976). Treatment with benzoylphenyl ureas also results in morphogenetic abnormalities such as the development of a

fragile cuticle, partially split exuvia, retention of old head capsule, and morphologically abnormal mouthparts causing starvation and death (Retnakaran et al., 1985).

In C. fumiferana, treatment with the benzoylphenyl ureas EL-494 and Dimilin® caused delayed molting effect, as well as morphogenetic effects such as retention of old cuticle, lethargy, loss of body fluids, and mortality (Brushwein and Granett, 1977). In field studies diflubenzuron was ineffective at economic dosage levels against the spruce budworm (Dimond, 1975; Retnakaran, 1978; Retnakaran and Smith, 1975; Retnakaran et al., 1978), and younger instars were shown to be less sensitive to the compound than older ones (Retnakaran and Smith, 1975; Granett and Retnakaran, 1977), providing little or no foliage protection. EL-494, an analog of diflubenzuron, was more effective on the spruce budworm than Dimilin® (Retnakaran, 1979), but shown to break down rapidly in the environment (Retnakaran, 1981). Several new chitin synthesis inhibitors have been screened against C. fumiferana: UC-62644, BAY SIR 8514, LY-127063, and LY-13125 (Retnakaran, 1980, 1981, 1982), with varying degrees of effectiveness.

2) Juvenile hormone analogs: The JHAs were developed as the result of research, in the 1960's, on the isolation, structure elucidation, and physiology of insect juvenile hormones. Schmialek (1961) was the first to elucidate the JH-active

farnesol and farnesal, both isoprenoids, in the frass of Tenebrio molitor. Slama and Williams (1966) reported that extracts of filter paper made from balsam fir inhibited metamorphosis of Pyrrhocoris apterus. This "paper factor", identified as juvabione (Bowers et al., 1966), and its closely related analog dehydrojuvabione (Cerny et al., 1967), has been found in the balsam fir tree A. balsamea. Williams (1967) suggested that such juvenile hormone mimics, i.e. JHAs, could be used as insect-specific control agents, referred to as "third-generation pesticides", to which the pest insect may be unable to develop resistance. These compounds were relatively weak mimics of JH, with limited range of activities against insect species.

Bowers (1969) synthesized several aromatic terpenoid ethers that were potent mimics of the natural hormone; these compounds were shown to be several hundred-fold more active than the natural hormone on Tenebrio molitor and Oncopeltus fasciatus. This evoked the interest of the pesticide industry, and led to syntheses of several hundred active compounds, with a wide variety of chemical structures (Henrick, 1982). The structure-activity relationship of JHAs was found to be extremely complex (Slama et al., 1974; Sobotka and Zabza, 1981; Henrick 1982; Henrick et al., 1976). While the insect juvenile hormones are epoxy methyl dodecadienoates (Richards, 1981), the JHAs synthesized to date are of various chemical

structures: alkyl trimethyl dodecatrienoates, alkyl trimethyl dodecadienoates, terpenoid phenyl ethers, aryl terpenoids, and related structures (Henrick, 1982). The only JHAs currently registered with the U.S. Environmental Protection Agency (EPA) for use in insect control are methoprene (ZR 515, Altosid®), kinoprene (ZR 777, Enstar®) (Staal, 1982), and to a limited extent fenoxycarb (RO 13-5223, Logic®) (Banks et al., 1988).

Methoprene and several other JHAs have been screened against diverse forest pests, with varying degrees of efficacy (Retnakaran et al., 1985). The spruce budworm has been found to be quite refractory to methoprene and several other JHAs that were initially developed, requiring high dosages to suppress development (Outram, 1975; Retnakaran, 1970, 1973; Retnakaran and Grisdale, 1970; Retnakaran et al., 1977, 1978). Two JHAs, GS42710 and CGA13353, were found to be moderately effective against C. fumiferana, but did not save any current year's foliage (Outram, 1975). There are several new JHAs currently being developed for insect control: S-71639, a phenoxyphenoxy pyridine (Sumitomo Chemical, Japan); ZR 8487, ZR 9582, ZR 9892, and ZR 10151, all carbamates (Staal, personal communication) (Zoecon Sandoz, U.S.A.); and fenoxycarb, a phenoxyphenyl carbamate (Maag Agrochemicals, U.S.A.).

3) Anti-Juvenile hormones: The synthesis and development of

potent JHAs, such as methoprene, as functional mimics of endogenous juvenile hormone (Staal, 1975) led to the concept that the reverse principle, anti-juvenile hormone action, could be explored to complement the use of JHAs (Staal, 1986). The application of JHAs against phytophagous insects in most field crop and forestry situations would be of little effect, since the immature feeding stages (e.g. last larval instar) responsible for the economic injury would be prolonged, and may even cause an increase in feeding damage (Sehnal, 1985). Bowers (1985) suggested that a chemical method (such as may be possible using anti-juvenile hormones) of interfering with JH biosynthesis, secretion, transportation, or action at a receptor site would overcome the above mentioned limitations of JHAs.

Bowers (1976) demonstrated that extracts of the bedding plant Ageratum houstonianum showed pronounced anti-juvenile hormone activity against the milkweed bug Oncopeltus fasciatus. The active factors, named "precocenes", were identified to be simple substituted chromenes, and synthesized in the laboratory (Bowers, 1976). Precocenes are selectively cytotoxic to the active corpora allata, causing a progressive degeneration of the glands (Unnithan et al., 1977). These compounds act as 'suicide substrates' for the epoxidase enzymes present only in the active corpora allata (Bowers, 1981; Pratt et al., 1980; Soderlund et al., 1981). The

epoxidase is also an important enzyme for the biosynthesis of juvenile hormone (Schooley and Baker, 1985). This membrane-bound enzyme reacts with precocene and forms a transient reactive epoxide, which then alkylates the proteins in the parenchyma cells of the corpus allatum, causing cellular death (Pratt et al., 1980). Precocene is generally active only on some Heteroptera and some Orthoptera (Bowers, 1985), albeit with a high dose requirement to induce precocious metamorphosis (Pener et al., 1978).

There are several other anti-juvenile hormones, with various modus operandi (Schooley and Baker, 1985): fluoromevalonate (Quistad et al., 1981), compactin (Monger et al., 1982), piperonyl butoxide (Kramer and Staal, 1981), ETB (Staal, 1977), and EMD (Staal et al., 1981). These compounds have been deemed to be not sufficiently active for practical control purposes (Staal, 1982), but remain useful experimental probes in the laboratory.

4) Ecdysone analogs: Ecdysone analogs, both naturally occurring and synthetic, cause disruption of growth and development in insects. Administration of ecdysone analogs to insects, in abnormal doses or when the endogenous ecdysteroid titre is low, results in morphogenetic abnormalities such as induction of supermolts and premature molting and pupation, and mortality (Wilson, 1987). Thus, theoretically, ecdysone

analogs could be considered as potential IGR pesticides. The discovery of several plant derived ecdysteroids (Horn and Bergamasco, 1985), may facilitate the synthesis and development of ecdysone analogs for insect control.

Incorporation of ponasterone A and cyasterone in the diets of Musca domestica (Singh et al., 1982), and Tribolium confusum (Robbins et al., 1968) caused an inhibition of larval development, while the inclusion of ponasterone A in the diets of Bombyx mori (Nakanishi, 1971), Ornithodorous monbata (Mango et al., 1976), Drosophila melanogaster (Fourche, 1967), and Kalotermes flavicollis (Lüscher and Karlson, 1958) induced supermolts as well as premature molting and pupation. The morphogenetic effects exerted by these compounds were shown to be dose dependent. Administration of ecdysteroids (or their analogs) also disrupted reproduction in Stomoxys calcitrans (Wright et al., 1971), and Musca domestica, Glossina morsitans and Tribolium confusum (Robbins et al., 1968). Ecdysteroids, or their analogs, have also been shown to possess antifeedant activity against Pieris brassicae, Chilo partellus, Phyllobius pyri, and Phyllobius organtatus (Wilson, 1987).

Ecdysone analogs, because of their high polarity, do not readily penetrate the insect cuticle (Wilson, 1987), and must be ingested in sufficiently large quantities by the insect in order to exert their effects on development and reproduction. Thus, pending the development of newer synthetic analogs with

better penetrating capacity, the ecdysone analogs are not considered at this time to be promising pesticides.

5) Metabolic inhibitors: Administration of the hypocholesterolemic agents 22,25-diazacholesterol and triparanol to Manduca sexta (Svoboda and Robbins, 1967), and Diatraea grandiosella (Chippendale and Reddy, 1973), caused an inhibition in the Δ^{24}-sterol reductase system and blocked the conversion of β-sitosterol to cholesterol. Several azasterols have been found to be potent inhibitors of the sterol reductase system in insects (Svoboda et al., 1972).

Other metabolic inhibitors that show various degrees of insect growth regulator activity include prostaglandins (Datta and Banerjee, 1978); caffeine and aminophylline (McDaniel and Berry, 1974); sclerin, a metabolite produced by the fungus Sclerotinia libertiana (Shimada et al., 1977); extracts of the plants Coleopsis lanceolata (Nakajima and Kawazu, 1980) and Mucuna mutisiana (Rehr et al., 1973); azadirachtin isolated from the neem tree Azadirachta indica (Rembold, 1987; Barnby and Klocke, 1990); and CGA 106630 (diafenthiuron), a thiourea (Ruder et al., 1991). With the exception of azadirachtin and CGA 106630, there has been no commercial interest in the development of metabolic inhibitors as IGR pesticides.

1.3 Effects of JHAs on Insects

Administration of JHAs to insects causes a wide array of effects on their growth, development, and metabolism. The effects of JHAs, in most cases, are similar to the effects of natural JH. Kramer and Staal (1981) suggested that JHAs can function as JH agonists or antagonists, or both. Thus, the mode of action of JHAs is complex, and may vary from one analog to another.

Application of JHAs to insect eggs has been shown to disrupt embryogenesis (Sehnal, 1983). Depending on the species, dose, and timing of application, effects ranging from ovicidal to delayed effects during postembryonic life have been reported (Riddiford, 1971). Death at hatching or during the first larval instar is also common in JHA treated insects (Staal, 1975). Studies on Hyalophora cecropia (Riddiford and Williams, 1967), Samia cynthia (Staal, 1975), Pyrrhocoris apterus (Matolin, 1970), Thermobia domestica (Rohdendorf and Sehnal, 1973), and C. fumiferana (Retnakaran, 1980) have shown that JHAs block embryonic development at blastokinesis, and are therefore ovicidal. The eggs were most sensitive to JHAs before the blastokinesis phase. If the JHAs were applied after blastokinesis, there was no ovicidal effect, but delayed effects occurred during metamorphosis (Riddiford, 1971). In C. fumiferana (Hicks and Gordon, 1992), treatment of eggs with

fenoxycarb at an early stage of embryogenesis (0-24 h old) prevented hatching, but the older eggs (48-72 h old) were found to be refractory. Disruption of embryogenesis by JHAs has been demonstrated in several other insect species (Retnakaran et al., 1985; Charmillot et al., 1985; Masner et al., 1987). In addition, the effect of terpenoid and non-terpenoid JHAs in impairing the fecundity of adult females has been documented for a variety of insect species (Retnakaran et l., 1985). The ability of JHA-treated males to affect fecundity in females, has also been reported in Pyrrhocoris apterus (Masner et al., 1968), Trogoderma granarium (Metawally and Landa, 1972), and C. fumiferana (Hicks and Gordon, 1992).

Juvenile hormone analogs also cause morphogenetic effects. Application of these compounds to insects causes abnormal morphogenesis of ' e integument. Juvenile hormone analogs do not interfere with the function and growth of insect epidermal cells, but prevent their imaginal differentiation (Sehnal, 1983). They adversely affect metamorphosis by disrupting the levels of JH at specific stages of the insect's development. In holometabolous insects, the titre of circulating JH in the hemolymph normally remains high during the early instars, drops during the last larval instar, and is below physiologically active levels in the pupa. When last instar larvae are treated with potent JHAs, the JH titre remains high and cellular programming of the

epidermis and possibly other tissues takes an abnormal course (Retnakaran et al., 1985). The extent and nature of the response to JHA treatment depends on a variety of factors such as species, timing of application, dose, mode of administration, and type of JHA (Staal, 1975). When JHAs are applied during the critical period for sensitivity to endogenous JH, usually the beginning of the ecdysteroid peak (Riddiford, 1985), the JH receptors become saturated with the compound (Slama, 1985), and lead to an inhibition of metamorphosis. This may also result in supernumerary molting (Sehnal, 1983). The JH receptors on cells of insects, however, are not sensitive in a synchronous fashion (Slama, 1985), allowing cells with refractory JH receptors to develop normally, and lead to the formation of larval-pupal intermediates as well as other morphologically deformed individuals (Slama, 1985). The precise mode of action of JHAs at the molecular level can only be elucidated once the mode of action of endogenous JH is clarified.

The biochemical/physiological effects of JHAs are incompletely known. Juvenile hormone analogs can function as JH agonists or antagonists, or both (Kramer and Staal, 1981), and may interfere with the regulation of JH secretion, transportation from the site of secretion to the target site, degradation, excretion, and feedback control (Retnakaran et al., 1985), as well as hormone-receptor interaction (Goodman

and Chang, 1985). In Diploptera punctata (Tobe and Stay, 1979), administration of the JHA hydroprene stimulated JH synthesis at low doses, but inhibited JH synthesis at higher doses, suggesting that the JHA interfered with the feedback control of the hormone titre. In Leptinotarsa decemlineata, methoprene and hydroprene exert their agonistic action by resisting degradation by the JH-specific esterase (Kramer, 1978), and by stimulating the esterase activity for the endogenous juvenile hormone (Kramer et al., 1977). Conversely, treatment of Manduca sexta larvae with hydroprene caused a decline in the levels of endogenous JH (Edwards et al., 1983), suggesting a JH antagonistic role for the compound in this insect. In the same insect, JH levels were also suppressed in a dose dependent fashion following topical treatment with fenoxycarb (Baker et al., 1986).

Juvenile hormone analogs also interfere with the normal pattern of neuroendocrine activity in insects (Retnakaran et al., 1985). In Mamestra brassicae, methoprene inhibits the release of the prothoracicotropic hormone from the brain, inhibits the prothoracic gland activity early in the last larval instar, but stimulates the gland prior to pupation (Hiruma et al., 1978). The possible role of JHAs in disrupting the normal neuroendocrine activity in insects receives support from the finding that RNA and protein synthesis in the brains of larval Calliphora vicina was modified, as the result of

methoprene injection (Scheller and Bodenstein, 1981), and from the observation that the corpus allatum of adult Aphis craccivora showed histological abnormalities following topical application of hydroprene (Elliott and McDonald, 1976). In Sarcophaga crassipalpis, methoprene was also found to inhibit the development and differentiation of the neuroendocrine system (Abou Halawa, 1981), and in Ephestia cautella (Shaaya et al., 1986), the JHA depressed the ecdysteroid titre and inhibited metamorphosis.

The effects of JHAs on metabolic homeostasis and energy metabolism in insects are poorly understood. The most common effect of JHA treatment is the disruption in the levels of hemolymph and fat body (or whole body) metabolites. The metabolic effects of JHAs may also be manifested as a result of the morphogenetic effects of the compound on the insect. Application of JHAs may also overwhelm the homeostatic mechanisms in the insect (Hammock and Quistad, 1981). Little information is available concerning the nature of physiological and biochemical effects of JHAs on insects.

In the last instar larvae of Spodoptera littoralis, topical application of methoprene caused a hypermetabolic response, as evidenced by abnormal O_2 consumption and CO_2 release by the treated insects (Kryspin-Sorensen et al., 1977). Downer et al. (1976) showed that whole pupae of Aedes aegypti became depleted in glycogen and lipid following

exposure to methoprene. Gordon and Burford (1984), in the same insect, reported that methoprene caused a depletion in fat body glycogen, and that glycogenolysis was suppressed due to an overall depletion of glycogen phosphorylase. The JHA also inhibited protein synthesis in the fat bodies of Aedes aegypti larvae and pupae, as demonstrated by their reduced capacity to synthesize proteins from radiolabelled leucine in vitro, causing an overall depletion of proteins in the hemolymph and the fat body (Gordon and Burford, 1984). Methoprene inhibited protein synthesis in larval Drosophila melanogaster homogenates (Breccia et al., 1976) and cultured Culex tarsalis cells (Himeno et al., 1979), but had no effect in a Calliphora vicina wing disc assay (Scheller et al., 1978). Topical application of methoprene has been shown to stimulate vitellogenin synthesis in Rhodnius prolixus (Chalaye and Lauverjat, 1985). Pyriproxyfen, a recently developed JHA, repressed the synthesis of larval-specific hemolymph proteins but stimulated vitellogenesis in Locusta migratoria (De Kort and Koopmanschap, 1991).

In Delia radicum (Young and Gordon, 1987), methoprene treatment caused a significant decrease in hemolymph trehalose levels. Treatment of Stomoxys calcitrans with a JHA structurally unrelated to methoprene, caused an increase in level of whole body glycogen (Wright and Rushing, 1973). Mansingh (1972), in Malacosoma pluviale, reported that

treatment with the JHA farnesyl methyl ether (FME) caused a depletion in whole body glycogen and trehalose, while lipid levels remained unchanged.

Fenoxycarb has been shown to modify protein synthesis by the fat body of Trichoplusia ni (Jones et al., 1988); the synthesis of a 76-kDa hemolymph protein was suppressed in vivo by topical application of the compound. In Bombyx mori, methoprene treatment depressed protein synthesis (Bosquet et al., 1989), and in Bombus terrestris (Roseler and Röseler, 1988), fat body lipid reserves were depleted as a result of synthetic JH-I treatment. Cotton and Anstee (1991), in Locusta migratoria, reported that methoprene treatment caused a depletion in the levels of fat body glycogen and lipid, when the tissue was examined structurally. Extraction and quantification of fat body lipid and glycogen showed that there was no significant difference from the controls. Cotton and Anstee (1991) reasoned that while the amount of fat body tissue was increased by the JHA treatment, total amounts of fat body lipid and glycogen remained unchanged, thus reducing the levels of lipid and glycogen per unit volume. Nonetheless, fat body wet weight was not significantly increased in treated insects (Cotton and Anstee, 1991). These studies, although scarce, demonstrate that administration of JHAs causes a wide array of metabolic effects in insects.

1.4 Lipid Metabolism in Insects

Lipids constitute a large number of structurally heterogenous compounds that are characterized by their solubility in organic solvents. They include fatty acids, fatty acid esters of glycerol (acylglycerols), sphingolipids, phospholipids, waxes, terpenes, steroids, prostaglandins, and hybrid molecules such as lipoproteins and glycolipids (Downer, 1985). Several reviews have appeared on insect lipids and their biochemistry (Fast, 1964, 1970; Gilbert, 1967a; Steele, 1976; Beenakkers et al., 1981; Downer, 1978, 1985).

The total lipid content of insect hemolymph has been found to range between 1.5 and 5.5 g % (Florkin and Jeuniaux, 1974). Hemolymph lipid levels may fluctuate under conditions such as muscular activity (Beenakkers et al., 1981), development (Downer and Matthews, 1976), starvation (Jutsum et al., 1975), and disease (Bennett et al., 1972). The processes of lipid absorption from the digestive system are incompletely understood in insects. Insects do not appear to utilize emulsifiers during digestion (Turunen, 1979), and chylomicral transport of lipids does not occur (Wyatt and Pan, 1978). Chino et al. (1981) suggest that triacylglycerols undergo partial hydrolysis in the gut and are transported as diacylglycerols complexed to lipoproteins. Transport of diacylglycerols from the midgut to the fat body, and from the

fat body to the flight muscle has been demonstrated in some insects (Beenakkers et al., 1981). Several lipid transporting hemolymph lipoproteins have been described in insects (Kanost et al., 1990). Recently, a very high density lipoprotein in the hemolymph of Manduca sexta has been isolated, and characterized as a lipid-transfer particle (Ryan et al., 1988).

While diacylglycerols are the mobile hemolymph lipids, triacylglycerols constitute the main storage form for fat body lipids (Downer, 1985). There is also evidence to suggest that lipids may be released from the fat body in several forms, which are then converted to diacylglycerols during hemolymph circulation (Keeley, 1985). Palmitate is esterified to triacylglycerols in the fat bodies of larval and adult saturniid silkmoths but to diacylglycerols in the pupal fat body (Stephen and Gilbert, 1969). The most common fat body fatty acids are palmitic, palmitoleic, stearic, oleic, linoleic, and linolenic acids (Stephen and Gilbert, 1969).

The fat body can incorporate radiolabelled acetate into fatty acids, as has been demonstrated in Periplaneta americana (Louloudes et al., 1961), Myzus persicae (Strong, 1963), Eurycotis floridana (Bade, 1964), Bombyx mori (Sridhara and Bhat, 1964), Anthonomus grandis (Lambremont, 1965), Calliphora erythrocephala (Brak et al., 1966), Oulema melanoplus (Lamb and Monroe, 1968), Trichoplusia ni (Nelson and Sukkestad,

1968), and Heliothis zea (Lambremont, 1971).

In addition to acetate, lipogenesis from carbohydrate precursors such as glucose also occurs in the fat body (Chino and Gilbert, 1965). The fatty acids then undergo esterification with the trihydric alcohol, glycerol, to form acylglycerols (Louloudes et al., 1961; Sridhara and Bhat, 1965; Lamb and Monroe, 1968; Turunen, 1973; Chino and Downer, 1979; Garcia et al., 1980).

The fat body forms diacylglycerol and releases it as the major hemolymph lipid. In Periplaneta americana, the triacylglycerol content declines in the fat body, with a concomitant increase in hemolymph diacylglycerol (Nelson et al., 1967). Chino and Gilbert (1965), in Hyalophora cecropia, Periplaneta americana, and Melanoplus differentialis, reported that fat bodies prelabelled in vivo with ^{14}C-Palmitate subsequently release radiolabelled diacylglycerol when the isolated tissues were incubated in hemolymph. Diacylglycerol synthesis and release was shown to be energy-dependent (Chino and Gilbert, 1965). The fat body also exhibits lipase activity for degradation of the storage triacylglycerol to circulatory lipids, as evidenced in Prodenia eridania (Stevenson, 1972), Locusta migratoria (Tietz and Weintraub, 1978), and Periplaneta americana (Hoffman and Downer, 1979).

Most of the potential metabolic energy available from stored triacylglycerol is contained within the fatty acid

component of the lipid, and these esterified fatty acids together with the non-esterified fatty acids constitute a major metabolic reserve (Downer, 1985). The pathway for fatty acid biosynthesis has been reviewed by Wakil (1970), Wakil et al. (1964), and Lehninger (1975). Two separable enzyme systems, acetyl-CoA carboxylase and fatty acid synthetase present in the cytosol, are involved in effecting the fatty acid synthesis. Acetyl-CoA carboxylase contains covalently bound biotin as a prosthetic group, and catalyzes the carboxylation of acetyl-CoA (derived from carbohydrate or amino acid sources via the tricarboxylic acid cycle) to malonyl-CoA (Downer, 1985). Malonyl-CoA, a 3-C compound, then condenses with acetyl-CoA in a series of reactions catalyzed by the fatty acid synthetase complex to yield a 4-C intermediate (Downer, 1985). After a series of similar condensations of malonyl-CoA with the newly formed acyl intermediate, an acyl chain of appropriate length is synthesized (Downer, 1985).

The primary products of de novo fatty acid synthesis are C16:0, C18:0, and C18:1, but the absolute amounts of these fatty acids may vary, depending on the time allowed for fatty acid synthesis (Sridhara and Bhat, 1965). The enzymes of the fatty acid synthetase complex are closely associated with an acyl carrier protein (ACP) that serves to bind the fatty acyl intermediate compounds through the formation of thiol esters

(Downer, 1985). The cytosolic enzymes for fatty acid synthesis have been demonstrated in Prodenia eridania (Zebe and McShan, 1959), Locusta migratoria (Tietz, 1963), Lucilia sericata (Thompson et al., 1975), Galleria mellonella (Thompson and Barlow, 1976), Periplaneta americana (Storey and Bailey, 1978), and Acyrthosiphon pisum (Ryan et al., 1982).

Following the synthesis of long chain saturated fatty acids, the monounsaturated homologues may be formed by direct dehydrogenation (Downer, 1985), as has been shown in the microsomal fractions from Locusta migratoria (Tietz and Stern, 1969), and Ceratitis capitata (Gonzalez-Buitrago et al., 1979). Most of the fatty acids synthesized from acetate and resulting from lipolysis of dietary acylglycerols undergo esterification with glycerol (Downer, 1985). Two biosynthetic pathways for acylglycerol synthesis, comprising several acyltransferases (Lehninger, 1975), have been isolated from Locusta migratoria (Tietz, 1969; Peled and Tietz, 1974; Tietz et al., 1975; Tietz and Weintraub, 1980), Hyalophora cecropia (Hirano and Gilbert, 1967), Galleria mellonella (Barlow et al., 1980), Glossina morsitans (Langley et al., 1981), Periplaneta americana (Hoffman and Downer, 1979), and Ceratitis capitata (Garcia et al., 1980).

Fatty acids play an important role as an energy rich fuel in insects, stored in the fat body cells in the form of triacylglycerols (Gilbert, 1967a). The mobilization of

acylglycerol reserves involves hydrolysis, catalyzed by lipases, of the acylester linkage. Insects have a variety of esterases in their hemolymph and tissues, that serve different metabolic functions (Chen, 1971). Lipases are a class of esterases that are defined in terms of their specificity for long-chain acylglycerols and their capacity to hydrolyze the acylester only at the interface formed between the emulsified substrate and the aqueous medium (Downer, 1985). Lipase-catalyzed triacylglycerol hydrolysis has been demonstrated in the gut, hemolymph, fat body, flight muscle, and other tissues of several insect species (Gilbert, 1967a; Downer, 1985). The complete hydrolysis of acylglycerol yields fatty acids and glycerol. Downer (1985) proposed that the liberated glycerol is transported to the fat body for esterification with free fatty acid produced by lipolysis (i.e. shuttle function), and/or converted to trehalose in the fat body.

The β-oxidation pathway for fatty acid oxidation, localized in the mitochondria, involves sequential removal of 2-C units in the form of acetyl-S-CoA, which undergo condensation with oxaloacetate to form citrate (Lehninger, 1975). The citrate is subsequently oxidized in the tricarboxylic acid cycle to carbon dioxide and water with concomitant generation of ATP (Lehninger, 1975). The units of acetyl-S-CoA are formed from the fatty acid molecule by oxidation at the β-carbon atom of the fatty acid. In addition

to providing acetyl-S-CoA, β-oxidation of fatty acids also generate reduced coenzymes $FADH_2$ and $NADH_2$, which are oxidized through the electron transport chain to yield additional molecules of ATP (Lehninger, 1975). The operation of the β-oxidation pathway in the tissue can be determined indirectly by measuring the emission of $^{14}CO_2$ from ^{14}C-labelled fatty acid by the tissue *in vitro*, in an airtight system.

In *C. fumiferana*, the mechanism(s) of lipid digestion, transport, synthesis, mobilization, and oxidation are unknown.

1.5 Hormonal Regulation of Metabolism

The hormonal regulation of metabolic processes in insects has been the subject of extensive study, and some excellent reviews are available on the topic (Steele, 1976, 1983, 1985; Keeley, 1985; Downer, 1981, 1985; Beenakkers, 1983). The hormonal aspects of carbohydrate, protein, and lipid metabolism in insects will be considered here in brevity.

Carbohydrates: Glycogen, a polymer of glycosyl residues derived from glucose and other carbohydrates, is the principal form of stored carbohydrate in insects. The glycogen reserves in the fat body are readily mobilized and distributed to other tissues (Friedman, 1985).

Glycogen synthesis in some Diptera and Orthoptera has

been shown to be controlled by a hormone originating in the medial neurosecretory cells (MNC) of the brain, and in some Lepidoptera, the suboesophageal ganglion (Steele, 1983). Ablation of the MNC in Aedes aegypti, Aedes taeniorhynchus, and Aedes solicitans causes an accumulation of glycogen in the tissues (Van Handel and Lea, 1970; Lea and Van Handel, 1970). This suggests that a hormone originating in the MNC inhibits glycogen synthesis in these insects. Similar results have been obtained in Calliphora erythrocephala (Thomsen, 1952), and Locusta migratoria (Goldsworthy, 1971). In Bombyx mori, the diapause hormone originating in the maternal suboesophageal ganglion, has been shown to cause an accumulation of glycogen in the diapause eggs (Yamashita and Hasegawa, 1970).

The role of the corpora allata (CA), i.e. juvenile hormone, in the regulation of glycogen synthesis has been studied extensively. Allatectomy of Carausius morosus (L'Helias, 1953), Pyrrhocoris apterus (Janda and Slama, 1965), Calliphora erythrocephala (Thomsen, 1952), Phormia regina (Orr, 1964), and Musca domestica (Liu, 1974) causes an accumulation of glycogen in the fat body or whole body, while implantation of CA or injection of synthetic JH in Drosophila melanogaster (Butterworth and Bodenstein, 1969) increased glycogen synthesis, suggesting that the role of JH in the regulation of carbohydrate metabolism remains to be clarified.

