STUDIES ON THE LIPIDS OF THE BLACK CUTWORM, AGROTIS IPSILON (HUFNAGEL) (LEPIDOPTERA: NOCTUIDAE)

By

SAMY MOSTAFA MOHAMED

B.Sc. Agric. (Biochemistry), 1975

1335.M

THESIS

SUBMITTED IN PARTIAL FULFILMENT
OF THE REQUIREMENTS
FOR THE DEGREE OF (M.Sc.)
Agric. Biochem.

FACULTY OF AGRICULTURE

AIN SHAMS UNIVERSITY

1986

APPROVAL SHEET

Title of Thesis: STUDIES ON THE LIPIDS OF THE BLACK

CUTWORM, AGROTIS IPSILON (HUFNAGEL)

(LEPIDOPTERA: NOCTUIDAE).

Name: - SAMY MOSTAFA MOHAMED

Degree: M.Sc. (Agric. Biochemistry)

Approved By:

Dr./. D. Colons

Dr./. R. El-gelinde

Committee in Charge

Date:



SON MO AT PROPERTY PINTO

The author wishes to express his profound gratitude, deep appreciation and infinite thanks to Prof. Dr. M. F. El-Sharawy, Dean of Faculty of Agriculture, Ain Shams University, for his supervision, guidance and encouragement during the course of this work.

The author wishes to express his profound gratitude, full appreciation to the help of Dr. Z. A. El-Hadidy, Prof. of Biochemistry Ain Shams University for his keen supervision, genius guidance, much valuable advice and his generous help throughout the whole time course of this investigation.

The author is greatly indebted to Dr. I.Z. Boctor,
Professor of Biochemistry, Laboratory of Plant Protection,
National Research Centre, for suggesting and supervising
this study and for her valuable discussions and criticism.

The author wishes to express his gratitude and thanks to Dr. M. Ibrahim, Lecturer of Biochemistry, Fac. of Agr., Ain Shams Univ., for his help during the course of this investigation.

CONTENTS

	Page
I.INTRODUCTION	7
II. REVIEW OF LITERATURE	3
1.Lipids as a source of energy	4
2. Lipids and metamorphosis	4
	6
3.Lipids and reproduction	9
4.Main components of lipids	11
a. Glycerides	1 1
b. Phospholipids	15
c. Sterols	•
d. Waxes	20
	23
e. Fatty acids	26
III. MATERIAIS AND METHODS	37
1. Rearing of the experimental animals	37
2. Moisture content	
	3 8
3. Extraction and separation of lipids	3 9
a. Fractionation, identification and	
quantitative determination of lipids	40
b. Preparation and identification of metnyl	
esters of fatty acids	42
IV. RESULTE	
	45
1. Moisture content	45
2. Fat content	46
3. Lipid classes	48

	Page
a. Lipid classes of Agrotis ipsilon larvae	48
b. Lipid classes of the prepupal and pupal	
stages of Agrotis ipsilon	53
c. Lip1d classes of male and female Agrotis	
moths	56
4. Fatty acids	59
a. Fatty acids of Agrotis ipsilon larvae	59
b. Fatty acids of the prepupal and pupal stages	
of Agrotis	68
c. Fatty acids of male and female moths	78
V. DISCUSSION	83
VI. SUMMARY	98
VII. REFERENCES	102

INTRODUCTION

I. INTRODUCTION

Insects form the most numerous class of living organisms and during their extensive evolutionary history a diversity of morphological, physiological and biochemical adaptations has arisen. Lipids have assumed considerable functional significance during the evolutionary history of the class Insecta. They facilitate water conservation both by the formation of an impermeable cuticular barrier and by yielding metabolic water upon oxidation. Fats, upon oxidation, yield almost two times more metabolic water than carbohydrates, and the storage of energy reserves in the form of fat provides a useful source of nonimbibed water. This additional water assumes particular importance during non-feeding stages in the insect's life history. The advantages of lipid over carbohydrate as a metabolic fuel include a higher caloric content/unit weight of substrate, and the fact that triglyceride may be stored in anhydrous form while glycogen is stored in the bulky hydrated form. Thus the use of lipid as a primary metabolic substrate permits accumulation of a large reservoir of energy which may be used during periods of prolonged energy demand. In insects the gneral energy-consuming processes are growth, metamorphosis, flight, movements, oogenesis and embryogenesis (Gilby, 1965; Gilbert, 1967a). As fats are the most economical energy reserves (Chapman, 1969), they form vital substrates for energy production required by these processes. Insects are not only capable of utilizing ingested lipids and synthesizing fatty acids from acetate (Lambremont, 1965), but are capable also of sonverting the non-lipoidal materials into lipids. The most important of the non-lipoidal components are carbohydrates (Chino and Gilbert, 1965).