Biosynthesis of trehalose, the predominant hemolymph

carbohydrate in most insects (Wyatt and Kalf, 1957), is controlled by the hypertrehalosemic hormone which was first described by Steele (1961) from the corpora cardiaca (CC) of Periplaneta americana. The hormone elevates the level of hemolymph trehalose, with a concomitant decrease in fat body glycogen (Steele, 1963). The hypertrehalosemic hormone has been demonstrated in Blaberus discoidalis (Bowers and Friedman, 1963), Phormia regina (Friedman, 1967), Carausius morosus (Dutrieu and Gourdoux, 1967), Calliphora erythrocephala (Normann and Duve, 1969), Manduca sexta (Ziegler, 1979), and Locusta migratoria (Moreau et al., 1982). The hypertrehalosemic response to CC has also been demonstrated in vitro for the fat bodies of Leucophaea maderae (Weins and Gilbert, 1967), and Periplaneta americana (McClure and Steele, 1981). The mode of action of hypertrehalosemic hormone has been elucidated (Steele, 1980). The hormone is responsible for the activation of glycogen phosphorylase in the fat body, by stimulating the conversion of phosphorylase b to a (Steele, 1963; Weins and Gilbert, 1967). The effect is apparently not mediated via cAMP, but has an absolute requirement for Ca^{2+} (Steele, 1987).

A hypotrehalosemic hormone, which stimulates the synthesis of glycogen at the expense of trehalose, has also been described in some insects (Steele, 1985). The hypotrehalosemic hormone was first demonstrated in Calliphora

erythrocephala (Normann, 1975), and has also been found in Phormia regina (Chen and Friedman, 1977), Calliphora vomitoria (Duve et al., 1979), Musca domestica (Liu, 1973), and Periplaneta americana (Spring et al., 1977). The hormone has been isolated from the MNC of the brains of Calliphora erythrocephala (Duve, 1978), and Calliphora vomitoria (Duve et al., 1979). The mode of action of the hypotrehalosemic hormone is unknown.

Proteins: Insects contain a variety of proteins in their hemolymph and tissues. However, the hormonal regulation of hemolymph and fat body proteins is incompletely known. The fat body is the principal synthetic source for hemolymph proteins (Agosin, 1978). The fat body produces many unique and physiologically significant proteins such as vitellogenins, storage proteins, diapause proteins, lipid-binding proteins, hormone-carrier proteins, and enzymes (Keeley, 1985).

Neurohormones have been shown to increase the general protein synthetic capacity in the fat body of several insects. In the adult females of Schistocerca gregaria, the rate of protein synthesis increases drastically within 4 hours after injection of CC extract (Osborne et al., 1968). Conversely, protein synthesis in the fat body was severely reduced in the adult female Schistocerca gregaria 8 days after removal of the CA and CC or cautery of neurosecretory cells (Hill, 1965).

Elliott and Gillott (1978) reported that total protein in the hemolymph, fat body, and ovaries of Melanoplus sanguinipes was reduced after ablation of the neurosecretory cells. A direct role for neurohormones in protein synthesis was demonstrated in Leucophaea maderae (Scheurer, 1969), where implantation of brains or CC increased fat body protein synthesis.

Juvenile hormone has been shown to regulate storage protein synthesis in some insects. Tojo et al. (1981) demonstrated that JH suppressed storage protein synthesis in Bombyx mori, while allatectomy caused the opposite effect. JH also induces the synthesis of specific diapause proteins in the larvae of Diatraea grandiosella (Turunen and Chippendale, 1980), and regulates the synthesis of hemoglobins in Chironomus thummi (Vafopoulou-Mandalos and Laufer, 1980). Synthesis of vitellogenins in insects has been shown to be regulated by JH. The hormone acts on the female fat body and ovaries to stimulate vitellogenesis (Keeley, 1985), as has been demonstrated in Rhodnius prolixus (Hill, 1972), Leucophaea maderae (Brookes, 1969), Periplaneta americana (Bell, 1969), and Locusta migratoria (Chen et al., 1979).

Synthesis of vitellogenins and other proteins in some insects is regulated by ecdysteroids. In Aedes aegypti (Hagedorn et al., 1975), ovarian ecdysteroids regulate the onset of vitellogenin synthesis after blood feeding. Treatment of Calliphora erythrocephala (Thomson et al., 1971), and

Drosophila melanogaster (Lepesant et al., 1978), with ecdysteroids causes an increase in fat body protein synthesis. The sequestration of storage proteins by the fat body has been shown to be mediated by the prepupal rise in ecdysteroid titres, as shown in Calpodes ethlius (Locke, 1980), Hyalophora cecropia (Tojo et al., 1978), Bombyx mori (Tojo et al., 1980), Galleria mellonella (Miller and Silhacek, 1982), and Sarcophaga peregrina (Ueno and Natori, 1982). More recently, the role of ecdysteroids in the synthesis of major hemolymph proteins in Bombyx mori (Plantevin et al., 1987), and storage proteins in Spodoptera litura (Tojo et al., 1985), has been demonstrated.

Lipids: Lipids are essential structural components of the cell membrane and cuticle in insects. They provide a substantial source of metabolic energy for peri ds of sustained energy demand, facilitate water conservation by the formation of waterprcofing cuticular waxes and yield metabolic water (Downer, 1978). Some insect hormones and pheromones are lipoidal in nature (Beenakkers, 1983).

The principal hormones that act directly on insect lipid metabolism are the juvenile hormone, the adipokinetic hormone, and the hypolipaemic hormone. The role of JH in lipid metabolism was first demonstrated by Pfeiffer (1945) in Melanoplus differentialis. Allatectomy of the adult females

resulted in an abnormal accumulation of lipid in the fat body. Similar results have been reported in Carausius morosus (L'Helias, 1953), Periplaneta americana (Bodenstein, 1953), Locusta migratoria (Minks, 1967; Strong, 1968), Schistocerca gregaria (Odhiambo, 1966; Hill and Izatt, 1974), Spodoptera littoralis (El-Ibrashy and Boctor, 1970); Drosophila melanogaster (Vogt, 1946), and Calliphora erythrocephala (Thomsen, 1952). However, fat body lipid content remained normal in allatectomized Aedes taeniorhynchus (Van Handel and Lea, 1970), and Blaberus discoidalis (Mannix and Keeley, 1980). In Schistocerca gregaria (Hill and Izatt, 1974), and Locusta migratoria (Beenakkers, 1969), the increase in lipid content of the fat body of allatectomized insects was prevented by reimplantation of the CA; and in insects which had not been allatectomized but had received CA implants, fat body lipid synthesis was diminished.

Adipokinetic hormone (AKH), produced by the corpora cardiaca (CC) stimulates the fat body to release diacylglycerols and increases the oxidation of lipids in preference to carbohydrates by the flight muscles in adult Schistocerca gregaria (Mayer and Candy, 1969; Robinson and Goldsworthy, 1974). AKH activity has also been demonstrated in Locusta migratoria (Beenakkers, 1969), Tenebrio molitor (Goldsworthy et al., 1972), Danaus plexippus (Dallmann and Herman, 1978), Manduca sexta (Beenakkers et al., 1978),

Carausius morosus (Gade, 1980), Melanoplus sanguinipes (Downer, 1985), and Heliothis zea (Jaffe et al., 1988).

The mode of action of AKH has been elucidated (Downer, 1985). The AKH-induced elevation of hemolymph diacylglycerol results from the mobilization of fat body reserves of triacylglycerol, with the lipolytic action modulated by the cAMP-linked second messenger system (Gade, 1979), via protein kinase(s) (Van Marrewijk et al., 1980). Recently, AKH has been demonstrated to regulate carbohydrate metabolism in the larvae of Manduca sexta, in addition to its role in the regulation of lipid metabolism in the adults of the same species (Ziegler et al., 1990).

A hypolipaemic hormone has been shown to depress hemolymph lipid levels in some insects. Downer and Steele (1969, 1972), in Periplaneta americana, reported that injection of aqueous extracts of corpora cardiaca reduced hemolymph lipid levels and increased lipogenesis in the fat body. The hypolipaemic hormone was demonstrated in Locusta migratoria (Orchard and Loughton, 1980). In this insect, injections of the storage lobe extracts of the corpora cardiaca resulted in depletion of hemolymph lipid levels. The significance of the hypolipaemic hormone in Locusta migratoria may be restoration of hemolymph lipid level following its elevation during flight (Orchard and Loughton, 1980). The mode of action of the hormone awaits elucidation.

MATERIALS AND METHODS

2.1 Insect Rearing

Choristoneura fumiferana were reared from second-instar hibernacula supplied by the Forest Pest Management Institute (Forestry Canada), Sault Ste. Marie, Ontario. Insects were reared according to the method described by Mulye and Gordon (1990). The diet was a wheat germ based meridic diet (McMorran, 1965), purchased from Bio-Serv Inc., Frenchtown, NJ (Appendix A).

All of the procedures involved in the setting up of larval cultures and transferring sixth-instar larvae into new diet cups were done under a laminar-flow sterile hood. The rearing incubators, working area, and the instruments were disinfected with Hinks-Byers solution (Hinks and Byers, 1976) to reduce the incidence of microbial contamination. Liquid diet (7-10 ml) was poured into 1-oz. clear plastic cups and allowed to cool to room temperature (25 °C). The diet cups were then sprayed with an antifungal agent consisting of 1.5 g sorbic acid and 0.6 g methylparaben (methyl p-hydroxybenzoate) in 100 ml of 95 % ethyl alcohol (Chawla et al., 1967). The diet cups were capped with unwaxed paper lids and stored in sealed plastic bags at 5 °C. Only diet that had

been stored for less than 2 weeks was used for insect rearing.

To reduce fungal contamination of the second-instar larvae, the cheesecloth-Parafilm® (American Can Co., Greenwich, CT) sheets containing hibernacula were dipped in 3 % sodium hypochlorite (v/v), then air-dried. Larvae were reared in groups of 25-30 per inverted diet cup in a Conviron® controlled environment chamber (Controlled Environments Ltd., Winnipeg, Manitoba) fitted with Indorsun® fluorescent lights (intensity 2.5 W/m^2), at 23 ± 1 °C, non-diapause 16L:8D photoperiod, and 65 ± 2 % relative humidity. It was noted that lower humidity or higher temperature, or both, caused the artificial diet to dry out and resulted in starvation of the larvae, with consequent impairment of larval development. Higher relative humidity promoted fungal growth that was detrimental to larval development. Diet cups were inspected daily. If any fungal or microbial contamination was observed in the diet cups or the larval galleries, the diet cup was discarded. Within 24 h after moulting, early sixth instar larvae were transferred to new diet cups (5 larvae per cup).

2.2 Dose-response Studies

The efficacy of seven insect juvenile hormone analogs (JHAs) in disrupting the metamorphosis of the spruce budworm was compared through dose-response studies. Fifth instar

larvae that were about to molt into sixth instars were identified by the characteristic transverse white band on the conjunctiva between the head capsule and the prothoracic tergite (Retnakaran, 1973). Sixth instar larvae were used in the experiments, 1 day after molting. The following JHAs were evaluated against the sixth instar: methoprene (1-100 μg/μl); fenoxycarb (0.0001-0.5 μg/μl); S-71639 (0.01-100 μg/μl); ZR 8487 (0.01-100 μg/μl); ZR 9582 (0.01-100 μg/μl); ZR 9892 (0.01-100 μg/μl); and ZR 10151 (1.0-100 μg/μl). Methoprene and the ZR compounds were supplied by Zoecon Sandoz Research, Palo Alto, CA; fenoxycarb was obtained from Elanco, Eli Lilly Canada Inc., Scarborough, Ontario; and S-71639 was donated by Sumitomo Chemical Co., Osaka, Japan.

Using a Gilson® micropipette, technical grade compounds, dissolved in acetone, were applied topically, to the mid-dorsal region, extending from the thoracic to the abdominal areas of the larvae. Controls consisted of acetone treated and untreated 1-day old sixth instar larvae. Larvae were monitored daily and developmental events such as molting, pupation, and adult eclosion were recorded.

2.3 Effect of JHAs on Metabolite Concentrations

Day 1, sixth instar larvae of C. fumiferana were treated

with fenoxycarb and methoprene, at the respective LD_{50} concentration. Controls consisted of solvent treated larvae. Biochemical assays were done at various intervals (24 h for hemolymph studies and 48 h for fat body assays) of the experimental period of 6 days, which was the time when the control insects had pupated.

2.3.1 Hemolymph Assays

Direct measurements of hemolymph volume were made in modified Pasteur pipets. Disposable, borosilicate glass Pasteur pipets were cut at the distal ends with a glass file, leaving about mid-?.5 cm of the pipet. The constricted end was flame-sealed, and the open end was flame-polished. A glass-wool plug was inserted inside to partition the modified pipet into a 'larval compartment' and a 'hemolymph receptacle'. Larvae were ligated in the head and the last abdominal segment with waxed dental floss, to prevent gut contents from contaminating the hemolymph, and placed, individually, in the modified pipets. Larvae were pricked with an insect pin to allow hemolymph flow. These pipets were then placed in microfuge tubes, and centrifuged at 200 r.p.m. for 5 minutes at room temperature (25 °C). Hemolymph volume was measured by drawing the collected hemolymph from the receptacle-end of the pipet with a Hamilton® microsyringe.

For the purpose of biochemical analyses, hemolymph was collected from individual larvae by piercing the thoracic region with an insect pin, and gently drawing the hemolymph in a micropipette (5-10 μl per insect). Only clear hemolymph was collected and care was taken to avoid contaminating the hemolymph with gut contents. Hemolymph was transferred into a centrifuge tube containing 5 ml of 5 % trichloroacetic acid (TCA) (w/v), on ice. After 15 minutes, the tubes were centrifuged at 800 x g for 10 minutes at 4 °C. This procedure was slightly modified for hemolymph total protein and total lipid determinations, by transferring 5-10 μl hemolymph into 1.0 ml of distilled water.

2.3.1.1 Carbohydrates

Total carbohydrates in the hemolymph were determined by the anthrone assay (Roe, 1955). The anthrone reagent was prepared by slow mixing of 0.05 % anthrone in 72 % sulfuric acid (w/v). The reagent was stored at 5 °C, and used within 2 weeks of preparation. Analysis of hemolymph total carbohydrates was done by mixing 0.5 ml of the supernatant with 5.0 ml of anthrone reagent on ice, and boiling in a water bath (100 °C) for 10 minutes. After cooling to room temperature (25 °C), absorbances of the mixtures were measured at 650 nm, against a reagent blank consisting of 0.5 ml of 5%

TCA and 5.0 ml of the anthrone reagent, in a Spectronic 601® spectrophotometer (Milton Roy Co., USA). Glucose was used as the reference standard. A standard curve is shown in Fig. 2.1.

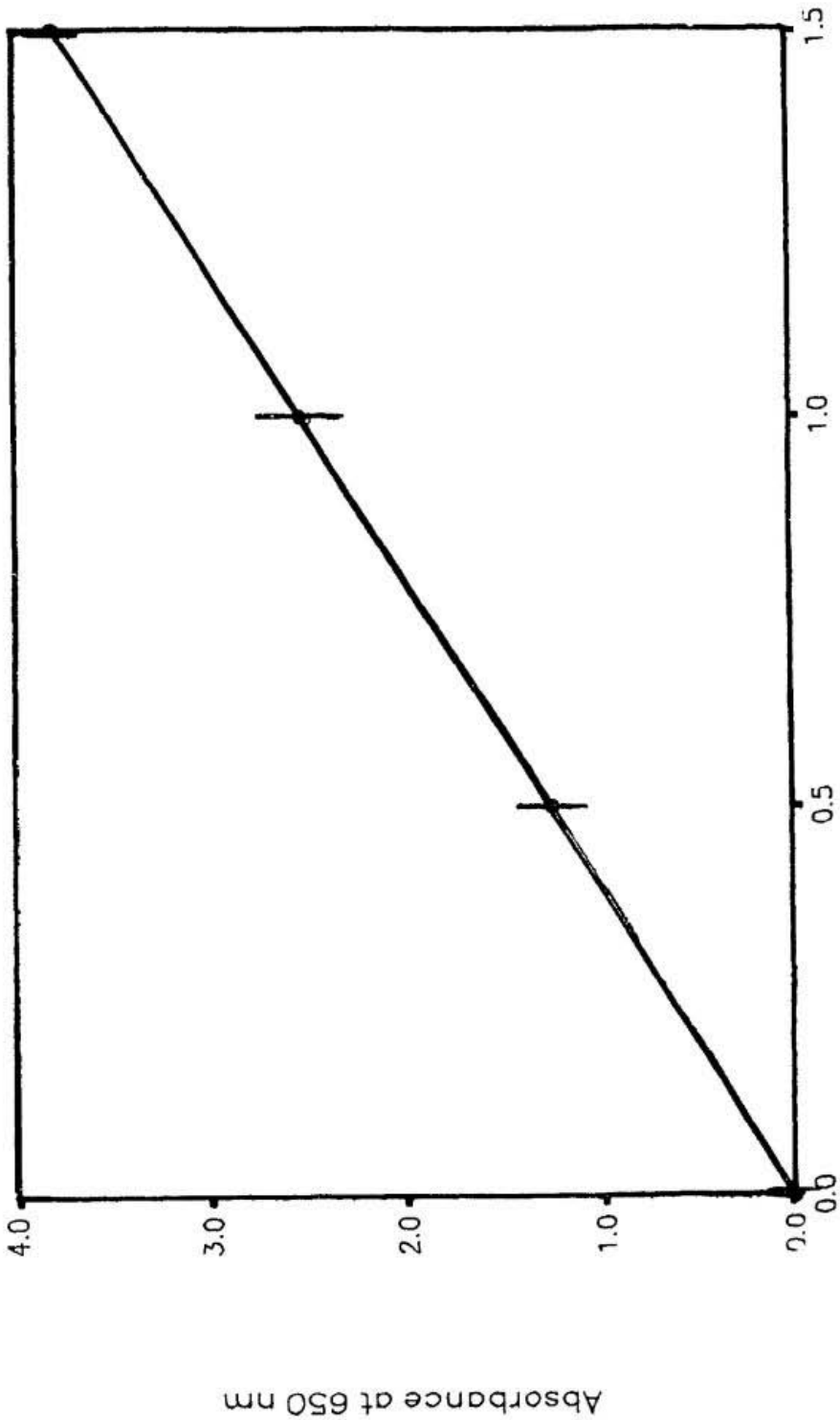
2.3.1.2 Proteins

Total protein concentration of the hemolymph was determined by the micro-Lowry assay (Lowry et al., 1951), as modified by Paterson (1977). A Sigma® protein assay kit was utilized for sake of consistency and convenience (Sigma Chemical Co., St. Louis, MO). Diluted hemolymph (1.0 ml) was treated with 0.1 ml of aqueous sodium deoxycholate (1.5 mg/ml), followed by thorough mixing. After 10 minutes at room temperature (25 °C), 0.1 ml of aqueous TCA (72 %, w/v) was added. Tubes were vortexed and centrifuged at 4000 x g for 5 minutes at 25 °C to precipitate the protein.

The supernatants were decanted, the pellets dissolved in 1.0 ml of 10 % sodium dodecylsulfate (w/v), 0.5 ml of 2.0 N Folin and Ciocalteu's phenol reagent was added and the mixture briefly vortexed. The mixture was allowed to stand at room temperature (25 °C) for 30 minutes to allow color development. The blank consisted of 1.0 ml of dist. water and the appropriate assay reagents. After 30 min., the absorbances of the mixtures were measured against the blank in a spectrophotometer at a wavelength of 750 nm. Protein

Figure 2.1. Plot of glucose concentration (mg/ml) vs. absorbance at 650 nm. Glucose was assayed by the anthrone procedure (Roe, 1955). Values are shown as Mean ± SE of 3 separate determinations.

Figure 2.1


Absorbance at 650 nm
4.0
3.0
2.0
1.0
0.0
0.0
0.5
1.0
1.5
Glucose Concentration (mg/ml)

concentrations were determined by comparing the absorbance values with a standard curve (Fig. 2.2) made from known amounts of bovine serum albumin (BSA).

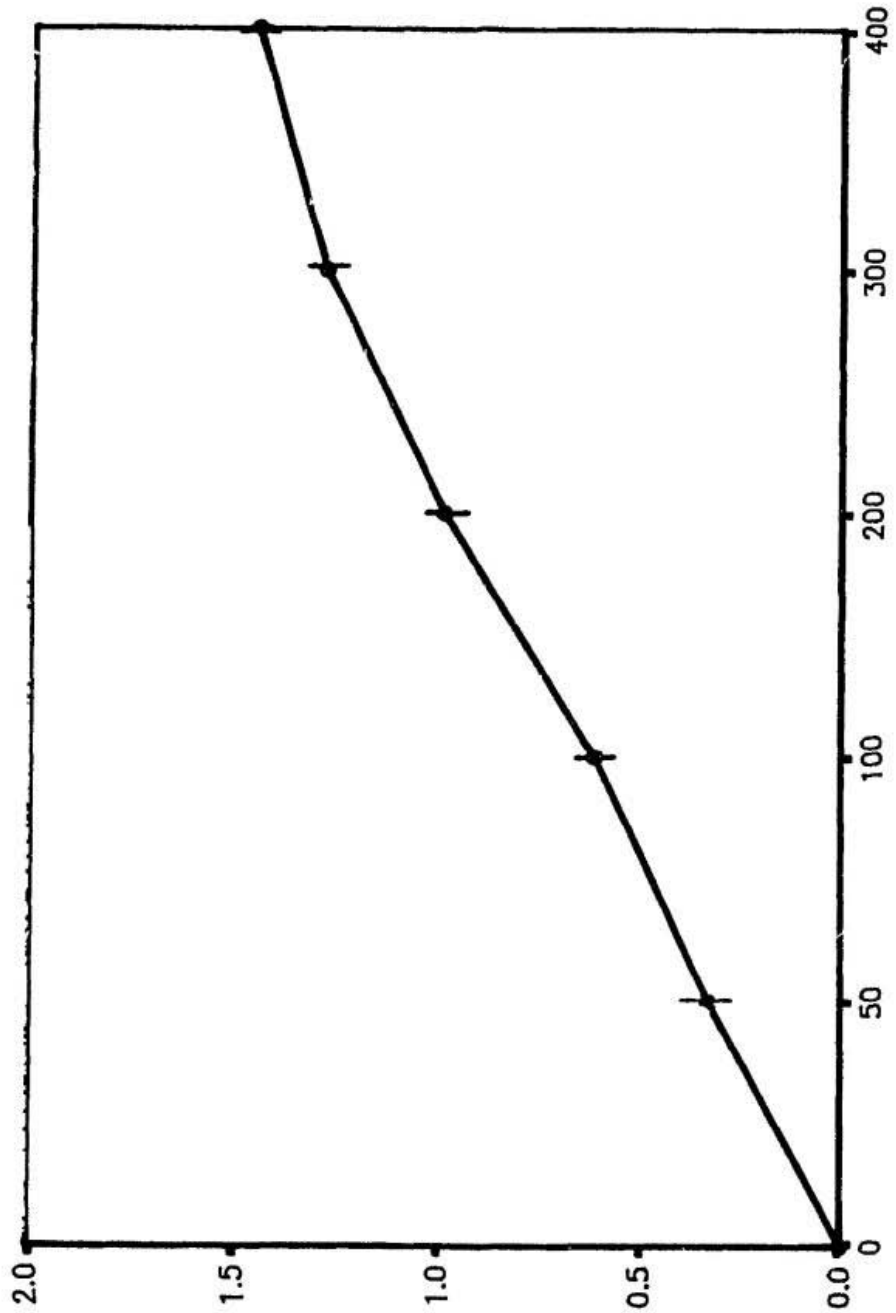
2.3.1.3 Lipids

Total lipid concentration in the hemolymph was measured by Folch extraction (Folch et al., 1957) of the hemolymph, followed by the Amenta procedure (Amenta, 1970). Diluted hemolymph (1.0 ml) was treated with 1.0 ml of chloroform:methanol (2:1, v/v, freshly prepared) in a Kolmer centrifuge tube, followed by thorough mixing. The mixture was allowed to stand for 15 minutes at 25 °C. After 15 minutes, 1.0 ml of 9 % NaCl (w/v) was added, and the mixture centrifuged at 1000 x g for 5 minutes at 25 °C. Using a Hamilton syringe, an aliquot (0.5-0.75 ml) of the lower solvent phase was transferred to culture tubes.

Extracts were dried under a stream of nitrogen. To this, 2.0 ml of the acid-dichromate reagent (5.0 g potassium dichromate ($K_2Cr_2O_7$) in 1000 ml of concentrated sulfuric acid) were added. The reagent blank contained distilled water extract and the appropriate reagents. Tubes were heated in an oven at 95 °C for 1 hour, and an aliquot (1.0 ml) of the solution was diluted with 3.0 ml water. Absorbances of the mixtures were read against a water blank at 430 nm in

Figure 2.2. Protein calibration curve. Bovine serum albumin was used as a standard (μg/ml). Protein was determined by the micro-Lowry assay (Lowry et al., 1951), as modified by Paterson (1977). Absorbances were measured at 750 nm. Values are shown as Mean ± SE of 3 separate determinations.

Figure 2.2


Absorbance at 750 nm
2.0
1.5
1.0
0.5
0.0
0
50
100
200
300
400
BSA Concentration (µg/ml)

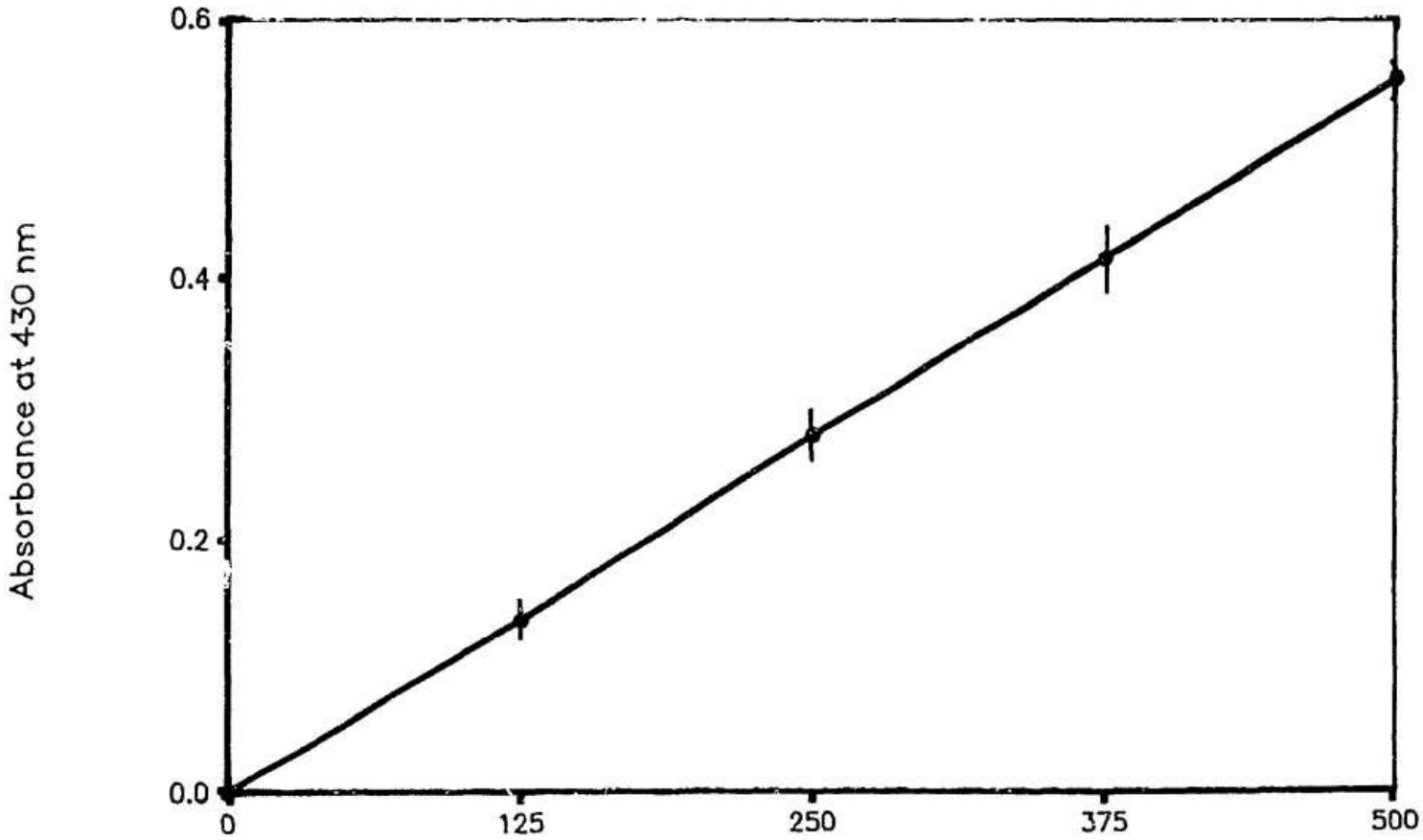
a spectrophotometer. A standard curve, constructed from known amounts of palmitic acid, was used to calculate lipid concentration of the hemolymph (Fig. 2.3).

2.3.2 Fat Body Assays

Fat bodies were dissected out under isotonic ice-cold Dulbecco's phosphate buffered saline (D-PBS), purchased from Gibco Laboratories, Grand Island, NY. Larvae were pinned in the head and the last abdominal segment, in a wax dish, and placed under a stereo-microscope (Wild Heerbrugg). An incision was made with a scalpel, along the mid-dorsal region of the body, extending from the prothoracic tergite to the last abdominal segment. The paired salivary glands, the alimentary canal, and, in the case of males, the paired testes, were then gently excised to avoid contamination of the fat bodies. The dissection was rinsed with ice-cold D-PBS, and the fat bodies were gently removed with a pair of fine forceps. Fat bodies were frozen rapidly in liquid nitrogen (-195 °C) and lyophilized for 8 hours in a Labconco® freeze-dryer (Labconco Corp., Kansas City, MO). Fat body preparations were stored at -15 °C, for a maximum of 2 weeks.

Figure 2.3. Plot of lipid concentration (μg) against absorbance at 430 nm. Palmitic acid was used as a reference standard. Lipid was assayed according to the Amenta method (Amenta, 1970). Values are shown as Mean ± SE of 3 separate assays.

Figure 2.3


0.6
0.4
0.2
0.0
Absorbance at 430 nm
0
125
250
375
500
Lipid Concentration (μg)

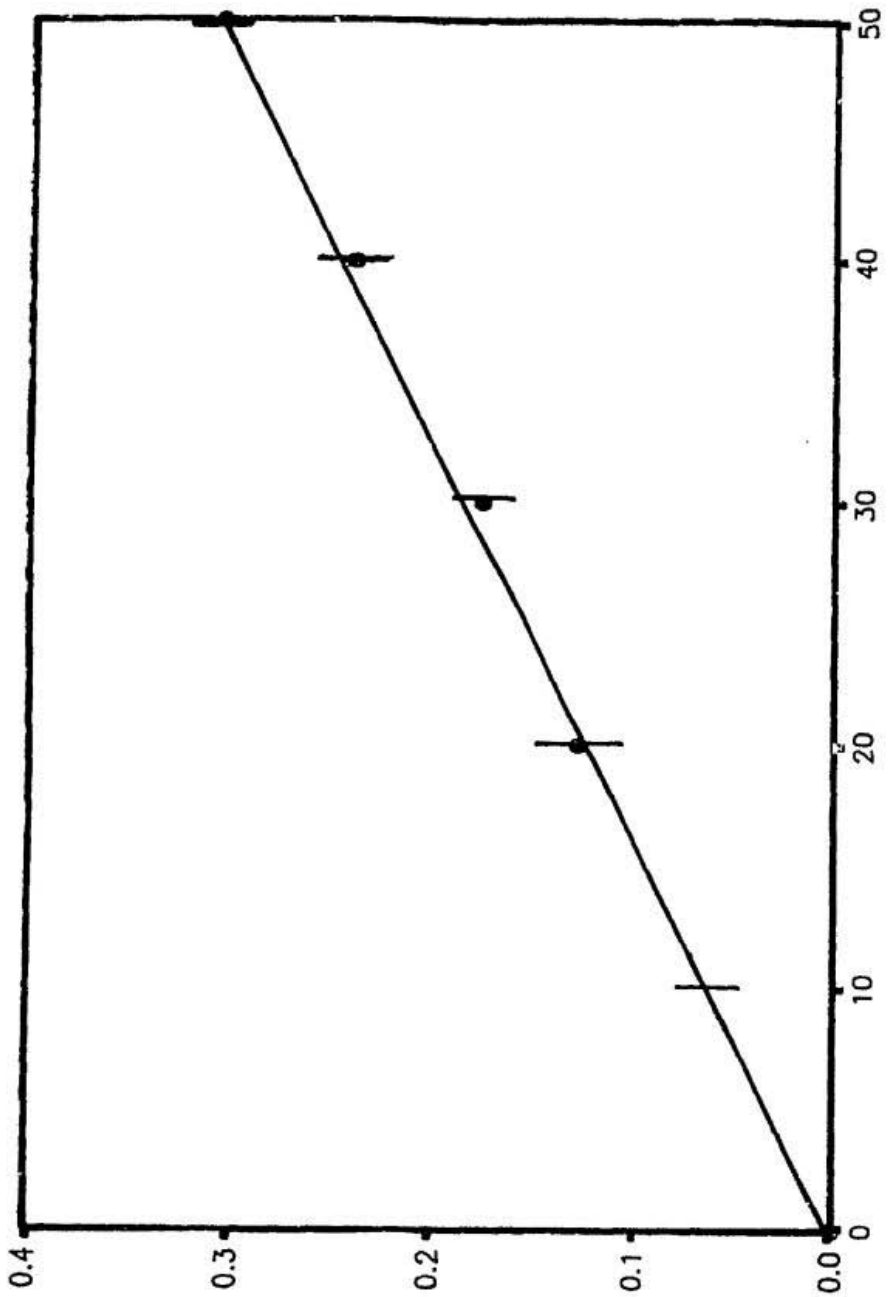
2.3.2.1 Glycogen

Fat body glycogen was extracted according to a method suggested by Dr. J.E. Steele, Department of Zoology, University of Western Ontario, London, Ontario. Lyophilized fat bodies (15-20 mg) were digested in 3.0 ml of 30 % potassium hydroxide (w/v) at 100 °C for 20 minutes, in a hot water bath. An aliquot (1.0 ml) of the digest was treated with 50 µl of saturated (40 %, w/v) sodium sulfate and 2.0 ml of 95 % ethyl alcohol (v/v), in a centrifuge tube.

The treated digests were vortexed, allowed to stand (15-20 minutes), centrifuged (800 x g for 5 minutes at 25 °C), the supernatant decanted, and the glycogen pellet resuspended in 1.0 ml of distilled water. To this, 5.0 ml of the anthrone reagent (0.05 % anthrone in 72 % H_2SC_4, w/v) were added, on ice (Roe, 1955). A reagent blank containing 1.0 ml distilled water and 5.0 ml of anthrone reagent was prepared. Tubes were heated in a water bath (100 °C) for 10 minutes. Upon cooling to room temperature (25 °C), absorbances of the mixtures were measured at 650 nm, against the reagent blank, in a spectrophotometer. Glycogen levels in the fat bodies were determined by comparing the absorbance values with a calibration curve made from known amounts of glycogen (Fig. 2.4).

Figure 2.4. Glycogen standard curve. Data are shown as glycogen concentration (µg/ml) plotted against absorbance at 650 nm. Glycogen was assayed by the anthrone procedure (Roe, 1955). Values are shown as Mean ± SE of 3 separate determinations.

Figure 2.4


Absorbance at 650 nm
0.0
0.1
0.2
0.3
0.4
0
10
20
30
40
50
Glycogen Concentration (µg/ml)

2.3.2.2 Proteins

Lyophilized fat bodies (15-20 mg) were homogenized in 1.0 ml of ice-cold insect saline, in a Potter-Elvehjem tissue grinder with a motor-driven pestle. The homogenate was centrifuged at 10,000 x g for 20 minutes at 4 °C. A 0.1 ml aliquot of the supernatant was made up to 1.0 ml (final volume) with distilled water and treated with 0.1 ml of aqueous sodium deoxycholate (1.5 mg/ml), then vortexed. After 10 minutes (25 °C), 0.1 ml of aqueous TCA (72 % w/v) was added. The samples were vortexed, then centrifuged at 4000 x g for 5 min. at 25 °C to precipitate protein.

The supernatant was decanted, and the pellet was solubilized in 1.0 ml of 10 % sodium dodecyl sulfate (w/v). To this, 0.5 ml of 2.0 N Folin and Ciocalteu's phenol reagent was added and mixed briefly. The reaction mixture was allowed to stand at 25 °C for 30 minutes to allow color development. A blank, consisting of 1.0 ml distilled water and the appropriate reagents, was set up. After 30 minutes, the absorbances of the mixtures were measured against the blank in a spectrophotometer, at 750 nm. Protein levels in the fat body extracts were determined by comparing the absorbance values with a calibration curve made from known amounts of BSA (Fig. 2.2).

2.3.2.3 Lipids

Neutral lipid levels in the fat bodies were determined by Folch extraction (Folch et al., 1957) of lyophilized fat bodies, followed by the Amenta procedure (Amenta, 1970). Lyophilized fat bodies (5-10 mg) were homogenized in 1.0 ml of ice-cold chloroform:methanol (2:1 v/v), in a Potter-Elvehjem homogenizer. The homogenate was transferred to centrifuge tubes and allowed to stand for 15 minutes at 25 °C. Then, 1.0 ml of 9 % NaCl (w/v) was added, the contents vortexed, then centrifuged (1000 x g; 10 minutes; 4 °C).

Using a Hamilton syringe, an aliquot (0.2 ml) of the lower solvent phase was transferred to culture tubes and the solvent evaporated under a stream of nitrogen. Then, 2.0 ml of the acid-dichromate reagent was added. The reagent blank contained 0.2 ml solvent, aspirated under nitrogen, and the appropriate reagents. Tubes were heated in an oven at 95 °C for 1 h, and an aliquot (1.0 ml) of the solution was diluted with 3.0 ml of distilled water. Absorbances of the mixtures were read against a water blank at 430 nm in a spectrophotometer. Lipid concentrations of the fat bodies were calculated from a calibration curve, plotted from known amounts of palmitic acid (Fig. 2.3).

2.4 Effects of Fenoxycarb on Lipid Metabolism

Treatment of day 1, sixth instar C. fumiferana larvae with fenoxycarb caused a highly significant depletion in the levels of hemolymph and fat body lipids. Accordingly, subsequent studies on the effects of JHAs on lipid metabolism of the spruce budworms were conducted only with fenoxycarb. Early sixth instar larvae were treated with fenoxycarb, at the LD_{50} concentration. Controls consisted of acetone-treated larvae. Physiological assays were performed at various intervals within the experimental period of 6 days, a time when the controls had pupated.

2.4.1 Qualitative Lipid Profile

Lipids were extracted from the hemolymph and the fat bodies of the spruce budworm as outlined (Section 2.3.1.3). Qualitative analysis of the lipid extracts was done by thin layer chromatography (TLC). The TLC procedure of Mangold (1969), as described in Fried and Sherma (1986), was employed to separate neutral lipids. Precoated Silica Gel G plates (20 x 20 cm, 250 μm thickness) were purchased from Fisher Scientific Co., Montreal, Quebec. TLC plates were heat activated at 70 °C for 30 minutes and stored in a desiccator

chamber (Fisher) prior to use. Each plate was divided into several lanes by scouring lines in the silica gel layer.

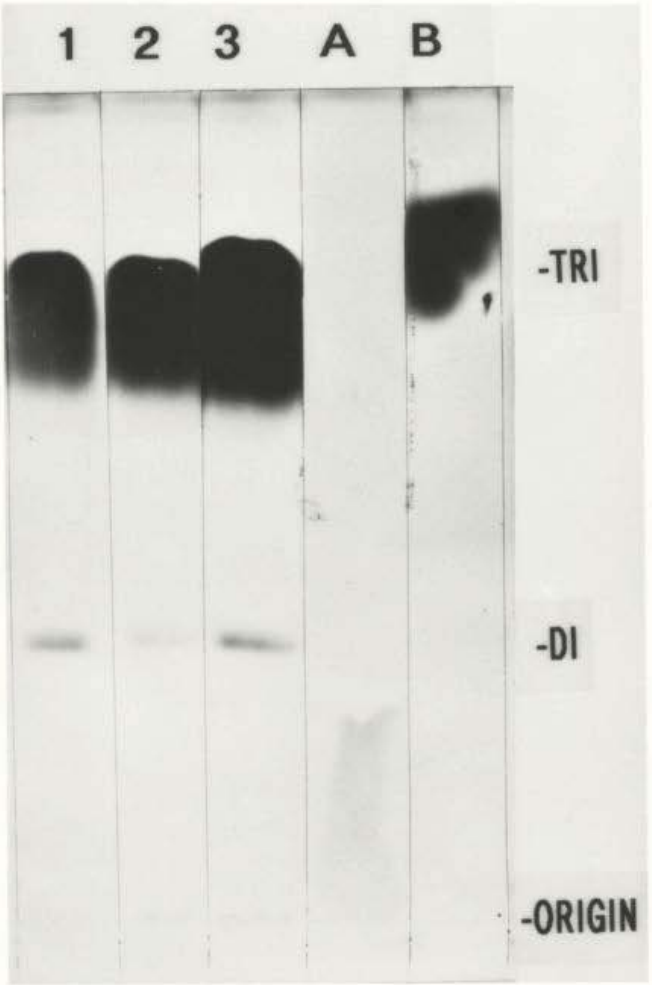
Lipid extracts (50-100 μl) were applied to the TLC plates, 2 3 cm from the bottom, with micropipets. A solvent front line was drawn 15 cm from the origin. The mobile phase consisted of N-Hexane: Diethyl ether: Acetic acid (70:30:1, v/v). The TLC plates were developed in a developing tank (Desaga, Heidelberg, Germany), pre-saturated (15-20 min.) by lining the inside with Whatman No.1 filter paper, pouring the mobile phase, and placing the lid on the tank. The solvent was allowed to ascend to the solvent front line, and the plates removed and dried under a stream of cool air from a hairdryer. Visualization of the separated lipids was done with iodine vapour, by placing the TLC plate in a separate developing tank containing crystalline iodine, placed in a warm water bath (40 °C). A sample chromatogram is shown in Fig. 2.5.

2.4.2 Fatty Acid Profile

The qualitative distribution of fatty acids in the hemolymph and fat body was determined by gas-liquid chromatography. Lipids were extracted from the hemolymph and the fat bodies, according to the procedure of Folch *et al.* (1957). Fatty acids were transmethylated according to the

Figure 2.5. Qualitative distribution of lipids in the fat body of Choristoneura fumiferana. Lipid was extracted according to the Folch procedure (Folch et al., 1957) and separated by the TLC procedure of Mangold (1969) and Fried and Sherma (1986). The mobile phase consisted of N-Hexane:Diethyl ether:Acetic acid (70:30:1 v/v). Lanes 1-3, Fat body lipid extract; Lane A, commercial diacylglycerol (DI) standard; Lane B, commercial triacylglycerol (TRI) standard.

Figure 2.5



procedure of Keough and Kariel (1987). Lipid extracts (0.5-0.75 ml) were transferred to a 6.0 ml conical vial and the solvent evaporated under a stream of nitrogen. Then, 2.0 ml of transmethylating reagent (6.0 ml of concentrated H_2SO_4 made up to 100.0 ml with 99.9 mol % methanol, and containing 15.0 mg of recrystallized hydroquinone as an antioxidant), was added. Vials were incubated for 4-5 hours at 70 °C and the sample, after dilution with 1.0 ml distilled water, extracted 3 times with 1.5 ml of pesticide grade hexanes. Fractions were combined and washed 2 times with 1.5 ml of distilled water. Hexane extracts, containing the fatty acid- methyl esters (FAMEs), were dried under nitrogen and stored at -20 °C. Prior to injection, samples were dissolved in carbon disulphide (10-20 μl).

Analysis of the fatty acid-methyl esters was accomplished by a Perkin-Elmer 8310 gas chromatograph (Perkin-Elmer, Norwalk, CT). A glass capillary column (30.0 m x 0.25 mm i.d.), packed with SP-2330 (Supelco Inc., Bellefonte, PA), was used to separate the FAMEs. Oven temperature was programmed to 180 °C for 11.6 min., then 199 °C for 10 min. The injection port and the flame ionization detector (FID) oven temperatures were 249 °C. Commercial standards of fatty acid methyl esters (FAMEs), obtained from Supelco Inc., were run under identical analytical conditions and the chromatograms evaluated with reference to the retention time of the standards.

2.4.3 Fat Body Lipid Synthesis

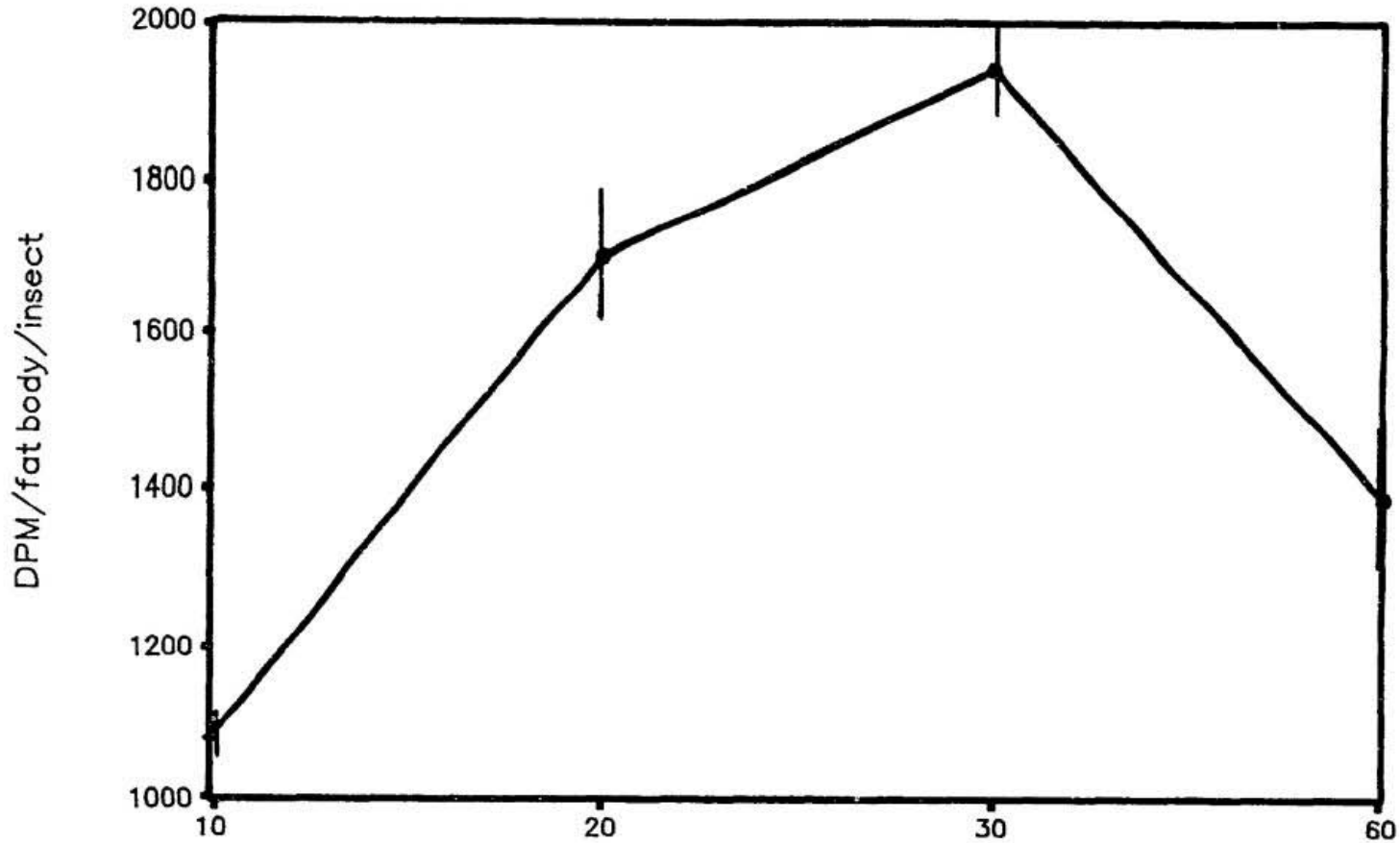
2.4.3.1 Overall Lipid Synthesis from ^{14}C-Acetate

The capacity of fat bodies isolated from sixth instar C. fumiferana to incorporate radiolabelled precursors into lipid was determined in vitro. Fat bodies were dissected out, as previously described, under ice-cold Dulbecco's phosphate buffered saline (D-PBS). The dissection was rinsed with D-PBS, and fat bodies were excised with a pair of fine forceps, weighed, rinsed with D-PBS, and transferred to borosilicate culture tubes (12 x 75 mm) containing 0.5 ml of the ^{14}C-acetate-culture medium (Grace's insect medium, Gibco Laboratories). [1-^{14}C]-sodium acetate (sp. activity 59.0 mCi/mmol; radiochemical purity 99 %) was purchased from New England Nuclear, Boston, MA.

Initially, a calibration curve for ^{14}C-acetate incorporation into fat body lipids was constructed; based upon fat body incubations at various concentrations of ^{14}C-acetate, and for various times. Based upon maximal linear acetate incorporation into fat bodies, 1.7 nM (0.1 μCi) ^{14}C-acetate/assay was utilized in subsequent experiments. A time course for ^{14}C-acetate incorporation into fat bodies using this dosage is given in Figure 2.6. All incubations were done at 30 °C for 30 minutes, in a Dubnoff metabolic shaking incubator

Figure 2.6. Incorporation of ^{14}C-Acetate into lipids by spruce budworm fat bodies *in vitro*, as a function of time. Values are expressed as Mean ± SE of 3 separate assays, DPM/fat body/insect (8.0 mg fresh wt.). Fat bodies were incubated in the culture medium containing 1.7 nM of ^{14}C-Acetate. Incubation temperature was 30 °C.

Figure 2.6


2000
1800
1600
1400
1200
1000
DPM/fat body/insect
10
20
30
60
Incubation Time (min.)

(Precision Scientific, Chicago, IL).

After incubation, the tissue was killed by addition of 0.5 ml 1.0 N HCl. Fat bodies were transferred to glass vials and rinsed 6 times with D-PBS to remove surface radioactivity. Initial trials revealed that, after rinsing 5-6 times, no radioactivity above background levels was evident in the washings. Following this, fat bodies were homogenized in 1.0 ml of ice-cold chloroform:methanol (2:1, v/v) in a Potter-Elvehjem homogenizer, to extract total lipids (Folch et al., 1957).

An aliquot (20.0 μl) of the lipid extract was transferred to scintillation vials (capacity 20.0 ml). The solvent was evaporated under a stream of cool air from a hairdryer, and 10.0 ml of Ready Safe® liquid scintillation cocktail (Beckman Instruments Inc., Fullerton, CA) was added. Vials were capped and shaken vigorously to solubilize and suspend the lipids in the scintillation cocktail. β-particle emission of the samples was counted in a LKB-Wallac 1214 Rackbeta LSC system, with a microcomputer processor (LKB-Wallac, Stockholm, Sweden). Radioactivity counts (cpm) were converted to disintegrations per minute (dpm), based on the quench curves, with the microcomputer processor.

2.4.3.1.1 Direct Effect of Fenoxycarb on Lipid Synthesis

The possibility that the JHA fenoxycarb may directly affect lipid synthesis by fat bodies of C. fumiferana was assessed by inclusion of the compound in the culture medium. Fat body preparations from untreated sixth instar larvae were incubated in 1.7 nM ^{14}C-acetate and 0.26 μg (LD_{50}) fenoxycarb in 0.5 ml of Grace's insect medium. After the incubation period (30 °C; 30 min.), fat body lipids were extracted (Folch et al., 1957). An aliquot (20 μl) of the lipid extract was transferred to scintillation vials, the solvent evaporated under a stream of cool air, and 10.0 ml of liquid scintillation cocktail were added. Scintillation counting of the samples was done in a liquid scintillation counter. Radioactivity counts (cpm) were converted into disintegrations per minute (dpm).

2.4.3.1.2 ^{14}C-Acetate Product Analysis

The qualitative distribution of fat body lipids synthesized from ^{14}C-acetate was determined by thin layer chromatography (TLC). Lipids were extracted from fat bodies that had been incubated (30 °C; 30 min.) in 1.7 nM of ^{14}C-acetate in 0.5 ml of Grace's insect medium. Lipid extracts were concentrated under a stream of nitrogen, and the neutral

lipids separated by TLC (Fried and Sherma, 1986). Precoated Silica Gel G plates (20 x 20 cm, 250 μm thickness) were heat activated at 70 °C for 30 minutes and 'blank-developed' in the mobile phase (N-Hexane:Diethyl ether:Acetic acid, 70:30:1, v/v), in a developing tank, to reduce background interference. This was followed by oven drying at 70 °C for 30 minutes.

Lipid extracts (50 μl) were then applied to the TLC plates, 2.5 cm from the bottom, with micropipets. Plates were developed in a pre-saturated developing tank. TLC plates were air dried and the lipid fractions were visualized with iodine vapour. Then, plates were heated in an oven at 50 °C for 12 hours to remove traces of iodine by sublimation. The appropriate areas of the silica gel were then scraped off the plates into scintillation vials, and suspended in 10.0 ml of scintillation cocktail. Samples were counted in a liquid scintillation counter. Radioactivity counts (cpm) were converted into disintegrations per minute (dpm).

2.4.3.2 Synthesis of Fatty Acids from ^{14}C-Acetate

The competence of the fat bodies from treated and control sixth instar C. fumiferana to biosynthesize fatty acids in vitro was investigated by assaying the cytosolic enzymes responsible for fatty acid synthesis from acetate precursor. The methods described by Tietz (1961, 1963) were modified for

spruce budworm studies.

The principle enzyme systems involved in cell-free fatty acid synthesis are the acetyl-CoA carboxylase (EC 2.3.1.9), which catalyzes the formation of malonyl-CoA from acetyl-CoA (carboxylation), and the fatty acid synthetase complex, which catalyzes the successive condensation of malonyl-CoA and acetyl-CoA units to palmitate (Lehninger, 1975).

Spruce budworm fat bodies were dissected out under ice-cold D-PBS, rinsed 3 times with D-PBS, and homogenized in 1.0 ml of enzyme buffer in a Potter-Elvehjem homogenizer, on ice. The enzyme buffer contained 85.0 mM dibasic potassium phosphate, 9.0 mM monobasic potassium phosphate, 10.0 mM potassium bicarbonate, and 2.0 mM glutathione (Tietz, 1961). The homogenate was centrifuged at 400 x g for 5 minutes at 5 °C, and filtered through a non-absorbent cotton pad to remove fat. Subcellular fraction from the filtrate was obtained by centrifugation at 20,000 x g for 20 minutes at 0 °C. The supernatant was decanted, and filtered through a non-absorbent cotton pad. The crude enzyme preparation was used within 2 hours of preparation. Protein estimation of the preparation was done by the micro-Lowry assay (Lowry et al., 1951).

Enzyme assays were performed according to the procedure of Wakil et al. (1957), with some modifications. The assay medium consisted of 5.0 μM (294.0 μCi) ^{14}C-acetate and cofactors (Table 2.1). Initially, a calibration curve was

Table 2.1. The composition of the Assay Medium for Cell-free Fatty acid Synthesis (from Tietz, 1961).

^{14}C-Acetate (294.0 μCi)	5.0 μM
ATP	5.0 μM
Coenzyme A	0.1 mg
Glutathione	5.0 μM
NAD	0.5 μM
NADP	0.5 μM
$MgCl_2$	10.0 μM
$MnSO_4$	0.5 μM
Malonate	20.0 μM
α-Ketoglutarate	10.0 μM
$KHCO_3$	10.0 μM

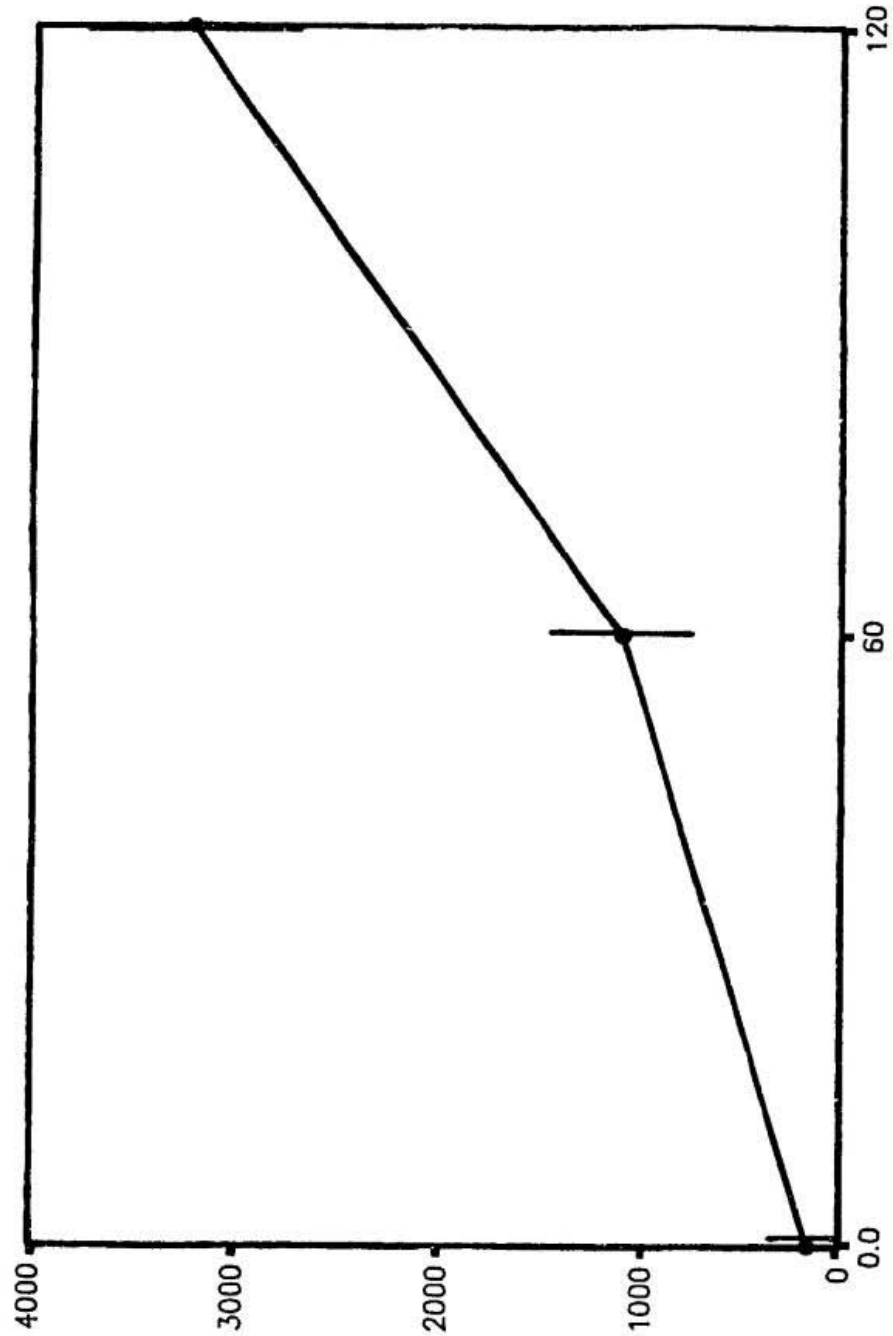
constructed for cell-free fatty acid synthesis from ^{14}C-acetate, based upon enzyme protein concentration and incubation time. Incubations were done at 30 °C in a Dubnoff metabolic incubator.

Maximum fatty acid synthesis was obtained with 5.34 mg enzyme protein/assay. A fatty acid synthesis-incubation time curve using this concentration of enzyme protein is shown in Figure 2.7. However, in order to obtain this amount of enzyme, it was necessary to pool fat bodies from several insects. Since my studies showed that 1.5-2.5 mg enzyme protein/assay allowed a measurable level of fatty acid synthesis to occur and that this amount of enzyme could be extracted from the fat bodies of individual insects, subsequent experiments comparing control and fenoxycarb-treated insects used the lower enzyme concentration (0.3-0.4 ml enzyme preparation; final assay volume 1.0 ml). An incubation time of 70 minutes was selected, because the amount of fatty acid synthesis that had occurred at this time was sufficiently high to allow measurable differences among samples to be discerned.

The reaction was started by addition of the enzyme preparation. After incubation, the reaction was terminated by addition of 0.5 ml of 10 % potassium hydroxide in ethyl alcohol (w/v). Tubes were then heated at 85 °C for 3 hours in a hot-water bath, to saponify the mixture. Following this, the tubes were allowed to cool to room temperature (25 °C) and

Figure 2.7. Cell-free fatty acid synthesis by fat bodies of C. fumiferana as a function of time, using an enzyme concentration equivalent to 5.34 mg protein. Each point is expressed as Mean DPM ± SE of 3 separate assays. Cell-free fat body preparations were incubated with the incubation medium containing 5.0 μM ^{14}C-Acetate and cofactors. Incubation temperature was 30 °C.

Figure 2.7


Fatty acid synthesis (DPM)
4000
3000
2000
1000
0
0.0
60
120
Incubation Time (min.)

0.5 ml aliquots of 2.0 N HCl were added to acidify the mixtures.

Samples were extracted by addition of 4.0 ml of petroleum ether (b.p. 40-60 °C) and the mixtures shaken for about 2 minutes. The two phases were allowed to separate and the petroleum ether layer was transferred by means of a Pasteur pipette into scintillation vials. The extraction procedure was repeated twice in exactly the same manner. The solvent fractions were evaporated under a stream of cool air. Then, 10.0 ml of scintillation cocktail were added, the vials capped and shaken vigorously to solubilize and suspend the radioactive lipid. Liquid scintillation counting was done in a scintillation counter. Radioactivity counts (cpm) were converted into disintegrations per minute (dpm).

The involvement of fat body cytosolic enzymes, particularly the biotin-dependent enzyme acetyl-CoA carboxylase, was determined by addition of avidin to the incubation medium. Avidin is an egg white protein which binds biotin very tightly, and inhibits the fatty acid synthesis by binding the enzyme-bound biotin (Wakil and Gibson, 1960). The enzyme preparation (0.3 ml) was preincubated for 5 minutes at 30 °C with 0.1 mg avidin. Then, the reaction was started by addition of the assay medium (0.4 ml) containing 5.0 μM of ^{14}C-Acetate and cofactors (Table 2.1). Incubations were done at 30 °C for 70 minutes, and the labelled fatty acids were extracted

and quantified, as outlined previously.

2.4.3.3 Synthesis of Complex Lipids from ^{14}C-Palmitate

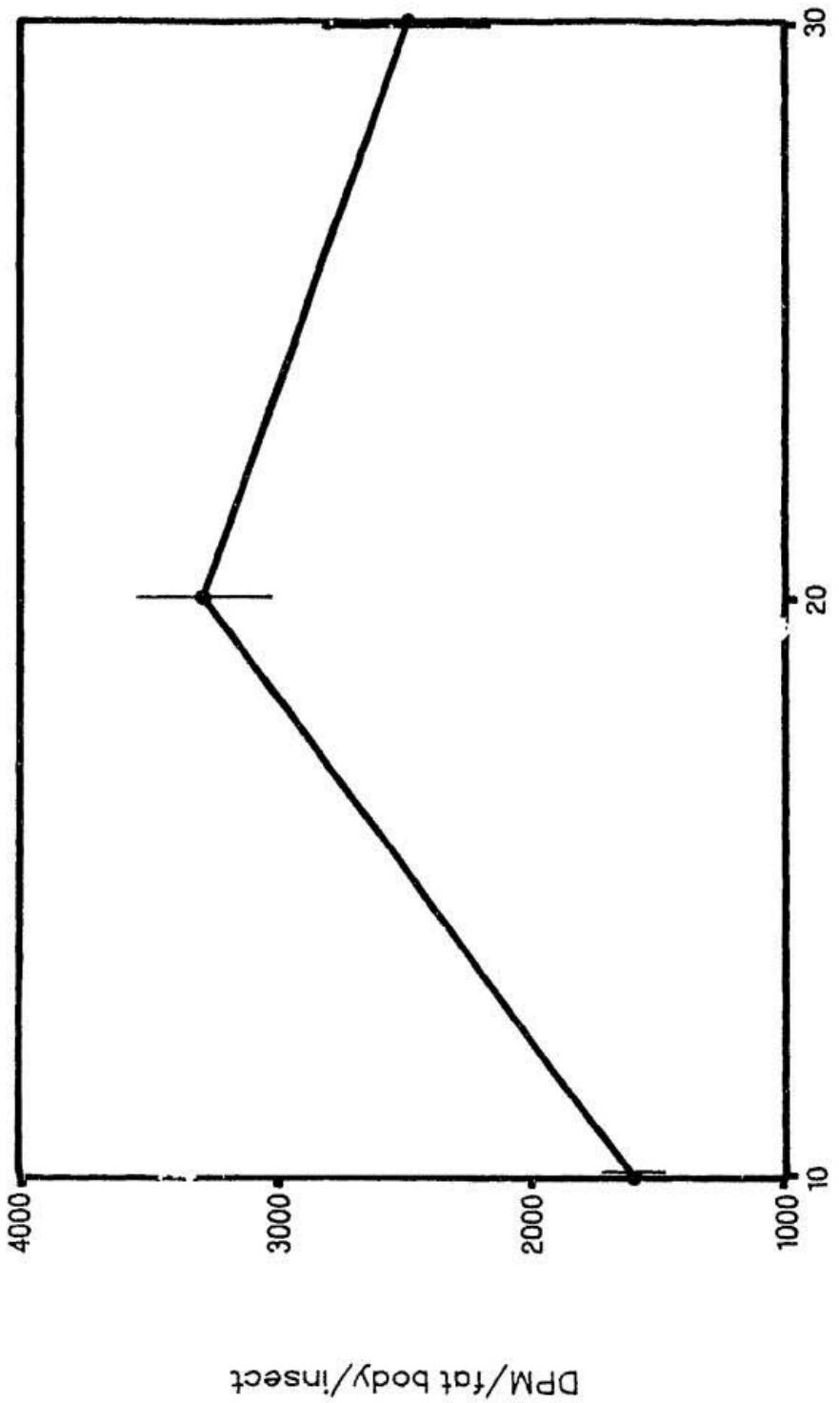
The capability of the spruce budworm fat bodies to incorporate pre-formed fatty acids into complex lipids was investigated by incubating the fat bodies, in vitro, with ^{14}C-palmitic acid. [U-^{14}C] Palmitic acid (sp. activity 819.0 mCi/mmol, radiochemical purity 99.0 %; Amersham Corp., Arlington Heights, IL) was converted into its sodium salt and complexed with bovine serum albumin, to allow solubilization in the aqueous culture medium (Spector et al., 1965). The methods described by Masironi and Depocas (1961) and Björntorp (1966) were employed. An aliquot (28 µl=1.96 µg) of ^{14}C-palmitic acid was transferred to a culture tube and the solvent aspirated under a stream of nitrogen. Then, 3.0-5.0 µl of 0.04 N sodium hydroxide in methyl alcohol was added, and the tube heated at 50 °C for 10 minutes in a water bath. The solvent was evaporated under nitrogen, and the labelled sodium palmitate was dissolved in a few drops of distilled water (50 °C). To this, 1.0 ml of the BSA stock, containing 192.4 µg bovine serum albumin (Fraction 5, fatty acid free) in 1.0 ml of D-PBS, was added with vigorous mixing. The final solution had a pH of 7.5, and contained 1.96 µg ^{14}C-palmitate and 192.4 µg BSA, i.e. a molar ratio of fatty acid to albumin of 3 to 1.

Fat bodies were dissected out under ice-cold D-PBS. Tissues were weighed (wet wt.), rinsed with D-PBS, and transferred to borosilicate culture tubes (12 x 75 mm) containing 0.5 ml of the ^{14}C-palmitate albumin complex-Grace's insect medium. First, a calibration curve for ^{14}C-palmitate uptake was constructed, based upon fat body incubations at several concentrations of labelled palmitate, and for various times. Based upon maximal linear palmitate incorporation into fat bodies, 0.335 nM (0.4 μCi) ^{14}C-palmitate/assay was utilized in subsequent experiments. A time course for incorporation into fat bodies using this dose of ^{14}C-Palmitate is given in Figure 2.8. Incorporation of palmitate into complex lipids was maximum at 20 minutes incubation (Figure 2.8). Thus, subsequent incubations comparing control and treated insects were performed at 30 °C for 20 minutes, in a Dubnoff metabolic incubator.

After incubation, the tissue was killed by addition of 0.5 ml 1.0 N HCl. Fat bodies were transferred to glass vials and rinsed 5-6 times to remove surface radioactivity. Then, fat bodies were homogenized in 1.0 ml of ice-cold chloroform:methanol (2:1, v/v) in a Potter-Elvehjem homogenizer to extract total lipids (Folch et al., 1957). A 20.0 μl aliquot of the lipid extract was added to the scintillation vials and the solvent evaporated under a stream of cool air. Then, 10.0 ml of scintillation cocktail was

Figure 2.8. Incorporation of ^{14}C-Palmitate into complex lipids by spruce budworm fat bodies *in vitro*, as a function of time. Data are expressed as Mean ± SE of 3 separate assays, DPM/fat body/insect (6.0 mg fresh wt.). Fat bodies were incubated in the culture medium containing 0.335 nM of ^{14}C-Palmitate-albumin complex. Incubation temperature was 30 °C.

Figure 2.8


DPM/fat body/insect
4000
3000
2000
1000
10
20
30
Incubation Time (min.)

added, the vials capped and shaken to solubilize the radioactive lipid. Liquid scintillation counting was done in a scintillation counter. Radioactive counts (cpm) were converted into disintegrations per minute (dpm).

2.4.3.3.1 ^{14}C-Palmitate Product Analysis

The qualitative profile of fat body lipids synthesized from ^{14}C-palmitate was determined by TLC. Fat body preparations were incubated in 0.335 nM (0.4 μCi) of ^{14}C-palmitate albumin in 0.5 ml of Grace's insect medium. After incubation (30 °C; 20 min.), fat body lipids were extracted according to the protocol outlined previously. Lipid extracts were concentrated under nitrogen, and the neutral lipids were separated by TLC (Fried and Sherma, 1986). Precoated Silica Gel G plates (20 x 20 cm, 250 μm thickness) were heat activated at 70 °C for 30 minutes and 'blank-developed' in the mobile phase (N-Hexane:Diethyl ether:Acetic acid, 70:30:1 v/v), to reduce background interference. Then, the plates were oven dried at 70 °C for 30 minutes.

Lipid extracts (50 μl) were then applied to the TLC plates, 2.5 cm from the bottom, with micropipets; and developed in a pre-saturated developing tank. Plates were air-dried, and the lipid fractions were visualized with iodine vapour. Then, the plates were heated in an oven at 50 °C for

12 hours to remove traces of iodine. The appropriate areas of the silica gel were scraped off the plate into scintillation vials and suspended in 10.0 ml of scintillation cocktail. Samples were counted in a scintillation counter, and radioactive counts (cpm) were converted into disintegrations per minute (dpm).

2.4.4 Fat Body ^{14}C-Palmitate Oxidation

The competence of the spruce budworm fat bodies to oxidize fatty acids *in vitro* via the β-oxidation pathway was measured indirectly by monitoring $^{14}CO_2$ emission from fat bodies incubated in the presence of ^{14}C-palmitate. Long chain fatty acids are degraded primarily by the sequential liberation of 2-carbon (Acetyl-CoA) fragments, by successive action of four enzymes- acyl-CoA dehydrogenase, enoyl hydratase, a second dehydrogenase, and a thiolase (Lehninger, 1975). The acetyl-CoA formed during these reactions is then oxidized to CO_2 and H_2O via the TCA cycle.

[U-^{14}C] Palmitic acid was converted into its sodium salt and complexed with BSA (Fraction 5). Spruce budworm fat bodies were dissected out under ice-cold D-PBS. The dissections were rinsed with ice-cold D-PBS, and fat bodies were removed with fine forceps. The tissues were weighed, rinsed with D-PBS, and transferred into culture tubes (15 x 60 mm) containing 0.5 ml

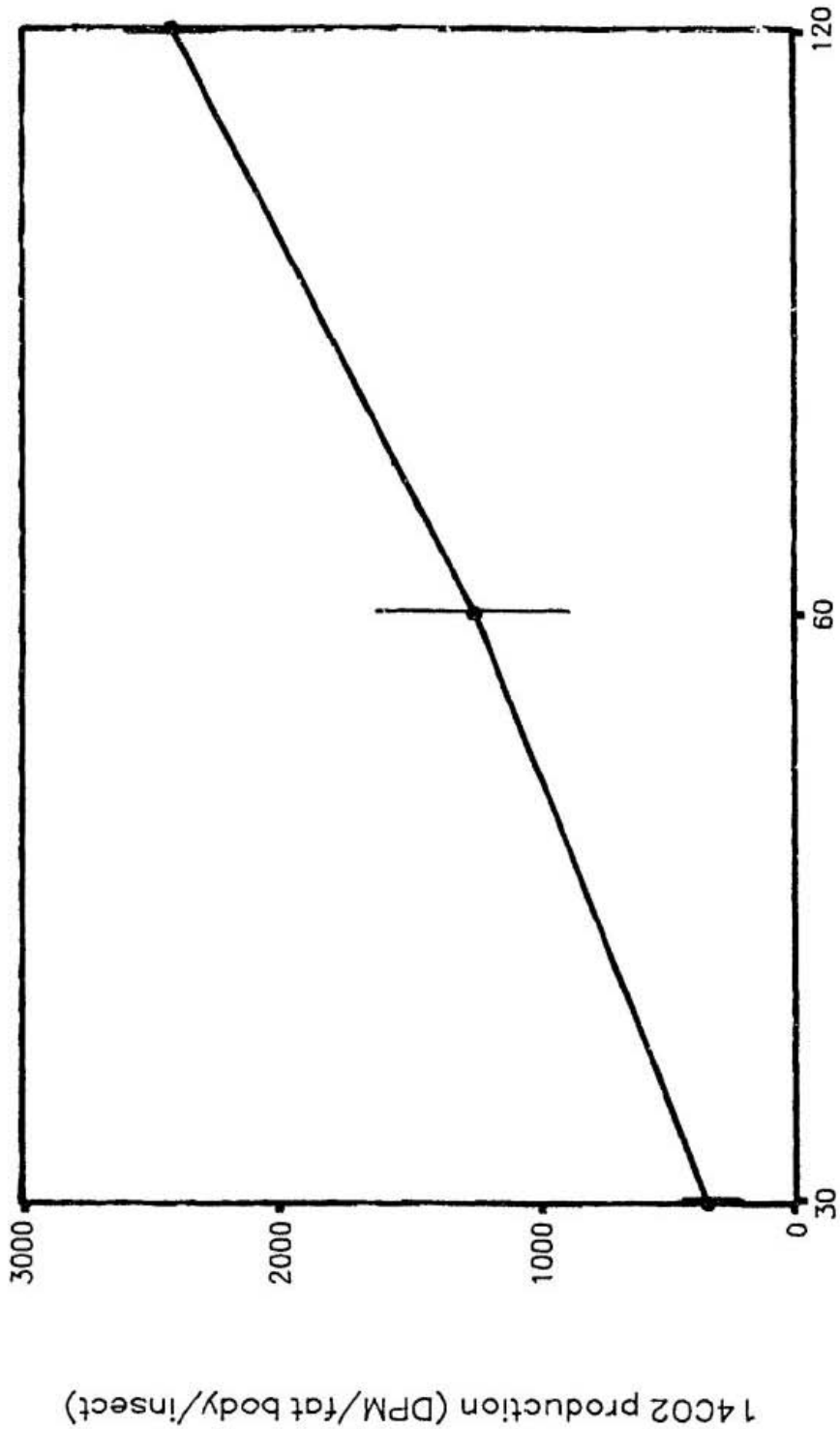
of the ^{14}C-palmitate albumin complex-Grace's insect medium, preincubated at 30 °C for 10 minutes. The tubes were closed with rubber stoppers, through which plastic centre-wells containing a small piece of fluted filter paper and 0.3 ml of 1.0 N potassium hydroxide (to absorb the $^{14}CO_2$) were inserted. The reaction tubes were then sealed with silicone grease.

Initially, a series of calibration curves for $^{14}CO_2$ emission from fat bodies incubated in ^{14}C-palmitate albumin-medium was constructed, based upon fat body incubations at several concentrations of ^{14}C-palmitate, and for different times. Based upon maximal-linear $^{14}CO_2$ emission from the fat bodies, 1.0 nM (1.65 μCi) ^{14}C-palmitate/assay was utilized in subsequent experiments. A time course for $^{14}CO_2$ emission from ^{14}C-palmitate by the fat bodies is given in Figure 2.9. All subsequent incubations in experimental samples were performed at 30 °C for 60 minutes, in a Dubnoff metabolic incubator. A 60 minute incubation time allowed sufficient $^{14}CO_2$ emission from ^{14}C-Palmitate for differences among samples to be discerned.

After incubation, the tissue was killed by the injection of 0.4 ml of 4.0 N HCl through the stopper, with a syringe. The reaction tubes were allowed to stand at 30 °C for 30 minutes to allow for the absorption of $^{14}CO_2$ that had evolved prior to the addition of the acid. Then, the stoppers were removed and the contents of the centre-wells were transferred

Figure 2.9. $^{14}CO_2$ emission from ^{14}C-Palmitate by spruce budworm fat bodies *in vitro*, as a function of time. Values are expressed as Mean ± SE of 3 separate assays, DPM/fat body/insect (5.0 mg fresh wt.). Fat bodies were incubated in the culture medium containing 1.0 nM of ^{14}C-Palmitate-albumin complex, at 30 °C.

Figure 2.9


14CO2 production (DPM/fat body/insect)
3000
2000
1000
0
30
60
120
Incubation Time (min.)

to scintillation vials and 0.5 ml of 1.0 N HCl was added to neutralize excess alkalinity in the samples. Following this, 10.0 ml of scintillation cocktail was added, the vials capped, and shaken vigorously to suspend the radioactive material. Liquid scintillation counting was done in a scintillation counter, and radioactive counts (cpm) were converted into disintegrations per minute (dpm).

2.5 Statistical Analysis of Results

Data collected from the dose-response studies were subjected to a Probit analysis, to calculate LD_{50} values (Finney, 1971), after allowing for the mortality in the controls (solvent-treated) using Abbott's formula (Abbott, 1925); the significance between treatment groups was determined using a one-way analysis of variance, followed by Duncan's multiple range test (Duncan, 1955). All other data were subjected to an Analysis of Variance, and the statistical significance of differences were estimated by Duncan's multiple range test (Duncan, 1955). A VAX 11/730 processor (M.U.N.) running the VMS operating system, and the SPSS/X software (Nie et al., 1975), were used to analyze the data.

RESULTS

3.1 Dose-response Studies

Larval mortality, expressed as per cent suppression of pupation, increased significantly in a dose-dependent fashion when early sixth instar larvae of C. fumiferana were treated with each of the juvenile hormone analogs. The most effective compound was fenoxycarb which, at the highest concentration tested (0.5 μg per insect), caused more than 70 % larval mortality and completely prevented adult eclosion (Figure 3.1). The JHAs S-71639, ZR8487, and ZR9892 were moderately effective in disrupting metamorphosis. At the highest dose tested, 100.0 μg per insect, these compounds caused 80-95 % mortality and prevented adult eclosion either entirely (S-71639 and ZR9892), or in more than 80 % of treated insects (ZR8487) that had pupated (Table 3.1).

Methoprene and ZR9582 were relatively ineffective insect growth regulators. At a concentration of 100.0 μg per insect, methoprene caused about 80 % larval mortality, but allowed less than 10 % of pupae to eclose (Figure 3.2). ZR9582, at this same concentration, caused approximately 50 % larval mortality and prevented eclosion to the adult stage in only 50 % of the insects that completed pupation (Table 3.1).

Figure 3.1. Dose-response curves for 1-day-old sixth instar larvae of Choristoneura fumiferana treated with fenoxycarb. Left ordinate (▲) represents percentage larval mortality, right ordinate (■) represents percentage adult eclosion from treated larvae that had pupated. Each point represents the mean of five replicates (20 insects per replicate). Standard errors were within ± 3 % of the means.

Figure 3.1

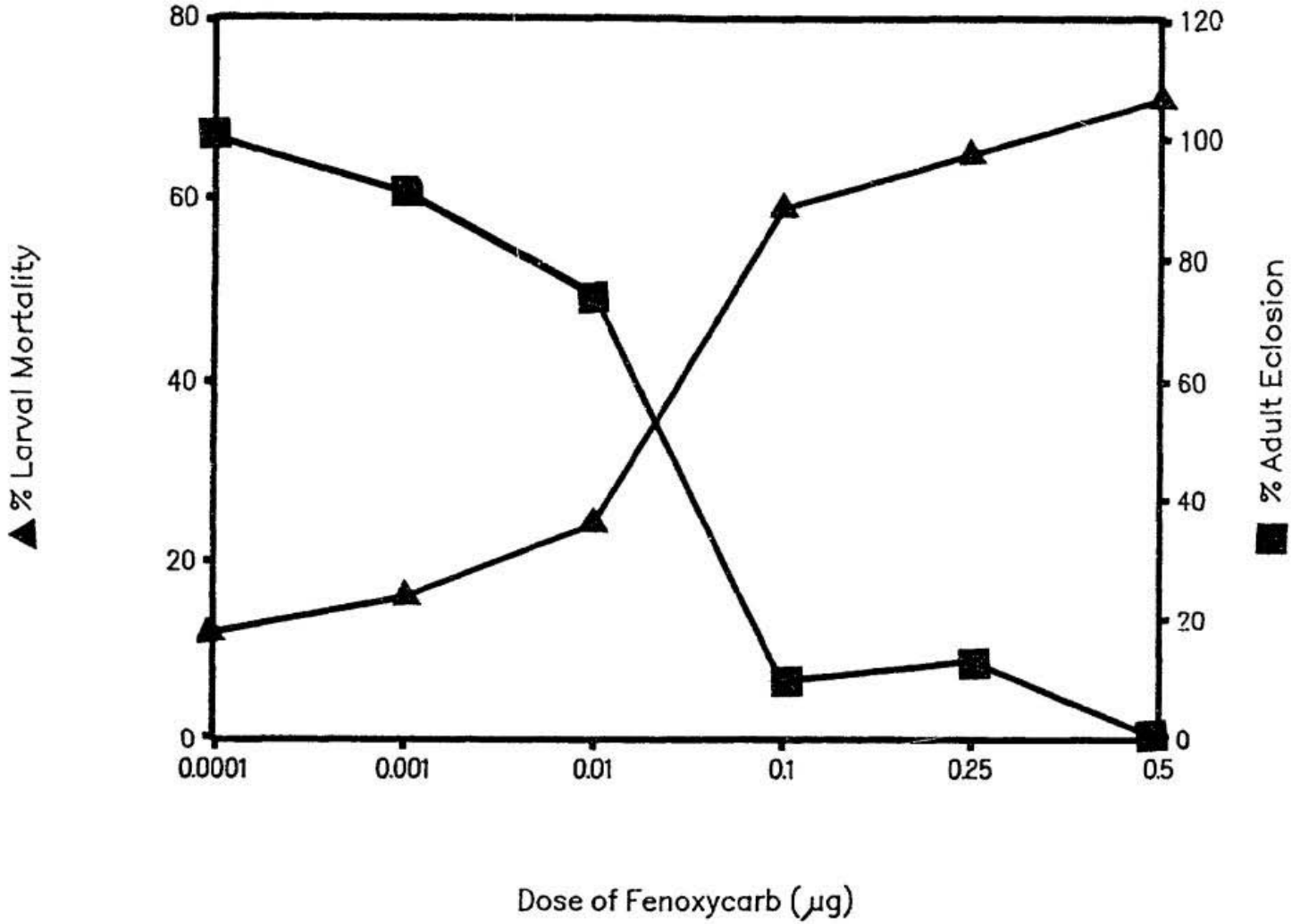

80
60
40
20
0
120
100
80
60
40
20
0
0.0001
0.001
0.01
0.1
0.25
0.5
▲ % Larval Mortality
■ % Adult Eclosion
Dose of Fenoxycarb (μg)

Figure 3.2. Dose-response curves for 1-day-old sixth instar larvae of Choristoneura fumiferana treated with methoprene. Left ordinate (▲) shows percentage larval mortality, right ordinate (■) represents adult eclosion from treated larvae that had pupated. Each data point represents the mean of five replicates (20 insects per replicate). Standard errors were within ± 2 % of the means.

Figure 3.2

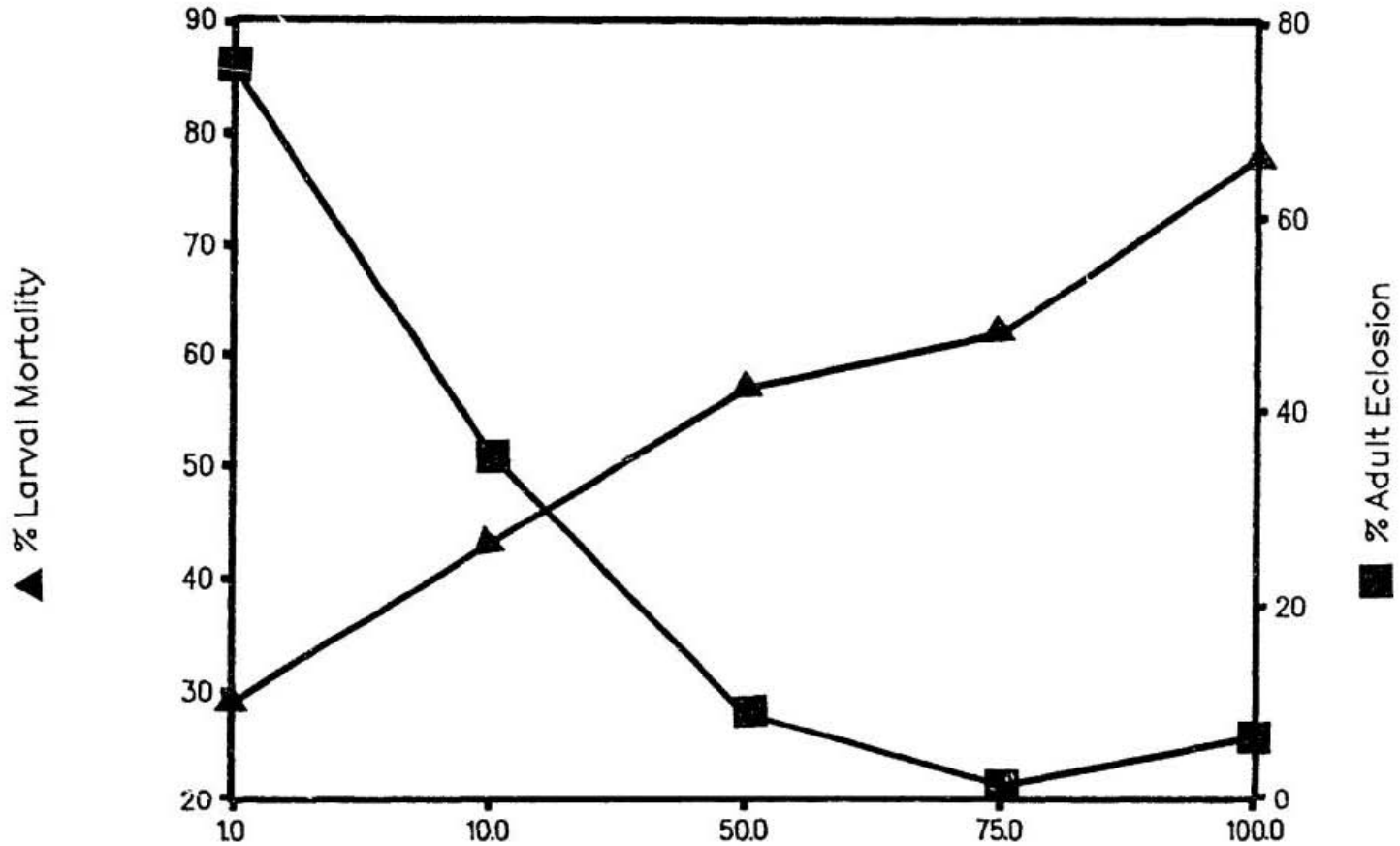

90
80
70
60
50
40
30
20
80
60
40
20
0
1.0
10.0
50.0
75.0
100.0
▲ % Larval Mortality
■ % Adult Eclosion
Dose of Methoprene (µg)

Table 3.1. Effect of selected juvenile hormone analogs (JHAs) on larval mortality and adult eclosion of Choristoneura fumiferana*.

Treatment (JHA)	Concentration of JHA (µg/insect)	n	% Larval Mortality	% Adult† Eclosion
ZR9892	0.01	100	28 ± 1.2[a]	90.7 ± 0.62[a]
	1.0	100	55 ± 0.31[b]	13.1 ± 1.63[b]
	100.0	100	94 ± 0.37[c]	0.0[c]
ZR8487	0.01	100	19 ± 1.15[a]	71.4 ± 0.42[a]
	1.0	100	73 ± 1.4[b]	3.3 ± 0.66[b]
	100.0	100	81 ± 0.97[c]	17.3 ± 2.58[c]
S-71639	0.01	100	15 ± 0.31[a]	93.9 ± 0.75[a]
	1.0	100	43 ± 1.83[b]	35.6 ± 1.16[b]
	100.0	100	92 ± 0.5[c]	0.0[c]
ZR9582	0.01	100	14 ± 0.49[a]	90.6 ± 0.47[a]
	1.0	100	19 ± 0.37[b]	93.7 ± 0.79[b]
	100.0	100	53 ± 0.87[c]	53.9 ± 1.04[c]
ZR10151	1.0	100	3 ± 0.4[a]	91.6 ± 0.42[a]
	5.0	100	8 ± 0.4[b]	91.0 ± 0.93[a]
	10.0	100	3 ± 0.4[a]	92.7 ± 0.4[a]
	25.0	100	7 ± 0.4[c]	97.8 ± 0.25[b]
	50.0	100	9 ± 0.37[d]	92.4 ± 0.43[a]
	100.0	100	30 ± 0.7[e]	91.6 ± 1.13[a]
Controls-Acetone	-	100	9 ± 0.58[a]	98.8 ± 0.22[a]
Controls-Untreated	-	100	7 ± 0.5[b]	97.9 ± 0.25[b]

* Values shown are Means ± SE. Values followed by the same letter for each of the JHA indicate no significant difference at the 0.05 level (Duncan's Multiple Range Test).

† Adult eclosion from treated 1 day-old sixth instar spruce budworm larvae that underwent pupation.

The median lethal dose (LD_{50}) values, obtained from Probit analysis of the mortality data (Finney, 1971), quantitatively reflects the order of effectiveness of the various JHAs (Table 3.2). Based on LD_{50} values, fenoxycarb, S-71639, ZR8487, and ZR9892 were, respectively, about 170-, 20-, 91-, and 123-times more effective than methoprene in causing mortality of C. fumiferana larvae. ZR10151, the least effective of all of the compounds screened, had an LD_{50} value of 705.3 μg per insect, almost 3000-fold that of fenoxycarb.

All of the JHAs, except ZR10151, caused a pronounced delay in molting. Although the controls (solvent-treated and untreated) molted in 6 days, the JHA-treated larvae molted 20-30 days post treatment.

3.1.1 Morphogenetic Effects

The application of various JHAs to early sixth instar larvae of C. fumiferana caused a wide range of morphogenetic deformities. The morphogenetic effects of fenoxycarb included the formation of larval-pupal intermediates, with precocious evagination of the wing imaginal-disks, and production of deformed pupae with hemolymph-filled blisters in the thoracic region (Figure 3.3 A-C).

Methoprene treatments caused supernumerary molting, with the eventual formation of larval-pupal intermediates, which

Table 3.2. Probit analysis of the lethal effects of juvenile hormone analogs (JHAs) to sixth instar larvae of Choristoneura fumiferana.

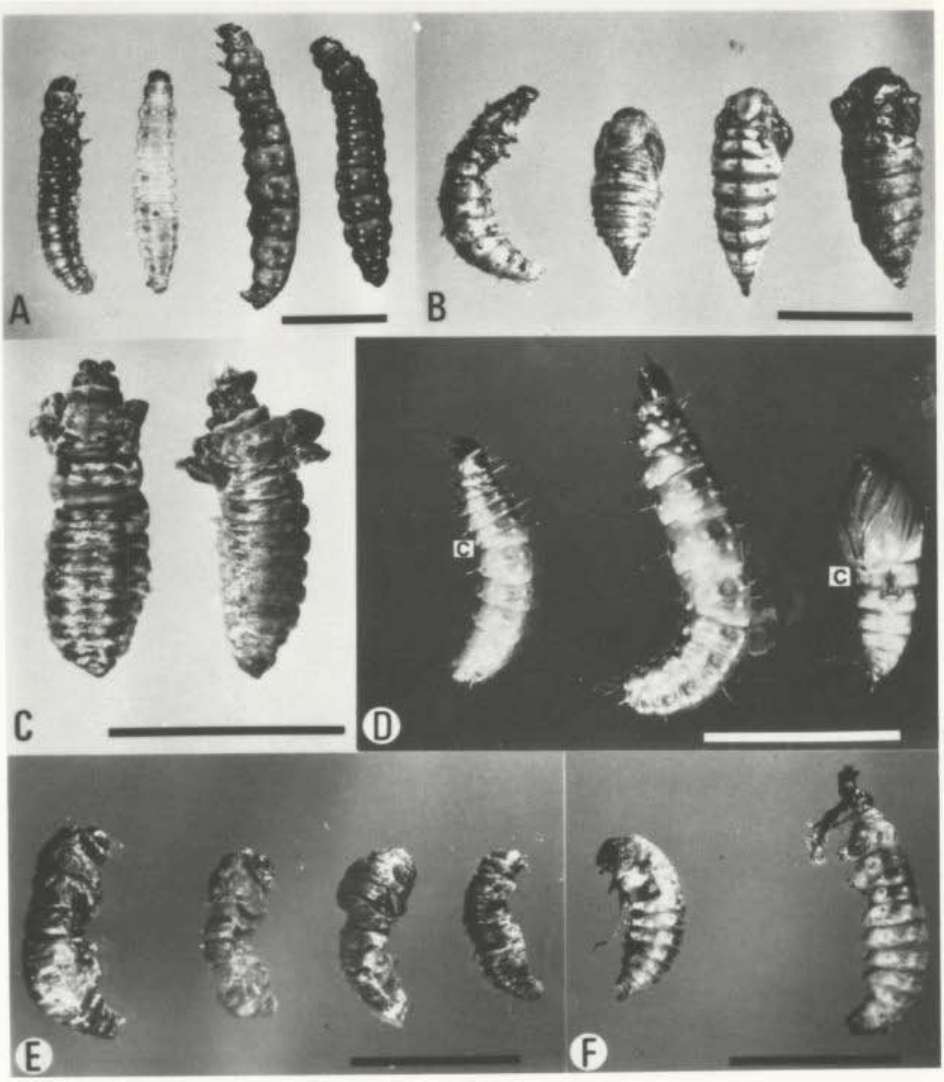
JHA	N*	Slope ± SE	LD_{50}†	95% C.L.
Fenoxycarb	800	0.477 ± 0.017	0.261	0.117-0.663
ZR9892	400	0.54 ± 0.025	0.36	0.140-0.863
ZR8487	400	0.475 ± 0.023	0.486	0.078-2.466
S-71639	400	0.766 ± 0.031	2.266	0.948-5.536
Methoprene	700	0.794 ± 0.037	44.275	25.812-98.017
ZR9582	400	0.366 ± 0.025	177.6	42.53-2004.76
ZR10151	600	1.15 ± 0.105	705.3	199.65-773724

* Total number of insects treated with test compound or acetone.

† In units of μg per insect.

Figure 3.3. Effects of juvenile hormone analogs (JHAs) on the sixth instar larvae of Choristoneura fumiferana. A-C, fenoxycarb; D-E, methoprene; F, S-71639 and ZR compounds. A, larvae that failed to pupate. Cuticle abnormally pigmented, (more heavily melanized) and/or leathery than in controls. B, larval-pupal intermediate (left) alongside pupae with hemolymph-filled blister formations. C, larval-pupal intermediates with precocious evagination of wing disks. D, control larva and pupa (c) on either side of a larva that had undergone supernumerary molting. E, mummified larval-pupal intermediates. F, larval-pupal intermediates induced by S-71639 (left) and ZR compounds (right). Scale line = 1.0 cm.

Figure 3.3



typically become mummified (Figure 3.3 D-E). The morphogenetic effects of S-71639 and the other ZR compounds, except ZR10151, were manifested as larval-pupal intermediates, followed by mortality of the deformed insects (Figure 3.3 F). The severity of the morphogenetic deformation was also concentration-dependent. The lower concentrations of each of the JHAs caused minor deformities, and thus lower mortalities.

3.2 Effect of JHAs on Metabolite Concentrations

3.2.1 Hemolymph Volume

The mean hemolymph volume of JHA treated and control sixth instar spruce budworms is given in Table 3.3. Statistical analysis of the data showed that volume of hemolymph in C. fumiferana larvae was significantly influenced by time ($F=26.71$, d.f.=5; $P < 0.05$). In the control insects, there was a gradual increase in the hemolymph volume, as the instar progressed. The hemolymph volume of spruce budworms treated with fenoxycarb and methoprene was similar to the respective controls.

Table 3.3. Effects of JHA treatment on the hemolymph volume of sixth instar larvae of Choristoneura fumiferana (μl)*.

Hours after treatment :	24	48	72	96	120	144
Control	12.43 ± 1.51[a]	18.10 ± 2.11[a]	25.94 ± 1.46[a]	29.92 ± 2.13[a]	26.40 ± 3.10[a]	22.63 ± 1.41[a]
Fenoxycarb	9.62 ± 1.18[a]	20.24 ± 3.92[a]	19.60 ± 3.66[a]	27.24 ± 3.25[a]	31.32 ± 1.78[a]	32.30 ± 2.21[a]
Methoprene	11.22 ± 0.90[a]	18.41 ± 1.83[a]	21.84 ± 1.73[a]	18.45 ± 1.96[a]	28.61 ± 2.74[a]	28.32 ± 2.40[a]

* Data are expressed as Mean ± SE of 5-15 separate measurements. Values followed by the same letter in the vertical columns indicate no significant difference at the 0.05 level (Duncan's Multiple Range Test).

3.2.1.1 Carbohydrates

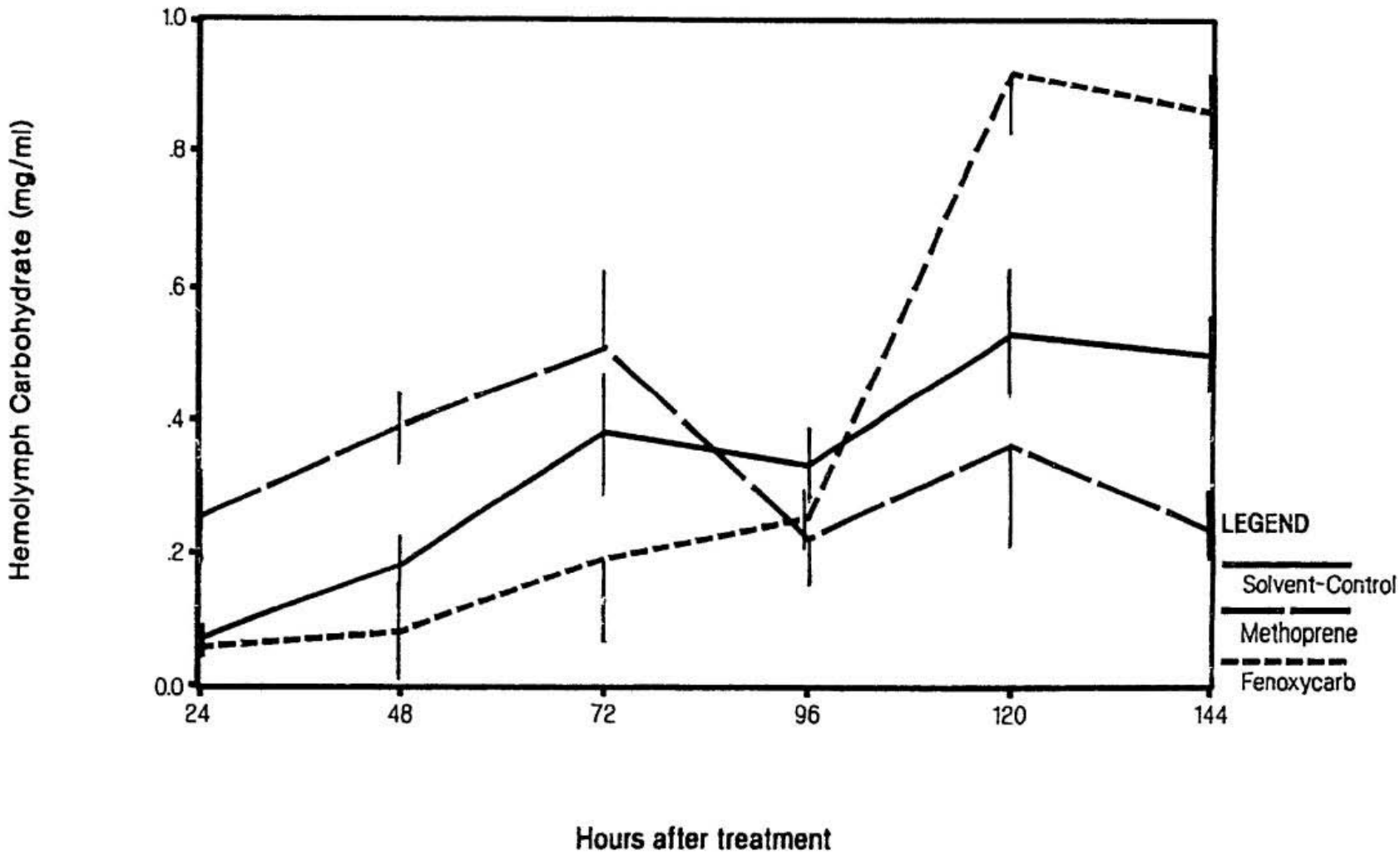
The mean total hemolymph carbohydrate concentration of JHA-treated and control larvae of C. fumiferana is shown in Figure 3.4. Statistical analysis of the data showed that carbohydrate concentration of the hemolymph was significantly influenced by the JHA treatments ($F=3.44$, d.f.=2; $P < 0.05$) and the time after treatment ($F=49.75$, d.f.=5; $P < 0.05$). Interaction between treatment and time after treatment was also statistically significant ($F=20.78$, d.f.=10; $P < 0.05$), indicating that JHA treatment altered hemolymph carbohydrate levels at certain times during the experimental period. In the control group, the hemolymph levels of carbohydrate rose gradually throughout the progression of the sixth larval instar.

In the fenoxycarb treated larvae, carbohydrate concentration of the hemolymph appeared to increase at a level lower than the controls, until 96 h after treatment. However, this increase was not statistically significant. After 96 h, levels of carbohydrate in the hemolymph increased rapidly, and remained significantly higher than the controls until 144 h post treatment (Figure 3.4).

In the methoprene treated larvae, there was an initial elevation in the hemolymph carbohydrate concentration (until

Figure 3.4. The mean hemolymph total carbohydrate concentration of JHA treated and control sixth instar larvae of Choristoneura fumiferana at various times after treatment. Values are shown as Mean ± SE of 10-22 independent determinations, mg/ml glucose equivalents.

Figure 3.4


Hemolymph Carbohydrate (mg/ml)
1.0
.8
.6
.4
.2
0.0
24
48
72
96
120
144
Hours after treatment
LEGEND
Solvent-Control
Methoprene
Fenoxycarb

72 h), and a decline thereafter until 144 h after treatment, a situation opposite to the fenoxycarb treated larvae. At 24 h and 48 h after treatment, the hemolymph carbohydrate concentration of methoprene-treated insects was significantly higher, while at 144 h after treatment it was significantly lower than in controls.

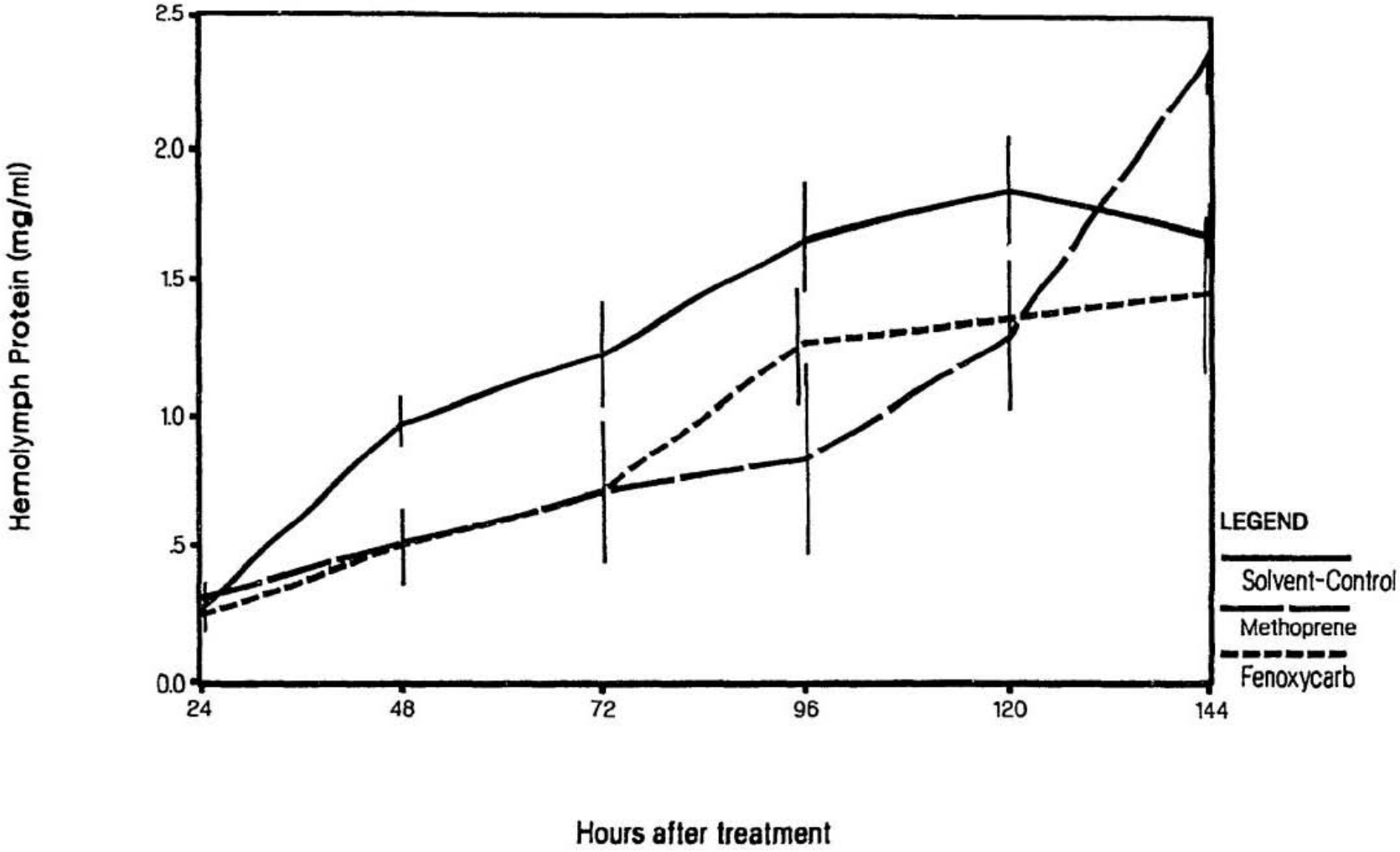
3.2.1.2 Proteins

The concentration of protein in the hemolymph of the JHA treated and control sixth instar larvae of C. fumiferana, is presented in Figure 3.5. Statistical analysis of the data revealed that total protein concentration in the hemolymph was significantly influenced by the JHA treatments ($F=17.85$, $d.f.=2$; $P < 0.05$), the time after JHA treatment ($F=80.5$, $d.f.=5$; $P < 0.05$), and the interaction between treatment and time ($F=6.9$, $d.f.=10$; $P < 0.05$), indicating that the effect of JHA treatment on hemolymph protein levels was evident at certain times during the experimental period. The concentration of hemolymph protein in the control group increased throughout the sixth instar, followed by a slight decline just prior to pupation (Figure 3.5).

In the fenoxycarb treated sixth instar spruce budworms, the total protein concentration in the hemolymph increased, at a level lower than the controls, in a similar manner until

Figure 3.5. The mean hemolymph total protein concentration of JHA treated and control sixth instar larvae of Choristoneura fumiferana at various times post treatment. Data are expressed as Mean ± SE of 10-20 separate measurements, mg/ml BSA equivalents.

Figure 3.5


Hemolymph Protein (mg/ml)
2.5
2.0
1.5
1.0
.5
0.0
24
48
72
96
120
144
Hours after treatment
LEGEND
Solvent-Control
Methoprene
Fenoxycarb

144 h post treatment. At 48 h, 72 h, and 120 h after treatment, the hemolymph protein concentration of fenoxycarb-treated insects was significantly lower than in controls.

Insects treated with methoprene also displayed an increase in their hemolymph protein titre similar to the control group; but had lower levels of blood protein, except at 144 h post treatment.

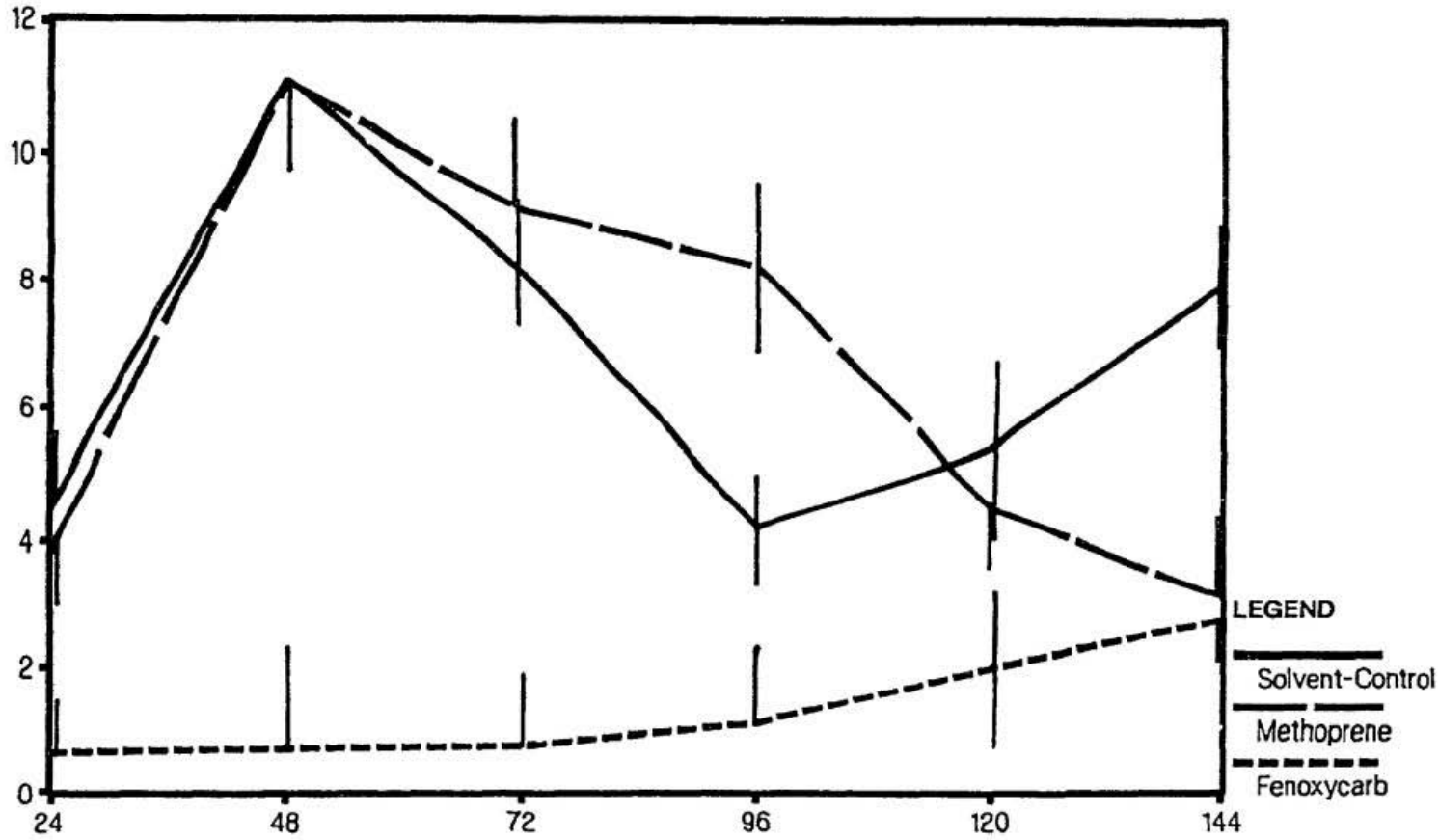
3.2.1.3 Lipids

The mean concentration of lipid in the JHA treated and control sixth instar spruce budworms are shown in Figure 3.6. Statistical treatment of the data showed that hemolymph lipid concentration was significantly affected by JHA treatments ($F=15.91$, d.f.=2; $P < 0.05$) and time after treatment ($F=3.94$, d.f.=5; $P < 0.05$). There was no statistically significant interaction between treatment and time post treatment. In the control group, hemolymph levels of lipid increased initially (48 h after treatment), but declined to almost the early (24 h after treatment) levels by 96 h post treatment. Then, the concentration of lipid in the hemolymph increased somewhat, and remained elevated until pupation (144 h).

By comparison, in the fenoxycarb treated insects, the concentration of lipids in the hemolymph remained significantly depressed throughout the instar, then increased

Figure 3.6. The mean hemolymph total lipid levels of JHA treated and control sixth instar spruce budworms at different times after treatment. Values are expressed as Mean ± SE of 5-10 independent analyses, mg/ml Palmitic acid equivalents.

Figure 3.6



Hemolymph Lipid (mg/ml)

Hours after treatment

slightly at 144 h post treatment (Figure 3.6). In sixth instar larvae of C. fumiferana treated with methoprene, lipid levels in the blood fluctuated concomitant with the control group, except in the 96 h post treatment larvae. After 96 h, levels of blood lipid continued to decrease until 144 h after treatment (Figure 3.6). However, with the exception of 96 and 144 h post treatment insects, there was no significant difference in the blood lipid titres between control and methoprene treated budworms.

3.2.2 Fat Body

Initial studies showed that fat body dry weight was unaffected by the JHA treatment.

3.2.2.1 Glycogen

The fat body glycogen levels of JHA treated and control larvae of C. fumiferana is shown in Table 3.4. Statistical analysis of the data showed that the glycogen content of the fat bodies was significantly influenced by JHA treatment ($F=523.88$, d.f.=2; $P < 0.05$) and time after treatment ($F=1329.81$, d.f.=2; $P < 0.05$). The interaction between treatment and time after treatment was also significant ($F=355.13$, d.f.=4; $P < 0.05$), indicating that JHA treatment

Table 3.4. Effect of JHA treatment on the Fat Body Glycogen content of the spruce budworm, Choristoneura fumiferana (g/100 g dry wt.)*.

Hours after treatment:	24	72	120
Control	1.181 ± 0.01^{a}	4.232 ± 0.07^{a}	1.354 ± 0.03^{a}
Fenoxycarb	0.156 ± 0.02^{b}	2.045 ± 0.03^{b}	1.607 ± 0.04^{b}
Methoprene	0.712 ± 0.005^{c}	1.407 ± 0.01^{c}	1.470 ± 0.02^{c}

* Data are shown as Mean ± SE of 3 separate analyses (10-15 insects each). Values followed by the same letter in the vertical columns indicate no significant difference at the 0.05 level (Duncan's Multiple Range Test).

affected fat body glycogen content at certain times during the experimental period. In the control group, the fat body glycogen levels increased, from the 24 h post treatment levels, by about 4-fold (72 h), then declined to near initial levels at 120 h.

In the fenoxycarb treated insects, glycogen content in the fat bodies was considerably lower, 24 and 72 h post treatment, than the controls, but was the same concentration as controls by 120 h after treatment. This resulted from the fact that in contrast to controls, the fat body glycogen content of treated insects did not decline between 72 h and 120 h after treatment. In the insects treated with methoprene, the fat body glycogen concentration was lower than in controls or fenoxycarb-treated insects throughout the 120 h post treatment period. As was the case in fenoxycarb-treated insects, the glycogen concentration of fat bodies of methoprene-treated insects increased up to 72 h post treatment, then stabilized.

3.2.2.2 Proteins

The fat body protein concentration of JHA-treated and control sixth instar larvae is given in Table 3.5. Statistical analysis of the data showed that protein concentration of the fat bodies was significantly influenced by the time after

Table 3.5. Effect of JHA treatment on the Fat Body Protein content of the spruce budworm, Choristoneura fumiferana, (g/100 g dry wt.)*.

Hours after treatment:	24	72	120
Control	8.06 ± 1.16[a]	17.15 ± 3.10[a]	16.71 ± 0.57[a]
Fenoxycarb	5.03 ± 0.24[b]	21.12 ± 1.00[b]	17.20 ± 0.80[a]
Methoprene	7.11 ± 0.62[a]	17.55 ± 2.08[a]	17.51 ± 0.53[a]

* Values are shown as Mean ± SE of 4-5 separate determinations (10-15 insects each). Values followed by the same letter in the vertical columns indicate no significant difference at the 0.05 level (Duncan's Multiple Range Test).

JHA treatment ($F=58.5$, d.f.=2; $P < 0.05$). There was no significant difference in the fat body protein content for the treatment groups and the interaction between treatment and time after treatment, indicating that JHA treatment, and the time after JHA treatment, did not affect fat body protein concentration significantly. In the control insects, the fat body protein content increased from about 8.0 g/100 g dry wt. at 24 h to approximately 17.00 g/100 g dry wt. at 72 h, then remained unchanged at 120 h.

In the larvae treated with fenoxycarb, protein levels in the fat body were lower at 24 h post treatment, as compared to their control counterparts. Protein levels in the fat bodies of fenoxycarb-treated insects increased almost 4-fold between 24 h and 72 h after treatment, then declined slightly at 120 h after treatment. Accordingly, the fat body protein concentration of fenoxycarb-treated insects was statistically higher than controls at 72 h, but the same as in controls at 120 h post treatment. Insects treated with methoprene showed a trend, with respect to fat body protein levels, similar to their control counterparts; there was no significant difference between the two groups.

3.2.2.3 Lipid

The lipid content of fat bodies from JHA treated and

control larvae of C. fumiferana is given in Table 3.6. Statistical analysis of the data showed that lipid levels in the fat bodies were significantly affected by time after JHA treatment ($F=17.7$, d.f.=2; $P < 0.05$) and the treatment-time post treatment interaction ($F=58.54$, d.f.=4; $P < 0.05$), indicating that JHA treatment affected fat body lipid levels at certain times during the post treatment experimental period. Lipid concentration in the fat bodies of control larvae more than doubled between 24 h and 72 h of the experimental period, then stabilized.

Lipid levels in the fat bodies of insects treated with fenoxycarb were initially (24 h post treatment) triple that of controls. In contrast to controls, however, the lipid content of the fat bodies of fenoxycarb treated insects declined about 40 % between 24 h and 72 h after treatment, then remained unchanged thereafter. Accordingly, the lipid concentration in the fat bodies of such treated insects was significantly less than in controls at both 72 h and 120 h after treatment.

Similar to fenoxycarb-treated insects, the lipid content of the fat bodies of methoprene-treated ones was initially (24 h post treatment) double, then subsequently (72 h post treatment) less than that of controls. The fat body lipid concentration declined by approximately 50 % between 24 h and 72 h after treatment to less than half the control levels. The lipid concentration in the fat bodies of methoprene-treated

Table 3.6. Effect of JHA treatment on the Fat Body Lipid content of the spruce budworm, Choristoneura fumiferana, (g/100 g dry wt.)*.

Hours after treatment:	24	72	120
Control	15.22 ± 1.75[a]	37.00 ± 0.10[a]	43.87 ± 2.73[a]
Fenoxycarb	44.71 ± 1.50[b]	27.94 ± 1.65[b]	26.80 ± 2.25[b]
Methoprene	33.58 ± 1.55[c]	17.30 ± 2.35[c]	37.88 ± 0.96[c]

* Data are shown as Mean ± SE of 4 separate determinations (15-20 insects each). Values followed by the same letter in the vertical columns indicate no significant difference at the 0.05 level (Duncan's Multiple Range Test).

insects subsequently doubled, however, with the result that it was only marginally less than control levels at 120 h post treatment.

3.3 Effect of Fenoxycarb on Lipid Metabolism

3.3.1 Qualitative Lipid Profile

The qualitative distribution of neutral lipids from the fat bodies of untreated (controls at 120 h) C. fumiferana is shown in Figure 2.5. The qualitative lipid profile of the treated insects was similar to the controls. The result demonstrated that the neutral lipid most abundant in the hemolymph was diacylglycerol (DG). Also present, were trace amounts of sterol ester (StE) and free fatty acids (FFA). In the fat body lipid extracts, triacylglycerol (TG) formed the major portion of the neutral lipids. When detected, phospholipids (PL), monoacylglycerol (MG), diacylglycerol (DG), and free fatty acids (FFA) were present in trace amounts.

3.3.2 Fatty Acid Profile

The qualitative distribution of total fatty acids from the budworm hemolymph and fat body lipid extracts is shown

Table 3.7 A. Qualitative distribution of fatty acids from the hemolymph neutral lipids of the spruce budworm, Choristoneura fumiferana. (Mol %)*.

	Fenoxycarb			Controls		
Hours after treatment:	24	72	120	24	72	120
Fatty acid						
C14:0	1.30	bld	bld	bld	bld	bld
C16:0	23.64	23.63	25.00	20.34	48.14	27.50
C16:1	1.40	bld	bld	bld	bld	bld
C18:0	11.24	12.22	17.85	12.50	10.62	7.86
C18:1	23.90	23.48	21.76	27.75	11.62	21.17
C18:2	17.43	18.48	16.15	19.42	12.17	22.00
C20:0	21.08	22.17	19.27	20.00	17.43	21.45

bld: below limits of detection.

* Lipid was extracted according to Folch procedure (Folch et al., 1957) and transmethylated according to the method of Keough and Kariel (1977). Fatty acid methyl esters (FAMEs) were analyzed in a Perkin-Elmer 8310 gas chromatograph.

Table 3.7 B. Qualitative distribution of fatty acids from the fat body neutral lipids of the spruce budworm, Choristoneura fumiferana. (Mol %)*.

	Fenoxycarb			Controls		
Hours after treatment:	24	72	120	24	72	120
Fatty acid						
C14:0	1.66	1.00	0.63	bld	1.11	0.64
C16:0	30.93	31.10	27.52	31.13	32.44	27.74
C16:1	1.38	1.11	1.37	bld	1.25	0.88
C18:0	5.54	2.80	3.30	6.71	3.33	3.05
C18:1	31.34	33.52	34.95	34.71	34.44	34.67
C18:2	12.48	13.12	13.76	12.32	12.00	14.42
C20:0	16.64	17.31	18.44	15.11	12.00	18.58

bld: below limits of detection.

* Lipid was extracted according to Folch procedure (Folch et al., 1957) and transmethylated according to the method of Keough and Kariel (1977). Fatty acid methyl esters (FAMEs) were analyzed in a Perkin-Elmer 8310 gas chromatograph.

in Table 3.7 A & B. The qualitative fatty acid profile of the hemolymph and fat body neutral lipids was similar in the treated and control insects. A wide array of saturated and unsaturated fatty acids with carbon chain length ranging from C14 to C20 were detected in the hemolymph and fat body lipids.

3.3.3 Fat Body Lipid Synthesis

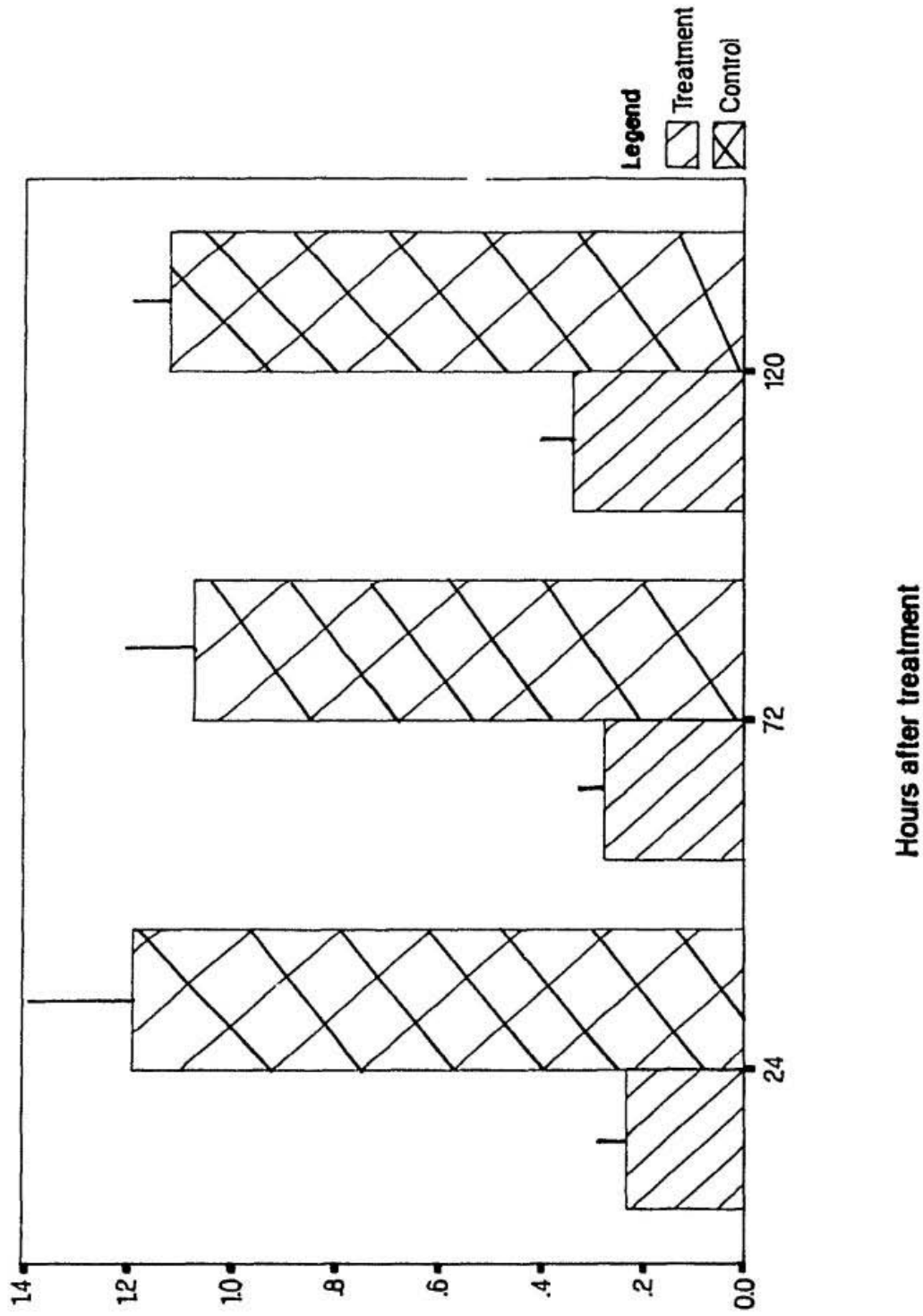
3.3.3.1 Overall Lipid Synthesis from ^{14}C-Acetate

The <u>in vitro</u> incorporation of ^{14}C-acetate into lipids by fat bodies of fenoxycarb treated and control larvae is given in Figure 3.7. Statistical analysis of the data showed that fat body lipid synthesis from ^{14}C-acetate was significantly affected by fenoxycarb treatment ($F=84.58$, d.f.=1; $P < 0.05$). In the control group, incorporation of acetate by the fat body into lipids remained unchanged during the 120 h experimental period.

In fenoxycarb treated larvae incorporation of ^{14}C-acetate by fat bodies increased gradually up to 120 h after treatment. However, the values were lower than their control counterparts; the incorporation of ^{14}C-acetate into fat body lipids was 3- to 5-fold lower than the respective control larvae.

Figure 3.7. The mean *in vitro* incorporation of ^{14}C-Acetate into lipids in fat bodies of fenoxycarb treated and control sixth instar larvae of *Choristoneura fumiferana* at 24 h, 72 h and 120 h post treatment. Data are shown as Mean ± SE of 10 independent measurements, nM/mg/h.

Figure 3.7


Legend
Treatment
Control
0.0
.2
.4
.6
.8
1.0
1.2
1.4
nM / mg / hour
24
72
120
Hours after treatment

3.3.3.1.1 Direct Effect of Fenoxycarb on Lipid Synthesis

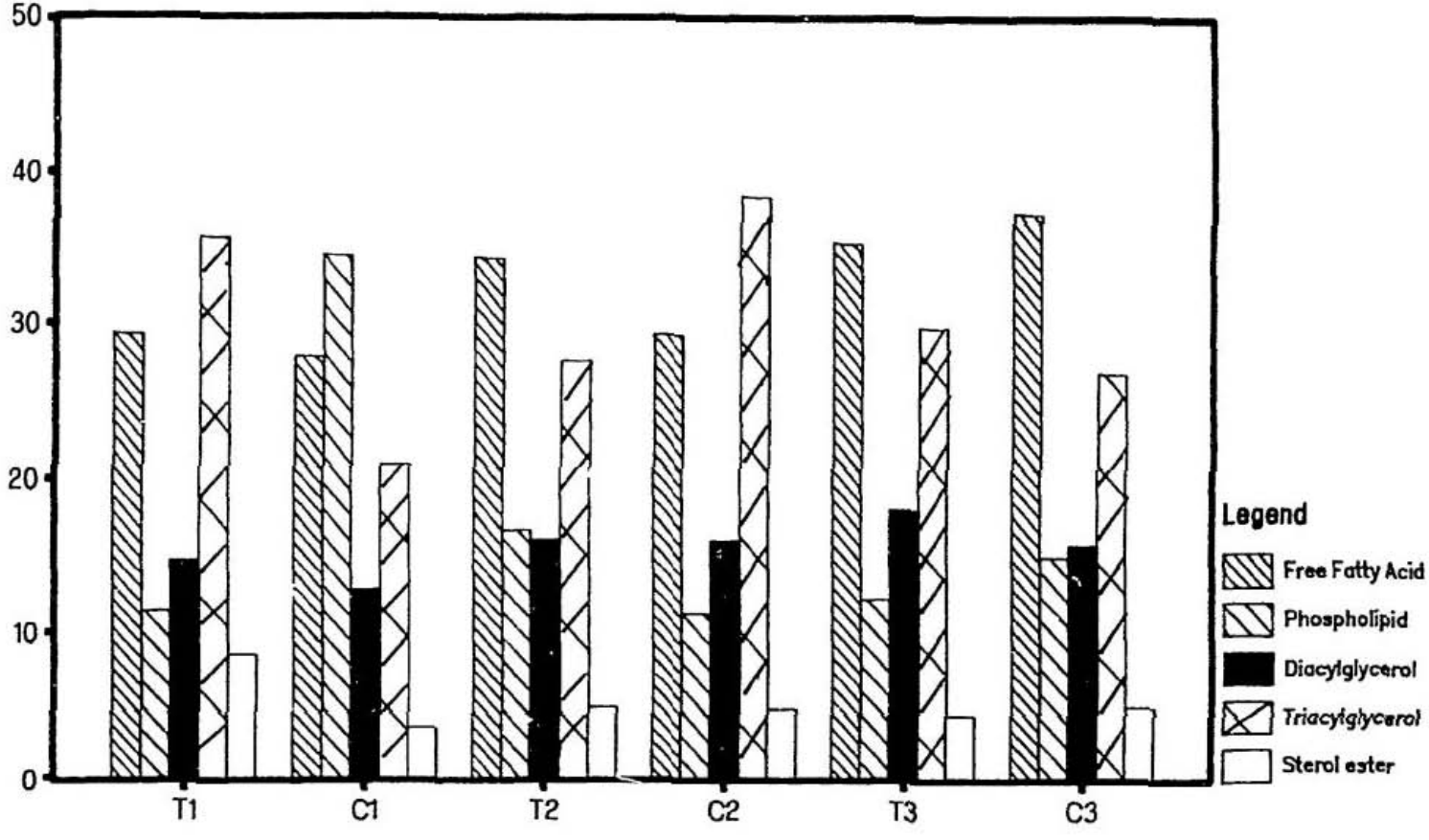
The addition of fenoxycarb to the ^{14}C-acetate culture medium caused a significant ($F=31.53$, d.f.=1; $P < 0.05$) inhibition in the incorporation of ^{14}C-acetate into fat body lipids of C. fumiferana. In the control fat bodies incubated with ^{14}C-acetate, the Mean ± SE ^{14}C-acetate incorporation into lipids was 1.187 ± 0.13 nM/mg fat body/h (n=10). In contrast, fat bodies incubated in the ^{14}C-acetate culture medium containing 0.26 μg fenoxycarb incorporated 0.365 ± 0.023 nM/mg/h (n=10) ^{14}C-acetate into lipids.

3.3.3.1.2 ^{14}C-Acetate Product Analysis

The qualitative distribution of fat body lipids synthesized from ^{14}C-acetate is shown in Figure 3.8. Statistical analysis of the data showed that treatment, time post treatment, as well as the treatment-time post treatment interaction, were the important factors which significantly influenced the fat body lipid profile of C. fumiferana, indicating that effect of JHA treatment was apparent at certain times after treatment. The analysis of variance of the main effects and the interaction between the main effects, are given in Appendix B.

Figure 3.8. The qualitative distribution of fat body lipids synthesized from ^{14}C-Acetate *in vitro*, in fenoxycarb treated and control larvae of *Choristoneura fumiferana* at various times after treatment. Values are expressed as % Mean ± SE of 6-9 determinations. T1, treatment 24 h; C1, control 24 h; T2, treatment 72 h; C2, control 72 h; T3, treatment 120 h; C3, control 120 h.

Figure 3.8



% Radioactivity from 14C—Acetate

Treatment/Control (Time post treatment)

In the 24 h old control larvae, the distribution of radiolabel from ^{14}C-acetate in the fat body lipids was found primarily in the phospholipid (PL) and free fatty acids (FFA) fractions, followed by lesser amounts in triacylglycerol (TG), diacylglycerol (DG), and sterol ester (StE). In the 72 h and 120 h old control larvae, the distribution of radiolabel was mostly found in the TG and the FFA fractions, followed by lower levels in PL, DG, and StE.

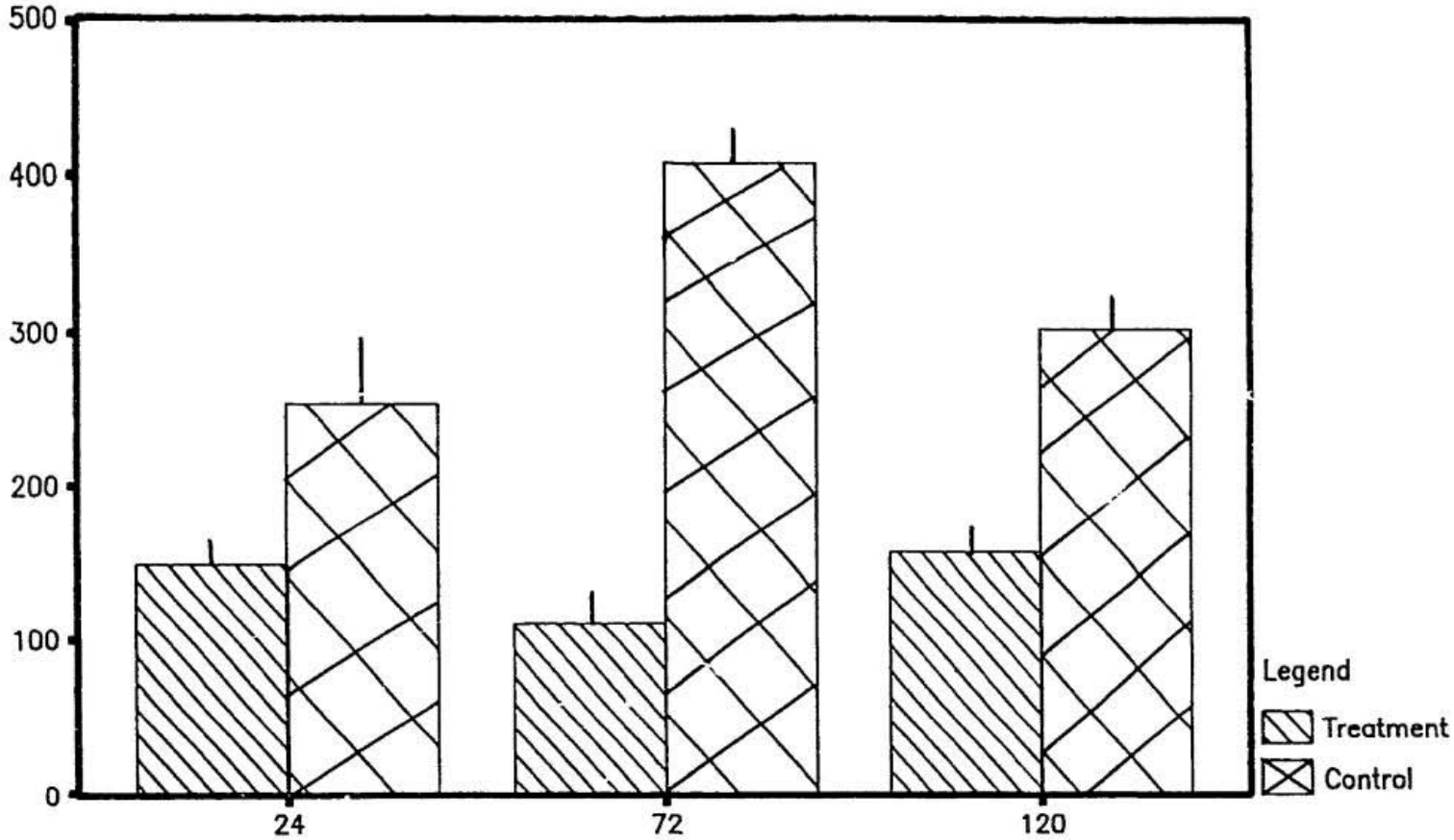
In contrast to the controls, in the 24 h post treatment budworms, TG and FFA were the principle lipid fractions, followed by PL, DG, and StE. At 72 h and 120 h after treatment, there was no further noticeable change in the lipid profile (Figure 3.8).

3.3.3.2 Synthesis of Fatty Acids from ^{14}C-Acetate

The average cell-free lipid synthesis from ^{14}C-acetate in fenoxycarb treated and control larvae is given in Figure 3.9. Statistical analysis of the data showed that cell-free lipid synthesis (i.e. synthesis of fatty acids from acetate) in the spruce budworms was significantly influenced by treatment ($F=39.25$, $d.f.=2$; $P < 0.05$) and the treatment-time after treatment interaction ($F=4.10$, $d.f.=2$; $P < 0.05$), indicating that JHA treatment affected fatty acid synthesis throughout the experimental period.

Figure 3.9. The mean synthesis of fatty acids from acetate (cell-free) in the fenoxycarb treated and control sixth instar larvae of Choristoneura fumiferana at 24 h, 72 h and 120 h after treatment. Data are expressed as Mean ± SE of 8-10 separate assays, DPM/mg protein/insect.

Figure 3.9


500
400
300
200
100
0
Lipid (DPM/mg protein/insect)
24
72
120
Hours after treatment
Legend
Treatment
Control

In control larvae, cell-free lipid synthesis from ^{14}C-acetate increased by approximately 60 % between 24 h and 72 h, then declined to near-initial levels by 120 h of the experimental period. By comparison, cell-free lipid synthesis in fenoxycarb treated insects declined by about 50 % between 24 h and 72 h post treatment, then increased to initial levels by 120 h. The degree of fatty acid synthesis by fat body extracts of fenoxycarb treated insects was 25 % to 50 % that of controls.

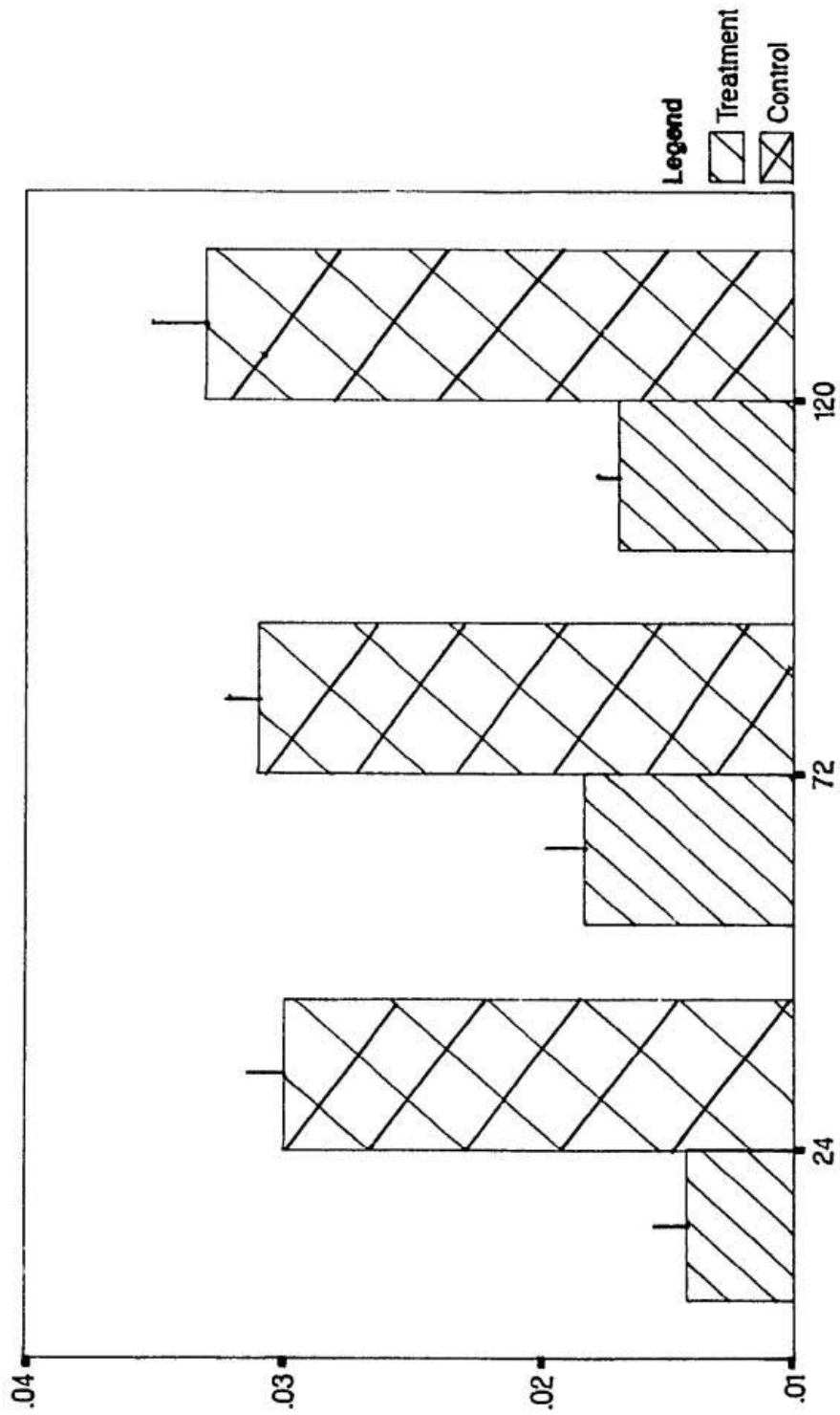
The fatty acids were synthesized by a pathway that included the biotin-dependent enzyme acetyl-CoA carboxylase. This was apparent because the addition of avidin, a biotin-binding protein, to the cell-free preparations of spruce budworm fat bodies caused a significant reduction in lipid synthesis from ^{14}C-acetate. In the cell-free system containing avidin, the Mean ± SE lipid synthesis was 92.6 ± 12.2 DPM (n=6), whereas in the controls it was 605.25 ± 55.72 DPM (n=8).

3.3.3.3 Synthesis of Complex Lipids from ^{14}C-Palmitate

The mean incorporation of ^{14}C-palmitate into fat body lipids <u>in vitro</u> in the fenoxycarb treated and control spruce budworms is given in Figure 3.10. Statistical analysis of

Figure 3.10. The mean incorporation of ^{14}C-Palmitate into fat body complex lipids *in vitro*, in fenoxycarb treated and control sixth instar larvae of *Choristoneura fumiferana* at various times after treatment. Data are shown as Mean ± SE of 10 independent measurements, nM/mg/h.

Figure 3.10


nM / mg / hour
.04
.03
.02
.01
24
72
120
Hours after treatment
Legend
Treatment
Control

the data showed that fat body lipid synthesis from ^{14}C-palmitate was significantly affected by fenoxycarb treatment ($F=61.86$, d.f.=1; $P < 0.05$). In controls, palmitate incorporation by fat bodies remained unchanged throughout the 120 h experimental period. Although the capacity of the fat bodies of treated insects to incorporate palmitate increased between 24 h and 72 h post treatment, then stabilized, the values were 50 to 60 % lower than their control counterparts.

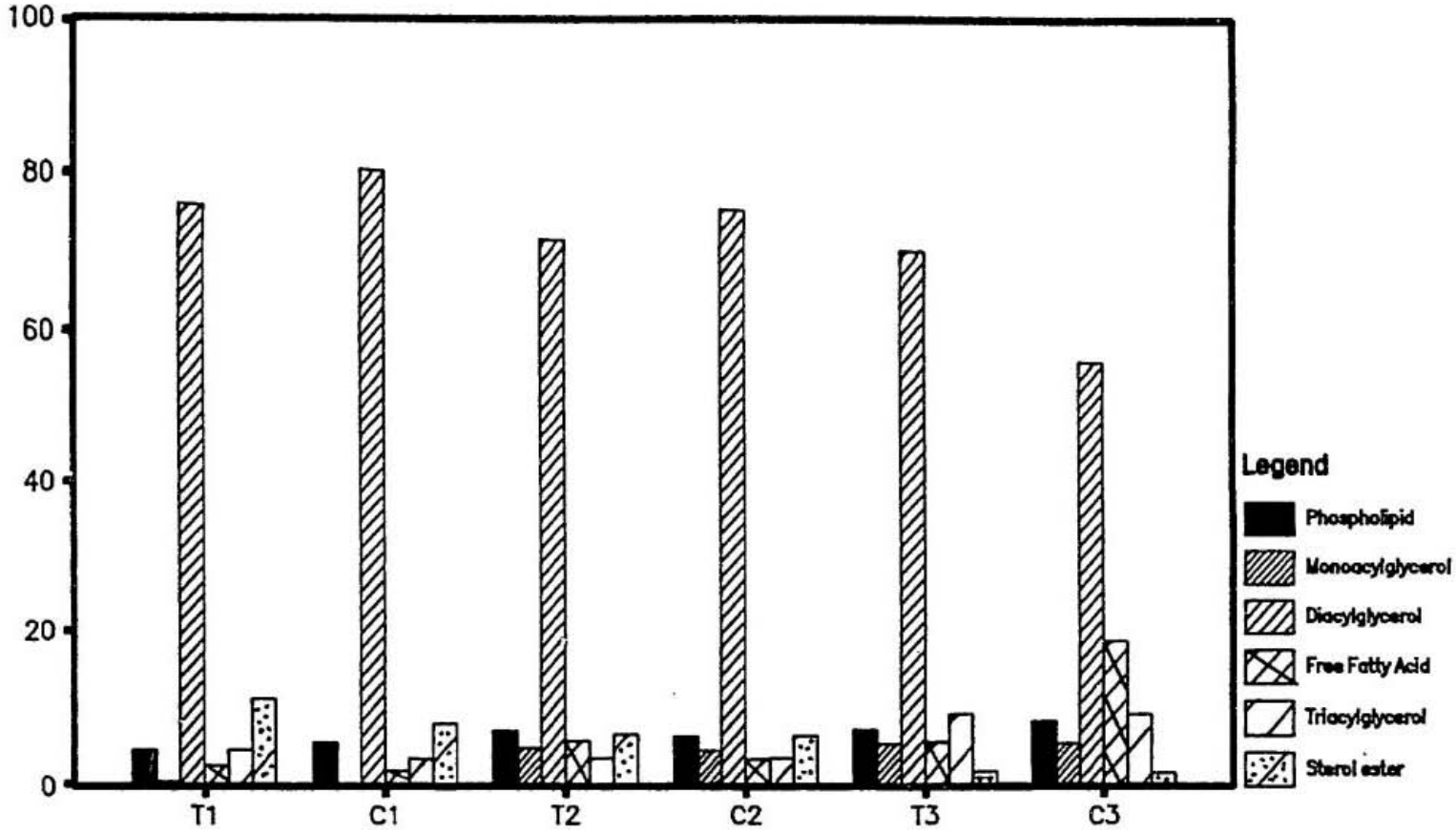
3.3.3.3.1 ^{14}C-Palmitate Product Analysis

The qualitative distribution of lipids synthesized from ^{14}C-palmitate by the spruce budworm fat bodies *in vitro* is given in Figure 3.11. Statistical analysis of the data demonstrated that time post treatment and the treatment-time interaction were significant factors which influenced the fat body lipid profile of *C. fumiferana*, indicating that effect of JHA treatment was apparent at certain times after treatment. However, treatment of larvae with fenoxycarb did not affect the type of lipids synthesized by the fat bodies from the palmitate precursor. The analysis of variance of data obtained is given in Appendix C.

In both the control and treated larvae at 24, 72, and 120 h, the distribution of the radiolabel from ^{14}C-palmitate in the fat body lipid extracts was found mainly in the diacylglycerol

Figure 3.11. The qualitative distribution of fat body lipids synthesized from ^{14}C-Palmitate *in vitro*, in fenoxycarb treated and control sixth instar spruce budworms, at various times post treatment. Results are expressed as % Mean ± SE of 8 measurements. T1, treatment 24 h; C1, control 24 h; T2, treatment 72 h; C2, control 72 h; T3, treatment 120 h; C3, control 120 h.

Figure 3.11


100
80
60
40
20
0
% Radioactivity from 14C—Palmitate
T1
C1
T2
C2
T3
C3
Treatment/Control (Time post treatment)
Legend
Phospholipid
Monoacylglycerol
Diacylglycerol
Free Fatty Acid
Triacylglycerol
Sterol ester

(DG) fraction, followed by negligible levels in the phospholipid (PL), monoacylglycerol (MG), free fatty acids (FFA), triacylglycerol (TG), and sterol ester (StE) fractions.

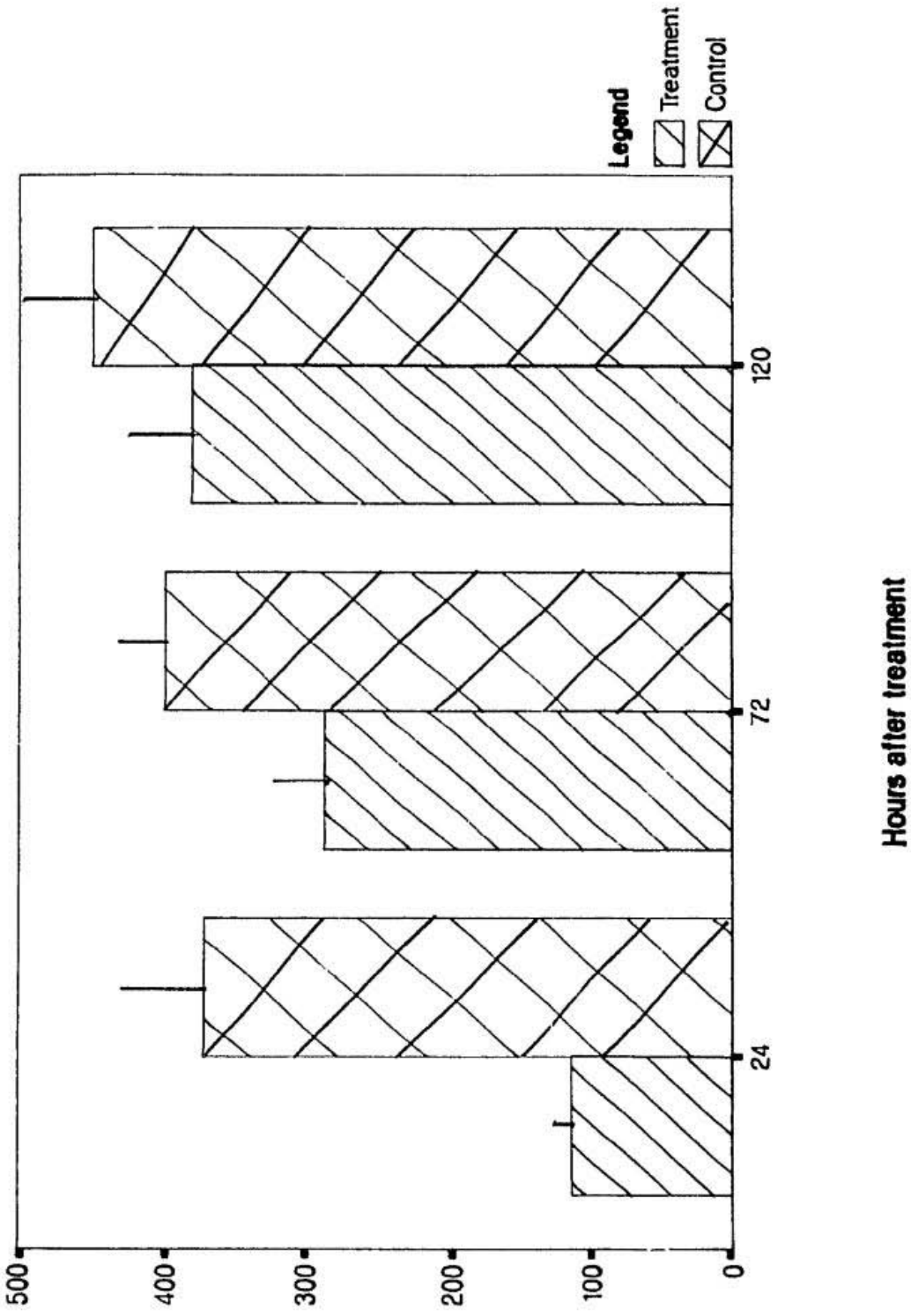
3.3.4 Fat Body ^{14}C-Palmitate Oxidation

The mean *in vitro* $^{14}CO_2$ emission from ^{14}C-palmitate by the fenoxycarb treated and control spruce budworm fat bodies is shown in Figure 3.12. Statistical analysis of the data showed that the ^{14}C-palmitate oxidation was significantly affected by fenoxycarb treatment ($F=16.87$, d.f.=1; $P < 0.05$), and the time after treatment ($F=7.92$, d.f.=2; $P < 0.05$).

$^{14}CO_2$ emission from ^{14}C-palmitate remained relatively stable for fat bodies of control insects. At 24 h post treatment $^{14}CO_2$ emission from ^{14}C-palmitate by fat bodies of treated insects was only about 30 % that of the controls. ^{14}C-Palmitate oxidation increased significantly at 72 h and 120 h after treatment, but the values were lower (72 h) than or similar (120 h) to their control counterparts.

Figure 3.12. The mean $^{14}CO_2$ emission from ^{14}C-Palmitate in the fenoxycarb treated and control spruce budworm fat bodies *in vitro*, at 24 h, 72 h and 120 h post treatment. Data are expressed as Mean ± SE of 9-10 independent determinations, DPM/mg/h.

Figure 3.12


DPM / mg / hour
500
400
300
200
100
0
24
72
120
Hours after treatment
Legend
Treatment
Control

DISCUSSION

Fenoxycarb was the most effective juvenile hormone analog of those that were screened for disruption of metamorphosis of the eastern spruce budworm, Choristoneura fumiferana. In addition to its low LD_{50} value against the sixth instar, fenoxycarb caused a wide array of morphogenetic deformities resulting in death of C. fumiferana larvae.

Fenoxycarb and methoprene treatment, at a sublethal dose, also caused a general disruption in the metabolism of the spruce budworm, as shown by altered levels of carbohydrate, protein and lipid in the hemolymph and fat bodies. Lipid levels in the hemolymph and fat bodies were severely depleted in fenoxycarb treated insects as compared to the controls.

The capability of fat bodies from treated larvae to synthesize lipids was impaired. It was found that impairment occurred both in the pathway leading to fatty acid synthesis, and the subsequent pathway leading to complex lipid synthesis.

Lastly, the ability of the fat bodies from treated spruce budworms to metabolize fatty acids in vitro via the β-oxidation pathway was impaired solely in the early (24 h post treatment) sixth instar spruce budworm larvae.

4.1 Dose-response Studies

The results demonstrated that early sixth instar larvae of C. fumiferana were highly sensitive to fenoxycarb, with an LD_{50} value of 0.261 μg per insect. This compound caused a wide array of morphogenetic deformities which resulted in death of the spruce budworm larvae. All of the effects were consistent with the juvenilizing action of potent JHAs on Lepidoptera (Retnakaran et al., 1985; Sehnal, 1983; Staal, 1975). Masner et al. (1981) reported that fenoxycarb was very effective against tortricids and homopterans in orchards and forests. Fenoxycarb, a phenoxy ethyl carbamate, has been shown to have favorable persistence on plants, stored products, and in water, with residual activity against several insect species (Dorn et al., 1981; deReede et al., 1984). Moreover, C. fumiferana eggs, at an early stage of embryogenesis (0-24 h old), as well as adults of both sexes, were found to be very sensitive to fenoxycarb treatment; treated eggs or eggs deposited by treated adults failed to hatch (Hicks and Gordon, 1992). It should be further evaluated against C. fumiferana under greenhouse and field conditions.

Fenoxycarb has also been found to be effective against other Lepidoptera: Adoxophyes reticulana and Adoxophyes orana (Masner et al., 1983; Charmillot and Blaser, 1985), Cydia pomonella (Charmillot, 1989; Charmillot et al., 1989),

Grapholita funebrana (Charmillot et al., 1986), Eupoecilia ambiguella and Lobesia botrana (Charmillot et al., 1987), Heliothis virescens (Masner et al., 1987; Mauchamp et al., 1989), Pandemis heparana (deReede et al., 1984), Ostrinia nubilalis (Gadenne et al., 1990), and Platynota idaeusalis (Hull et al., 1991).

By contrast, relatively high doses of methoprene, ZR9582, and ZR10151 were required to interrupt the development of early sixth instar larvae of C. fumiferana. High doses of methoprene also were required to cause supernumerary molting and attendant mortality of C. fumiferana (Retnakaran, 1973) and a related species, C. occidentalis (Robertson and Kimball, 1979). In laboratory, greenhouse, and field screening of methoprene against the sixth instar larvae of C. fumiferana, high doses of the JHA were required to achieve mortalities of control significance (Retnakaran et al., 1977).

Various developmental stages of C. fumiferana have also been reported to be refractory to several of the earlier JHAs tested. Applications of high doses of juvabione, ethyl-aromatic terpenoid ether, and farnesyl methyl ether were required to cause ovicidal effects (Retnakaran, 1970) and high ED_{50} (i.e. median effective dose to elicit a specific effect) values were obtained for AY22,342 (a mixture of eight isomers of JH-I), topically applied to spruce budworm pupae (Outram, 1973). The biochemical and physiological mechanisms

responsible for differential susceptibility of C. fumiferana to JHAs remains to be elucidated.

The JHAs S-71639, ZR8487, and ZR9892 may be considered as moderately effective against the early sixth instar spruce budworm larvae, as indicated by their LD_{50} (i.e. median lethal dose of the JHA) values and the associated morphogenetic deformities induced. Topical application of S-71639 was found to disrupt molting in fifth instar larvae of Spodoptera litura (Hatakoshi et al., 1986). This compound was also reported to be 32 times more active than methoprene in causing supernumerary molting when applied to fifth instar larvae of Manduca sexta (Hatakoshi et al., 1988). Therefore, it seems reasonable to explore their field efficacy against forest pests such as the spruce budworm.

4.1.1 Morphogenetic Effects

Juvenile hormone analogs adversely affect metamorphosis by disrupting the titre of juvenile hormone (JH) at specific stages of the insect's development. In a holometabolous insect, such as C. fumiferana, the level of circulating JH normally remains high during the initial instars, drops during the last larval instar, and is below physiologically active levels in the pupa. When last instar larvae are treated with effective JHAs, the JH titre remains high and cellular

programming of the epidermis and imaginal disks takes an abnormal course (Retnakaran et al., 1985).

Larval death, however, may also be attributed to a combination of secondary factors, which are not directly related to the hormonal activity of the JHAs (Sehnal, 1983): suffocation, bleeding, and dessication due to imperfect exuviation, starvation due to morphogenetic defects, failure of vital homeostatic processes, and metabolic impairment. As illustrated in Figure 12 A-C, treatment of day 1, sixth-instar larvae of C. fumiferana with all JHAs, except ZR10151, caused a wide range of morphogenetic deformities- formation of larval-pupal intermediates, with precocious evagination of the imaginal-wing disks, production of deformed pupae with hemolymph-filled blisters, supernumerary molting, mummified larval-pupal intermediates, and inhibition of molting. The severity of the morphogenetic aberrations were also concentration-dependent. These morphogenetic deformities contributed directly or indirectly to the mortality of the spruce budworm larvae.

The elucidation of the mode of action of JHAs, and thus the explanation of their morphogenetic effects, will depend upon the clarification of the mode of action of endogenous JH in insects. It is generally accepted that the molting process and metamorphosis in insects is under the control of hormones. The hormonal control of molting and cuticle deposition during

a molt is under the control of ecdysteroids, secreted by the prothoracic glands. The prothoracic glands are stimulated by the prothoracicotropic hormone (PTTH), secreted from the neurosecretory cells of the brain. The type of cuticle produced, however, is under the control of JH, produced by the corpora allata. JH, as the name implies, is required in order to maintain larval characteristics and its disappearance must be ensured for metamorphosis to occur (Riddiford, 1985).

Exogenous application of JHAs to insects causes an elevation in the endogenous JH titre. The extent and nature of the response to JHA application, however, depends on the species, the time of application, the dose, the mode of administration, and the type of compound (Staal, 1975). If JHAs are applied during the critical period for sensitivity to JH, usually the beginning of the ecdysteroid peak (Riddiford, 1985), the JH receptors (located on the epidermal cells and possibly cells of other tissues) become saturated with the JHA (Slama, 1985), and lead to an inhibition of metamorphosis. This may also result in supernumerary molting (Sehnal, 1983). Moreover, the JH receptors on cells of insects are not sensitive at exactly the same time (Slama, 1985), i.e. asynchronous JH-sensitivity, allowing cells with insensitive JH receptors to develop normally. Such a situation will lead to the formation of larval-pupal intermediates as well as other morphologically deformed individuals (Slama, 1985).

Direct experimental evidence for this theory is still lacking.

Hemolymph and cellular JH-binding proteins have been reported in several insect species (Goodman and Chang, 1985; Roberts and Jefferies, 1986) and, in Leucophaea maderae, cytosolic and nuclear JH receptors in the fat body have been characterized (Engelmann et al., 1987). Moreover, two high-affinity nuclear binding proteins for JH-I and JH analogs (methoprene and iodovinylmethoprenol) have been demonstrated in the larval epidermis of Manduca sexta (Riddiford et al., 1987). Factors such as the chemical structure of the JHA, its rate of entry into the cuticle, in vivo metabolism, and excretion are also important factors governing its efficacy. The mode of action of fenoxycarb, the most potent JHA against the sixth instar C. fumiferana, remains to be elucidated at the cellular and molecular level.

4.2 Effect of JHAs on Metabolite Concentrations

In addition to their morphogenetic effects on the sixth instar C. fumiferana larvae, the fenoxycarb and methoprene, at a LD_{50} concentration, caused an alteration in the hemolymph and fat body levels of carbohydrate, protein and lipid as compared to the controls. Since there was no effect of JHA treatment on the hemolymph volume and fat body dry weight of sixth instar C. fumiferana, the observed alterations in metabolite levels

resulted from changes in the mobilization and/or utilization of carbohydrate, protein and lipid.

4.2.1 Hemolymph and Fat Body Carbohydrates

In the control sixth instar larvae of C. fumiferana, blood carbohydrate levels, on an average, increased throughout the progression of the instar. This finding is consistent with observations that the levels of blood trehalose, the principal carbohydrate in the hemolymph, increased during the last larval instar in several insect species: Hyalophora cecropia (Wyatt and Kalf, 1957), Antheraea pernyi (Egorova and Smolin, 1962), Leucania separata (Liu and Feng, 1965), Bombyx mori (Duchateau-Bosson et al., 1963), and Samia cynthia ricini (Chang et al., 1964).

This increase in the blood carbohydrate (trehalose) levels appears to be associated with active feeding. There may also be hormone-mediated regulation of blood trehalose levels, associated with the growth, molting and development of the insects (Chen, 1971). In the control spruce budworms, the increase in the levels of hemolymph carbohydrate during the last larval instar may be associated with feeding and growth, in preparation for the metamorphic molt. The role of the hypertrehalosemic hormone (Steele, 1961), if any, in the carbohydrate regulation of C. fumiferana remains to be

investigated.

In the fat bodies of control insects, the levels of glycogen, the predominant reserve carbohydrate in insects (Wyatt, 1967), increased almost 4-fold at 72 h from the initial levels, then declined to near-initial levels towards the end of the instar. Similarly, a substantial rise in the glycogen levels, both per individual and as percentage of body weight, during larval growth have been reported in several insect species: Tenebrio molitor (Rousell, 1955), Antheraea pernyi (Smolin and Gudalina, 1957), Bombyx mori (Zaluska, 1959), Hyalophora cecropia (Bade and Wyatt, 1962), Samia cynthia ricini (Chang et al., 1963), Anthrenus vorax (George and Nair, 1964), Musca domestica (Ludwig et al., 1964), Acyrthosiphon pisum (Srivastava, 1965), and Anthonomus grandis (Nettles and Benz, 1965). The increase in glycogen levels during larval growth was interrupted by a temporary decline at each molt. In the final larval instar, the accumulation of glycogen was increased in preparation for metamorphosis (Wyatt, 1967). A similar situation appears to occur in C. fumiferana; however, the metabolic and homeostatic pathways remain to be studied in more detail.

In some Diptera and Orthoptera, glycogen synthesis is controlled by a hormone originating in the medial neurosecretory cells (MNC) of the brain, and in some Lepidoptera, the suboesophageal ganglion (Steele, 1983).

Glycogenolysis, on the other hand, is mediated by the hyperglycaemic (hypertrehalosaemic) hormone secreted by the corpora cardiaca (Steele, 1963); this hormone causes an elevation of hemolymph trehalose, with a concomitant decline in the fat body glycogen.

After 96 h, fenoxycarb treatment, at a sublethal dose, caused blood carbohydrate levels to elevate significantly in the JHA treated insects compared to the controls. There was a substantial decrease in glycogen levels in the fat bodies of fenoxycarb-treated C. fumiferana larvae, at 24 h and 72 h post treatment; at 120 h after treatment, levels were slightly higher than the controls. However, this apparent depletion in glycogen did not result in an increase in hemolymph carbohydrate levels, as might be anticipated from accelerated glycogenolysis or reduced glycogen synthesis, until after 96 h post treatment. This paradoxical result is difficult to explain, since biochemical/enzymatic resolution of the effect was not attempted.

The concomitant depletion in fat body glycogen and hemolymph carbohydrate prior to 96 h post treatment may be a result of accelerated glycogenolysis and/or depressed glycogen synthesis, followed by energy mobilization for the synthesis of proteins or other biomolecules. Toward the end of the experimental period, the elevated levels of carbohydrates in the hemolymph of fenoxycarb-treated insects, concomitant with

high levels of fat body glycogen (cf. controls), suggest an increase in glycogen synthesis and/or reduction in glycogenolysis accompanied by decreased energy mobilization for biosynthetic purposes. Such postulated effects on glycogenolysis and/or glycogen synthesis may be induced by the JHA indirectly effecting changes in the insect's neurohormonal system responsible for regulating carbohydrate metabolism.

Alternatively, the JHA may target the fat body and its constituent pathways for carbohydrate metabolism directly, as appears possible from the direct effect of fenoxycarb on lipid synthesis that was demonstrated in this study. While the precise role of JH in carbohydrate metabolism remains to be elucidated, there is evidence that the natural hormone, or its analogs, modulate fat body glycogen levels. Allatectomy of Carausius morosus (L'Helias, 1953), Pyrrhocoris apterus (Janda and Slama, 1965), Calliphora erythrocephala (Thomsen, 1952), Phormia regina (Orr, 1964), and Musca domestica (Liu, 1974) caused an accumulation of whole body or fat body glycogen, while injection of synthetic JH or implantation of corpora allata in Drosophila melanogaster (Butterworth and Bodenstein, 1969) increased glycogenesis.

In the methoprene-treated larvae of C. fumiferana, there was an initial increase in the hemolymph carbohydrate levels (72 h post treatment), and a decline thereafter until 144 h post treatment, compared to the controls. Thus, the effect of

methoprene on the hemolymph carbohydrate concentration was opposite to that of fenoxycarb treatment. In the fat bodies of methoprene-treated spruce budworms, glycogen levels were altered in a fashion similar to the fenoxycarb treated insects.

Methoprene treatment, initially, caused a significant depletion in fat body glycogen (24 h and 72 h post treatment), followed by an elevation at 120 h after treatment, as compared to the respective controls. The hemolymph carbohydrate levels in methoprene-treated insects show a trend opposite to the fat body glycogen concentration, suggesting a relationship between hemolymph carbohydrate mobilization and the depletion of glycogen in the fat body.

Disruption of hemolymph and fat body carbohydrate levels have been reported for certain other insect species. Exposure of early fourth-instar larvae of Aedes aegypti to methoprene significantly increased the carbohydrate levels in the hemolymph of late fourth-instar larvae, but reduced the hemolymph carbohydrate concentration of 24 h old pupae (Gordon and Burford, 1984). Additionally, treatment of post-feeding larvae of Delia radicum with the JHA caused a significant decrease in the hemolymph trehalose levels (Young and Gordon, 1987). Thus, it is apparent that methoprene causes varied effects on carbohydrate mobilization, depending on the species, instar, and age of the insect.

Data obtained in this study are explicable in terms of increased glycogenolysis/reduced glycogen synthesis, followed by diminished glycogenolysis/enhanced glycogenesis, resulting from methoprene treatment.

There is evidence obtained from other insect species that methoprene can modify glycogenolysis and glycogenesis. In the mosquito Aedes aegypti, Gordon and Burford (1984) reported that methoprene caused a reduction in fat body glycogen, and that glycogenolysis was suppressed due to an overall depletion of glycogen phosphorylase. Downer et al. (1976) showed that whole pupae of Aedes aegypti became depleted in glycogen and lipid following exposure to methoprene. However, treatment of Stomoxys calcitrans with an aromatic terpenoid ether, a JHA unrelated to methoprene, caused increased level of whole body glycogen (Wright and Rushing, 1973).

In a subsequent study, Wright et al. (1973) reported that JHA treatment had no effect on the glycogen phosphorylase activity in this insect, suggesting that the increased glycogen levels resulted from enhanced synthesis rather than impaired glycogenolysis. The precise mechanism of action of methoprene on anabolic and catabolic carbohydrate pathways in C. fumiferana is still not known. As has been postulated for fenoxycarb, methoprene-induced perturbation in carbohydrate metabolism of C. fumiferana may be the result of a direct or indirect effect of the JHA on the fat body or the

neuroendocrine system.

4.2.2 Hemolymph and Fat Body Proteins

Hemolymph protein concentration of the control sixth instar larvae of C. fumiferana increased during the instar, and declined slightly, just before pupation. Hemolymph protein levels in many Lepidoptera (Wyatt and Pan, 1978) are lower in early instars, rise steeply in the last larval instar, and may fall before the larval-pupal ecdysis. The findings reported here are consistent with the trend for blood protein levels in the last larval instar reported in several other Lepidoptera: Deilephila euphorbiae (Heller and Moklowska, 1930), Hyalophora cecropia (Chefurka, 1953), Popillia japonica (Ludwig, 1954), Galleria mellonella (Denucé, 1958), Bombyx mori (Wyatt et al., 1956), Samia cynthia ricini (Laufer, 1960), and Pieris brassicae (Van Der Geest, 1968; Chippendale and Kilby, 1969). The increase in blood protein levels during the last instar is associated with active feeding, and is a result of active protein synthesis in the fat body (Chen, 1978). The results suggest that a similar situation exists in C. fumiferana larvae.

The larval fat body has been shown to serve as a major site of synthesis of hemolymph proteins in Bombyx mori (Shigematsu, 1958, 1960; Faulkner and Bheemeshwar, 1960),

Hyalophora cecropia (Laufer, 1960; Telfer and Williams, 1960; Skinner, 1963), Pieris brassicae (Chippendale and Kilby, 1969), and several other insect species (Chen, 1978; Keeley, 1985).

In the fat bodies of the control spruce budworm, the protein content increased about 2-fold (at 72 h and 120 h), from the initial levels (24 h). This increase in the levels of fat body protein may be the result of enhanced protein synthesis during the active feeding period or storage of protein, or both, in preparation for the upcoming metamorphic molt. These data parallel the respective hemolymph protein levels, as might be anticipated, given that the hemolymph proteins are derived from the fat body.

In the larvae of M. sexta, the increased concentration of hemolymph protein may serve as a nutritional source of amino acids for protein synthesis and energy (Greene and Dahlman, 1973). The stored protein may also be utilized for the biosynthesis of adult structures (Kanost et al., 1990). The hormonal regulation of hemolymph and fat body proteins is incompletely known.

In the Holometabola, such as C. fumiferana, the fat body synthesizes and releases proteins into the hemolymph during the earlier larval stages, then sequesters these proteins from the hemolymph in the prepupal stage (Kanost et al., 1990), suggesting that 20-hydroxyecdysone or a declining titre of JH

stimulate protein synthesis/uptake. In larvae of B. mori, synthesis of major hemolymph proteins is regulated by JH (Plantevin et al., 1987); there being a reverse correlation between protein synthesis and JH titre. In the same insect, storage protein uptake was induced by ecdysone after a decline in JH levels (Tojo et al., 1981). Also, protein storage granule formation in the Calpodes ethlius larvae (Locke and Collins, 1965, 1968) has been shown to be stimulated by 20-hydroxyecdysone (Collins, 1969). Similar results have been reported in Spodoptera litura (Tojo et al., 1985). The hormonal regulation of protein levels in C. fumiferana hemolymph and fat body remains to be elucidated.

In the fenoxycarb treated sixth instars, hemolymph protein concentration increased in a pattern similar to controls, albeit at a lower level, throughout the last instar. In the fat body of sixth instar C. fumiferana treated with fenoxycarb, the protein content was reduced at 24 h post treatment as compared to the controls. Then, the protein levels increased about 4-fold, so that levels were higher than or comparable to the controls at 72 h and 120 h, respectively. These data suggest that fenoxycarb caused an initial reduction in protein synthesis by the fat body.

The finding that protein levels in the fat bodies of treated insects became higher than, or similar to the controls, while hemolymph protein levels were below normal,

suggests that the JHA may interfere with release of synthesized proteins into the hemolymph. There is evidence to suggest that fenoxycarb modifies protein synthesis by the fat body of certain insect species. In larvae of Trichoplusia ni, the appearance of a 76-kDa hemolymph protein was suppressed in vivo by topical application of fenoxycarb (Jones et al., 1988). Using an antibody specific for the 76-kDa protein, Jones et al. (1988) reported that translation of poly(A) RNA from untreated larvae yielded the protein whereas mRNA from fenoxycarb-treated larvae did not. Grzelak and Kumaran (1985, 1986) have shown that, in Galleria mellonella, several ecdysteroid-induced storage proteins could be suppressed by JH treatment. The degree to which fenoxycarb modifies the separate processes of protein synthesis and release, as well as the molecular mode of action, remains to be determined for C. fumiferana.

Methoprene treatment caused a significant depletion in the hemolymph protein levels of sixth instar C. fumiferana until 120 h post treatment, as compared to the controls. Then, the blood protein titre increased significantly. However, the protein content of the fat bodies of methoprene-treated insects was comparable to controls throughout the experimental period. These findings are in agreement with those of Tojo et al. (1981) and Bosquet and Calvez (1985), who reported that treatment of Bombyx mori larvae with methoprene caused a

significant reduction in the hemolymph proteins. In the same insect, allatectomy-induced storage protein synthesis was reversed by methoprene injection (Izumi et al., 1981, 1984). The result is also in agreement with Gordon and Burford (1984) who, in Aedes aegypti, reported a depletion in hemolymph protein as a result of methoprene treatment. The depletion in hemolymph protein of methoprene-treated insects was the result of impaired capacity of the fat bodies to synthesize proteins (Gordon and Burford, 1984).

More recently, the inhibitory effect of methoprene on protein synthesis in Bombyx mori has been studied at the molecular level (Bosquet et al., 1989). These authors reported that, the JHA treatment decreased major hemolymph protein synthesis without any accumulation of untranslated mRNA, suggesting that methoprene treatment affected translation of mRNA for protein synthesis. Interpretation of the data obtained for C. fumiferana can only be done once the effects of the JHA on the separate processes of protein synthesis and release by the fat body have been elucidated. It is possible, for example, that the JHA could impair both processes, leading to lower than normal hemolymph protein levels concomitant with normal, or above normal, fat body concentrations. Alternatively, the lower than normal hemolymph protein levels may be the consequence of increased utilization and uptake by other tissues rather than reduced production by the fat body.

More in depth studies are required to determine which of these hypotheses is tenable and the nature of the endocrinological events attendant with such disrupted protein metabolism.

4.2.3 Hemolymph and Fat Body Lipid

In the control sixth-instar larvae of C. fumiferana, hemolymph lipid levels were characterized by an initial steep increase (48 h), followed by a gradual decline to near-initial levels until 96 h, and a subsequent increase until pupation. In the fat bodies, there was a concomitant increase in the lipid content at 72 h, from the initial (24 h) levels. These broad fluctuations in the lipid levels may be associated with active feeding as well as developmental events during the last larval instar, in preparation for the metamorphic molt.

Many holometabolous insects have been shown to accumulate large amounts of lipid during larval development, as energy reserves for later nonfeeding stages (Fast, 1964). This has been demonstrated in several Lepidoptera: Bombyx mori (Niemierko et al., 1956), Malacosoma americana (Rudolfs, 1926), Heliothis zea (Lambremont and Graves, 1969), Heliothis virescens (Wood et al., 1969), Hyalophora cecropia (Gilbert and Schneiderman, 1961); and other insect species (Beenakkers et al., 1981).

The hemolymph lipid levels in a developing larva may be

the net result of feeding, digestion, absorption, transport to and from fat body and other tissues, and metabolism (Beenakkers et al., 1985). Thus, in the controls, the initial rapid elevation in blood lipid levels followed by a gradual decline until 96 h, suggests that dietary lipid may appear in the hemolymph, for transport to the fat body for storage and turnover. However, direct experimental evidence for this hypothesis is still required.

After 96 h, there was a gradual rise in the blood lipid levels until pupation (144 h). The fat body lipid content at 120 h also increased over the 72 h levels, suggesting that fat body lipid may not be mobilized at this time. The elevation in blood lipid may be associated with the metamorphic developmental program, or metabolic turnover, or both, in these insects. The possibility that some of the increase in hemolymph lipid toward the end of the experimental period may have resulted from carbohydrate to lipid conversion in the fat body (Steele, 1976) appears unlikely, since fat body glycogen levels were not depleted at this time. An examination of the enzyme systems involved in this process would seem to be a promising avenue to follow.

In spruce budworms treated with fenoxycarb, the hemolymph lipid levels were severely depleted throughout the progression of the instar, although there was a slight increase at 144 h after treatment. In the fat bodies, there was an initial

elevation in lipid content (24 h post treatment), followed by a depletion at 72 h and 120 h post treatment. These data demonstrate that hemolymph and fat body lipid turnover is severely perturbed by the JHA treatment, either by reducing synthesis, transport or increased metabolism. Indeed, as discussed in the subsequent section, lipid synthesis and metabolism in the fat body was severely impaired in fenoxycarb treated insects. The digestion and/or absorption of dietary lipid may also have been disrupted.

The results presented here are in agreement with reported effects of corpora allata (CA) implantation or exogenous application of JH in some insects. Using in vitro techniques, Gilbert (1967a) has shown unequivocally that CA depress the rate of neutral lipid synthesis in the fat body of Leucophaea maderae. The inhibitory effect of JH on lipid synthesis in the fat body has also been confirmed in Schistocerca gregaria (Hill and Izatt, 1974). There are numerous studies which demonstrate abnormal accumulation of lipid in the fat body following allatectomy: Carausius morosus (L'Helias, 1953), Periplaneta americana (Bodenstein, 1953), Locusta migratoria (Minks, 1967; Strong, 1968), Schistocerca gregaria (Odhiambo, 1966; Hill and Izatt, 1974), Spodoptera littoralis (El-Ibrashy and Boctor, 1970), Drosophila melanogaster (Vogt, 1949), and Calliphora erythrocephala (Thomsen, 1952); a result that is opposite to CA implantation or application of JH. In

Schistocerca gregaria (Hill and Izatt, 1974) and Locusta migratoria (Beenakkers, 1969), the increase in lipid content of the fat body of allatectomized insects was prevented by reimplantation of the CA; and in insects which had not been allatectomized but had received CA implants, fat body lipid synthesis was reduced compared to controls.

In the sixth-instar larvae of C. fumiferana treated with methoprene, lipid levels in the blood corresponded to controls until 96 h after treatment, then declined relative to controls until 144 h post treatment. As was the case in fenoxycarb-treated insects, fat bodies from methoprene treated insects initially (24 h post treatment) had lipid levels higher than the controls. Then, lipid levels in the fat bodies were depleted at 72 h post treatment, followed by an increase at 120 h after treatment to near-initial levels. However, the lipid content of the fat bodies of methoprene-treated insects was still lower than in controls. These data on hemolymph and fat body lipids demonstrate a methoprene-induced disruption in lipid metabolism. The changes in fat body lipids (initially higher, then lower than in controls) does not correlate with the hemolymph lipid titres, however, which are generally similar to the controls. A detailed examination of lipid metabolism by the fat body and other tissues that avail of fat body generated lipids in methoprene-treated insects is needed to explain the observed effects.

The depletion in hemolymph lipid of methoprene-treated larvae of C. fumiferana, albeit after 96 h post treatment, appears to be in agreement with the results of Downer et al. (1976). These authors reported that treatment of fourth-instar larvae of Aedes aegypti with the JHA caused a depletion in lipid content of the pupae. However, these results should be viewed with caution, since whole body homogenates were utilized. More recently, in a structural study of the Locusta migratoria fat body, Cotton and Anstee (1991) reported depletion in the levels of fat body lipid and glycogen, as a result of methoprene treatment. Paradoxically, extraction and quantification of the fat body lipid and glycogen showed that there was no significant difference from the controls. Cotton and Anstee (1991) reasoned that while the amount of fat body tissue was increased by the JHA treatment, the total amounts of fat body lipid and glycogen remained unchanged; thus reducing the levels of lipid and glycogen per unit volume. Yet, fat body wet weight was not significantly increased in treated insects (Cotton and Anstee, 1991). In the bumblebee Bombus terrestris, fat body lipid reserves were depleted as a result of synthetic JH-I treatment (Röseler and Röseler, 1988).

4.3 Effects of Fenoxycarb on Lipid Metabolism

4.3.1 Qualitative Lipid Profile

The predominant class of neutral lipid in the hemolymph of sixth-instar C. fumiferana was diacylglycerol (DG). When detected, sterol esters (StE) and fatty acids (FA) were present in trace concentrations. By contrast, triacylglycerol (TG) was the major constituent of the fat body neutral lipid, followed by trace amounts of phospholipid (PL), DG, and monoacylglycerol (MG). The major repository of TG is the fat body, as demonstrated in Periplaneta americana (Downer, 1981), Leucophaea maderae (Gilbert, 1967b), Pyrrhocoris apterus (Martin, 1969), Diatraea grandiosella (Chippendale, 1971), Sitotroga cerealella (Chippendale, 1973), Manduca sexta (Fernando-Warnakulasuriya et al., 1988), Acheta domesticus (Grapes et al., 1989), and Lymantria dispar (Clark et al., 1990). Diacylglycerol is the dominant hemolymph lipid in most insects (Gilbert and Chino, 1974; Downer and Matthews, 1976; Chino et al., 1981; Van der Horst et al., 1981; Mwangi and Goldsworthy, 1981; Turunen and Chippendale, 1981; Chino and Downer, 1982; Chino, 1985). In C. fumiferana, the qualitative lipid profile of the treated insects was similar to the controls; and the overall lipid picture was in agreement with the reported data on hemolymph and fat body lipids in several

insects.

4.3.2 Fatty Acid Profile

Qualitative analysis of the fatty acids from the hemolymph and fat body neutral lipids in C. fumiferana revealed that the major proportion of the fatty acid complement was represented by seven fatty acids: the saturated fatty acids, myristic acid (C14:0), palmitic acid (C16:0), and arachidic acid (C20:0), the monounsaturated fatty acids, palmitoleic acid (C16:1) and oleic acid (C18:1), and the polyunsaturated fatty acid, linoleic acid (C18:2).

This result is in agreement with the profile of fatty acids reported in several insects (Thompson, 1973; Fast, 1964; Gilbert, 1967a); with the exception of linolenic acid (C18:3), which was below the limits of detection in C. fumiferana. Most of the potential energy available from neutral lipids in insects is contained within the fatty acid component of the molecule(s).

In the spruce budworm, the qualitative fatty acid profile of the hemolymph and fat body neutral lipids was similar at various times of the last larval instar, and there was no significant difference between the treated and control insects. Thus, it appears that the fatty acid complement in C. fumiferana may be maintained at a qualitatively steady level,

irrespective of the lipid concentration of the hemolymph and fat bodies of control and treated insects. The mechanism(s) by which this 'qualitative steady-state' is accomplished remains to be studied.

4.3.3 Fat Body Lipid Synthesis

4.3.3.1 Overall Lipid Synthesis from ^{14}C-Acetate

The capacity of fat bodies from treated C. fumiferana larvae to synthesize lipids in vitro was significantly impaired, as evidenced by reduced incorporation of ^{14}C-acetate precursor into lipid; in contrast to the respective controls. This result is also consistent with the observed depletion in lipid levels of fenoxycarb treated budworms in the hemolymph throughout the entire experimental period and in the fat bodies at 72 h and 120 h after treatment. The reduced incorporation of ^{14}C-acetate precursor in the fat bodies of treated insects suggest that the enzyme complement involved in lipid synthesis may be affected by the JHA.

That the fat body can incorporate radiolabelled acetate into fatty acids has been established in several insect species: Periplaneta americana (Louloudes et al., 1961), Eurycotis floridana (Bade, 1964), Bombyx mori (Sridhara and Bhat, 1964), Trichoplusia ni (Nelson and Sukkestad, 1968),

Heliothis zea (Lambremont, 1971), Calliphora erythrocephala (Brak et al., 1966), Oulema melanopus (Lamb and Monroe, 1968), Anthonomus grandis (Lambremont, 1965), and Myzus persicae (Strong, 1963). The fatty acids then undergo esterification with the trihydric alcohol, glycerol, to form acylglycerols (Louloudes et al., 1961; Sridhara and Bhat, 1965; Lamb and Monroe, 1968; Turunen, 1973; Chino and Downer, 1979; and Garcia et al., 1980).

In C. fumiferana, as discussed elsewhere, fatty acid synthesis via the fat body cytosolic enzymes was reduced as a result of fenoxycarb treatment. This would cause a deficiency of fatty acids for subsequent esterification and complex lipid synthesis. Moreover, the demonstrated decrease in the capacity of the fat bodies from JHA treated larvae (discussed elsewhere) to incorporate preformed fatty acid (i.e. ^{14}C-palmitate) into complex lipids, may have contributed to depleted lipid in the treated insects.

The effect of JHA treatment on lipid synthesis from ^{14}C-acetate appears to be at least partially direct, since addition of fenoxycarb to the incubation medium of fat bodies from untreated larvae resulted in reduced lipid synthesis. The possible involvement of the neuroendocrine system in lipid synthesis of C. fumiferana remains to be researched.

4.3.3.1.1 ^{14}C-Acetate Product Analysis

A thin layer chromatographic (TLC) analysis of lipids synthesized from ^{14}C-acetate by the fat bodies of treated insects revealed that, at various times after treatment, most of the radioactivity was present in the TG and FA fractions, followed by PL, DG, and StE. In the controls, initially (24 h), PL and FA were the principal lipids synthesized from ^{14}C-acetate, with lesser amounts of TG, DG, and StE. Then, at 72 h and 120 h, the radiolabel from ^{14}C-acetate was found mostly in the TG and FA fractions, with lower levels in PL, DG, and StE. These results suggest that fenoxycarb treatment also caused an alteration in the lipid synthetic pattern, as compared to the controls.

The synthesis of PL and FA in the control early sixth-instar budworms may be associated with active growth, since PL comprise an essential component of biological membranes, i.e. structural function (Downer, 1985); and FA may be utilized for energy metabolism and/or TG synthesis. The high proportion of TG and FA fractions in the later period may be interpreted as a switchover towards lipid storage in the fat body. In the treated insects, a disruption in the growth pattern may be associated with a preponderance of TG over PL synthesis. The high levels of FA concomitant with TG in the treated insects suggest that FA may be utilized for TG synthesis or energy

metabolism. However, the possibility of metabolic interconversions between the various lipid classes cannot be discounted, and should be explored by characterizing the enzyme systems involved.

4.3.3.2 Synthesis of Fatty Acids from ^{14}C-Acetate

The ability of cytosolic enzymes, i.e. cell-free preparations, from the fat bodies of treated budworms to synthesize fatty acids *in vitro*, was also significantly impaired, as demonstrated by reduced incorporation of ^{14}C-acetate into fatty acids. This result corroborates the observed reduction in the incorporation of ^{14}C-acetate into lipids in intact fat bodies, and may explain the overall depletion in lipid levels of fenoxycarb treated *C. fumiferana* in the hemolymph throughout the entire experimental period and in the fat bodies at 72 h and 120 h post treatment. The data suggest that the enzyme complement involved in fatty acid synthesis is affected by JHA treatment. Perhaps, the biosynthesis of the enzyme complex, as a composite of total protein, was affected; or the capacity to utilize the precursor and/or cofactors was impaired as a result of fenoxycarb treatment.

There is compelling evidence that fatty acid biosynthesis in insects proceeds by a pathway similar to that described for

bacteria and other animal groups (Wakil, 1961, 1970; Wakil et al., 1964; Municio et al., 1977; Lizarbe et al., 1980; Tietz, 1961, 1963). Two separable cytoplasmic enzyme systems, acetyl-CoA carboxylase and fatty acid synthetase are responsible for fatty acid synthesis (Downer, 1985). Acetyl-CoA carboxylase contains covalently bound biotin as a prosthetic group, and catalyzes the carboxylation of acetyl-CoA to the 3-carbon compound malonyl-CoA. Malonyl-CoA then condenses with acetyl-CoA in a series of reactions catalyzed by the fatty acid synthetase complex to yield a 4-carbon butyryl intermediate (Downer, 1985). After a successive sequence of similar condensations of malonyl-CoA with the newly formed acyl intermediate, an acyl chain of appropriate length is formed. The enzymes of the fatty acid synthetase complex are closely associated with an acyl-carrier protein (ACP) that serves to bind the fatty acyl intermediate compounds through the formation of thiol esters with the active 4'-phosphopantetheine group of ACP (Downer, 1985). This pathway results in the production of fatty acids containing mostly an even-number of carbon atoms. The cytosolic enzymes for fatty acid synthesis have been demonstrated in Prodenia eridania (Zebe and McShan, 1959), Locusta migratoria (Tietz, 1963), Periplaneta americana (Storey and Bailey, 1978), Ceratitis capitata (Lizarbe et al., 1980), Lucilia sericata (Thompson et al., 1975), Galleria mellonella (Thompson and Barlow, 1976),

and Acyrthosiphon pisum (Ryan et al., 1982).

In C. fumiferana, the involvement of cytosolic enzymes, particularly acetyl-CoA carboxylase, is suggested by the observation that addition of avidin, a potent inhibitor of biotin, to cell-free preparations of the fat body inhibited the synthesis of fatty acids.

4.3.3.3. Synthesis of Complex Lipids from ^{14}C-Palmitate

The competence of fat bodies from treated spruce budworms to synthesize complex lipids in vitro from preformed fatty acid was significantly diminished, as demonstrated by the reduced incorporation of ^{14}C-palmitate into lipid, compared with the respective controls. This result, in conjunction with the impaired capacity of the fat bodies from treated insects to synthesize fatty acids, may account for the overall depletion in hemolymph and fat body lipids of treated budworms. The results are in agreement with Gilbert (1967b) who, in Leucophaea maderae, demonstrated that JH (i.e. CA implantation) depressed the rate of incorporation of preformed fatty acids into fat body lipids.

It is generally accepted that insect fat body has the capacity to incorporate long-chain fatty acids into acylglycerols (Gilbert, 1967a). Short-term in vitro experiments have demonstrated that ^{14}C-palmitate was rapidly

esterified by fat bodies from several developmental stages of Hyalophora cecropia (Bhaktan and Gilbert, 1970). Similar results were obtained with Philosamia cynthia, Antheraea polyphemus, Hyalophora gloveri, and Leucophaea maderae (Bhaktan and Gilbert, 1970).

In C. fumiferana, the data suggest that the enzyme system(s) responsible for the esterification of fatty acids in the fat bodies may be disrupted as a result of JHA treatment. However, the type(s) of lipid synthesized from ^{14}C-palmitate appear to be unaffected by the JHA treatment; possibly because the enzymes involved are affected quantitatively, rather than qualitatively.

In Locusta migratoria, the fat body homogenates have been shown to contain various acyltransferases of the monoacylglycerol and α-glycerophosphate pathways for triacylglycerol synthesis (Tietz, 1969; Peled and Tietz, 1974; Tietz et al., 1975; Tietz and Weintraub, 1980). The involvement of acyltransferases in lipid synthesis has also been demonstrated in Glossina morsitans (Langley et al., 1981), Ceratitis capitata (Municio et al., 1980), Galleria mellonella (Barlow et al., 1980), and Periplaneta americana (Hoffman and Downer, 1979). Further studies on the characterization of the acyltransferases in C. fumiferana are required.

4.3.3.3.1 ^{14}C-Palmitate Product Analysis

TLC analysis of the lipids biosynthesized from ^{14}C-palmitate by the fat bodies of JHA treated spruce budworms showed that substantial radiolabel from ^{14}C-palmitate was localized in the DG fraction, followed by smaller amounts in the PL, MG, TG, FA, and StE fractions. However, this lipid profile in the treated insects was similar to the respective controls. These results indicate that, while overall fat body lipid synthesis from labelled palmitate was reduced, the classes of lipid synthesized was unaffected as the result of fenoxycarb treatment. The enzyme system(s), principally acyltransferase(s), involved in the esterification of fatty acids may be disrupted quantitatively, rather than qualitatively, in the treated insects.

Alternately, it is possible that the incubation period employed in this study (20 minutes, see Materials and Methods), although allowing maximal incorporation of ^{14}C-palmitate into fat body lipids, was not sufficient to esterify the fatty acid to TG and other lipids. In Hyalophora cecropia and other saturniid silkmoths (Bhaktan and Gilbert, 1970), the larval fat bodies incorporate palmitate into triacylglycerol in vitro, in an experiment of 3 hours incubation period; indicating that the longer period of incubation would allow almost all the label to accumulate in the TG. Further studies

on the esterification of preformed fatty acids into fat body lipids in C. fumiferana are needed.

4.3.4 Fat Body ^{14}C-Palmitate Oxidation

The β-oxidation pathway for fatty acid oxidation, localized in the mitochondria, involves sequential removal of 2-carbon units in the form of acetyl-S-CoA, which undergo condensation with oxaloacetate to form citrate (Lehninger, 1975). The citrate is subsequently oxidized in the tricarboxylic acid cycle to carbon dioxide and water with concomitant generation of ATP (Lehninger, 1975). In C. fumiferana, the emission of $^{14}CO_2$ from ^{14}C-palmitate by fat bodies *in vitro* is indicative of palmitate oxidation, and suggest that the β-oxidation pathway is operative in this insect.

The capacity of fat bodies from fenoxycarb treated budworms to oxidize preformed fatty acids *in vitro*, via the β-oxidation pathway, was significantly impaired, as demonstrated by reduced emission of $^{14}CO_2$ from ^{14}C-palmitate, compared with the respective controls. The effect of JHA treatment was most pronounced at 24 h post treatment, when the rate of palmitate oxidation was about 30 % that of controls. At 72 h and 120 h after treatment, palmitate oxidation increased, albeit at a

level lower than (72 h) or similar to (120 h) the respective controls. This result indicates that fatty acid oxidation, as monitored by $^{14}CO_2$ emission from ^{14}C-palmitate indirectly via the β-oxidation pathway, was impaired initially; and may account for the observed accumulation of lipid in the fat bodies of fenoxycarb treated insects at 24 h post treatment. Then, the rate of fatty acid oxidation appears to have been restored to near-control levels. Thus, it is conceivable that, in treated budworms, there may be initial disruption in the β-oxidation pathway, either by impaired enzymatic activities or incapacity to utilize substrate/cofactors or both; followed by a return to near-control levels.

This result, in conjunction with the effects of JHA treatment on lipid synthesis from acetate and palmitate, demonstrate that lipid metabolism is severely perturbed in C. fumiferana as a result of fenoxycarb treatment. The net result of JHA treatment on lipid metabolism in C. fumiferana was an overall depletion in hemolymph and fat body lipid, evidently caused by reduced lipid synthesis and continued oxidation of lipid. The initially high level of fat body lipids in fenoxycarb-treated insects may be attributed to the pronounced inhibition of the β-oxidation pathway evident at this time.

That fat bodies can oxidize fatty acids via the β-oxidation pathway has been unequivocally demonstrated, enzymatically, in Apis mellifera (Hoskins et al., 1957),

Hyalophora cecropia (Domroese and Gilbert, 1964), Locusta migratoria (Beenakkers, 1969; Beenakkers et al., 1967, 1975, 1981), Periplaneta americana (Storey and Bailey, 1978), Eurosta solidaginis (Storey and Storey, 1981), Schistocerca gregaria (yer et al., 1960), Prodenia eridania (Stevenson, 1966), and Lucilia cuprina (D'Costa and Birt, 1967, 1969a,b). The enzyme systems involved in the β-oxidation pathway for fatty acid oxidation in C. fumiferana remain to be elucidated.

SUMMARY AND CONCLUSIONS

The present study has focused on the efficacy of several juvenile hormone analogs (JHAs) against the sixth instar C. fumiferana, and on the effects of selected JHAs on carbohydrates, proteins, and lipids in the hemolymph and fat bodies of the insect. Detailed studies were conducted on the effects of the JHA fenoxycarb on lipid metabolism of the spruce budworm.

1) Dose-response Studies: Fenoxycarb, a phenoxy ethyl carbamate, was the most effective JHA, of those that were evaluated, in disrupting the metamorphosis of C. fumiferana; with a LD_{50} of 0.261 μg/insect.

2) Morphogenetic Effects: Fenoxycarb caused a wide array of morphogenetic deformities resulting in death of C. fumiferana larvae.

3) Effects of JHAs on Metabolite Concentrations: Fenoxycarb and methoprene, at a sublethal dose, caused a general disruption in the metabolism of C. fumiferana, as shown by altered levels of carbohydrate, protein, and lipid in the hemolymph and fat bodies. Lipid levels in the hemolymph

and fat bodies were severely depleted in fenoxycarb treated insects.

4) Neutral Lipid and Fatty Acid Profile: The predominant class of neutral lipid in the hemolymph of C. fumiferana was diacylglycerol; in the fat body triacylglycerol was abundant. Trace amounts of sterol esters and fatty acids (hemolymph), and phospholipid, diacylglycerol, and monoacylglycerol (fat body) were also detected. The fatty acid complement of the hemolymph and fat body lipids was represented by C14:0, C16:0, and C20:0 (saturated fatty acids); C16:1 and C18:1 (monounsaturated fatty acids); and C18:2 (polyunsaturated fatty acid). These profiles were similar in controls and fenoxycarb treated insects.

5) Overall Lipid Synthesis: The capacity of fat bodies from fenoxycarb treated C. fumiferana larvae to biosynthesize lipids in vitro was significantly impaired, as evidenced by reduced incorporation of ^{14}C-Acetate precursor into lipid, compared to controls.

6) ^{14}C-Acetate Product Analysis: A TLC analysis of lipids synthesized from ^{14}C-Acetate by the fat bodies in vitro showed that fenoxycarb treatment caused an alteration in the types of complex lipids synthesized, compared to controls.

7) Fatty Acid Synthesis: The ability of cytosolic enzymes (cell-free preparations), from the fat bodies of treated insects to synthesize fatty acids *in vitro*, was also significantly impaired, as demonstrated by reduced incorporation of ^{14}C-Acetate precursor into fatty acids. The involvement of cytosolic acetyl-CoA carboxylase, a biotin-dependent enzyme, in fatty acid synthesis was demonstrated in the cell-free preparations of fat bodies from *C. fumiferana*.

8) Synthesis of Complex Lipids: The capability of fat bodies from treated *C. fumiferana* to biosynthesize complex lipids *in vitro* from preformed fatty acid was significantly diminished, as shown by the reduced incorporation of ^{14}C-Palmitate into lipid.

9) ^{14}C-Palmitate Product Analysis: A TLC analysis of lipids synthesized from ^{14}C-Palmitate by the fat bodies *in vitro* showed that, while overall fat body lipid synthesis from labelled palmitate was reduced in fenoxycarb-treated insects, the classes of lipid synthesized was unaffected, compared to controls.

10) ^{14}C-Palmitate Oxidation: The capacity of fat bodies from fenoxycarb treated budworms to oxidize preformed fatty acids *in vitro*, via the β-oxidation pathway, was significantly

impaired, as demonstrated by reduced emission of $^{14}CO_2$ from ^{14}C-Palmitate. This effect was most pronounced in the early (24 hours post treatment) sixth instar C. fumiferana larvae.

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Appendix A

The constitution of the wheat-germ based meridic diet used to rear laboratory cultures of spruce budworms (from Bio-Serv Inc., Frenchtown, N.J.).

Ingredient	Weight (g)
Agar	25.3
Dry Mix	150.8
Wheat germ, toasted	
Casein	
Fiber	
Wesson's salt mixture	
Methyl parahydroxybenzoate	
Aureomycin	
Ascorbic acid	
Choline chloride	
Sucrose	
Linseed oil	
Vitamin mixture #722	
4 M KOH solution	
Formaldehyde	
Water	835.0 ml

Appendix B

Summary of the analysis of variance of data obtained for the lipid profile of fenoxycarb treated and control larvae of Choristoneura fumiferana. Values shown are F values ($P < 0.05$).

Lipid	Treatment (d.f.=1)	Time post treatment (d.f.=2)	Treatment-Time Interaction (d.f.=2)
Free Fatty Acids	7.97	59.83	14.13
Phospholipid	23.81	33.80	66.36
Diacylglycerol	15.21	23.77	4.33
Triacylglycerol	2.09*	37.41	186.72
Sterol ester	12.46	7.04	29.78

* indicates 'Not Significant'.

Appendix C

Summary of the analysis of variance of the data obtained for the lipid profile of the fat bodies of Choristoneura fumiferana. Values shown are F values ($P < 0.05$).

Lipid	Treatment (d.f.=1)	Time post treatment (d.f.=2)	Treatment-Time Interaction (d.f.=2)
Phospholipid	3.10*	45.25	7.52
Monoacylglycerol	0.80*	288.07	0.50*
Diacylglycerol	0.94*	19.37	9.31
Free Fatty Acids	3.61*	12.11	8.00
Triacylglycerol	2.22*	155.06	2.50*
Sterol ester	14.45	214.62	10.68

* indicates 'Not Significant'.