Lipids also facilitate the process of embryogenesis by providing an efficient source of metabolic energy and an essential source of precursors for the synthesis of cellular and subcellular membranes (Babcock and Rutschky, 1961). During vitellogenesis appreciable quantities of lipid are deposited in the eggs (Dutkowski and Ziajka, 1972), and in at least some species the source of this yolk lipid is the fat body (Dutkowski and Ziajka, 1970, 1972).

The present work was carried out to investigate in detail the changes that take place during the development and metamorphosis of the lepidopterous greasy cutworm, Agrotis ipsilon (Hufnagel) (Lepidoptera: Noctuidae) concerning the total lipid content, the classes of lipids and the fatty acid composition in whole tissue extracts.

Agrotis ipsilon is one of the most destructive soil pests

in Egypt. It is a serious pest of cotton and other plants. It is a widely spread species all over the cotton cultivating areas and usually infests the early summer crops including essentially the seedlings of cotton, as well as the winter crops including clover, wheat, barley and bean.

Most of the investigations on Agrotis ipsilon dealt with the biology, ecology and toxicology of the pest.

Nonavailiability of any information regarding lipid metabolism of Agrotis ipsilon a lepidopterous and an insect of economic importance in Egypt, induced us to study the lipid changes during various stages of development. It is hoped that the results arrived at may share and pave the way for an effective control of this pest.

REVIEW OF LITERATURE

- 4 -

II- REVIEW OF LITERATURE

Lipids

The study of insect lipids has received increasing attention in recent years, and a number of reviews have appeared on various aspects of the topic (Scoggin and Tauber, 1950; Strong, 1963; Fast, 1964; Gilby, 1965; Gilmour, 1965; Sridhara, 1966; Gilbert, 1967a; Fast, 1970; Gilbert and O'Conner, 1970; Thompson, 1973; Thompson et al., 1973; Turunen, 1974a; Gilbert and Chino, 1974; Svoboda et al., 1975; Downer and Matthews, 1976; Jackson and Blomquist, 1976; Downer, 1978). The lipoidal nature of most growth hormones, pheromones and sex attractants has greatly stimulated the study of lipid biochemistry in insects (Gilbert, 1967a).

1. Lipids as a source of energy

Lipids serve as an important source of fuel for various phases of physiological activity in all organisms. Lipids are also of vital importance to many insects as substrates for embryogenesis, metamorphosis and flight. Many insects can use either (or both) carbohydrate or fat as major fuels for flight (Bailey, 1975). In the flying locust, Schistocerca, about 80% of the energy for flight

is derived from fat (Meyer et al ., 1960) . Glycogen is utilized together with fat at the beginning of flight. whereas during the next several hours of flight fat is the sole source of energy. Adults of several Lepidoptera have been shown to utilize fat for energy in their flight muscles (Domroese and Gilbert, 1964; Sacktor, 1970; Stevenson, 1972) . Indeed, in butterflies and moths, even when glucose was injected into the blood it had to be converted into fat prior to being used for energy production in flight. Thus, it appears that members of the order Lepidoptera are unable to use carbohydrate as a direct fuel for flight and must be converted to fat and this conversion is especially rapid in Noctuidae (Kozhantshikov, 1938 Zebe . 1954; Wigglesworth, 1960; Gilbert & Schneiderman, 1961 a; Domroese & Gilbert, 1964) . Where fat is the major fuel, it is usually stored mainly in the fat body and transported to flight muscles during flight. However, it has been observed that the flight muscles of some Hemiptera (Lethocerus species, Ashurst and Luke, 1968; Aphis fabae Cockbain, 1961; and Rhodnius prolixus, Ward et al ., 1982) contain considerable numbers of lipid droplets. Zebe(1959 a&b) has already suggested that insect flight muscle is of three physiological types, the exclusive carbohydrate users, the

flies, the exclusive lipid users, the moths and butterflies; and that can use both fat and carbohydrate, the locusts. Stevenson (1968) working with Prodenia eridania and found that the small amount of glycogen in newly emerged moth could not sustain flight for a significant period of time. According to the author; the role of carbohydrate metabolism in the flight muscle of this insect, which oxidizes fat for flight energy, is probably to convert ingested carbohydrate to fat for storage. Van Handel (1974) working with Spodoptera frugiperda and Heliothis zea reported that both species use lipids as the flight substrate. After the flight period, about half the lipid content had been used.

2. Lipids and Metemorphosis

There is a wide variation in lipid content of insects of different orders and even within a single family. This is because of the large diversity of insects, their modes of living and varying habitats. In most insects the female contains more lipid than the male, as lipid components are most efficient substrate for egg development. Among Lepidoptera, however, the male moth commonly has a higher lipid content than the female (Vaney and Maignon, 1906;

Yamafuji, 1937; Niemierko et al., 1956; Demyanovsky and Zubova, 1957; Gilbert and Schneiderman,1959). This appears also to hold true in the larval and pupal stages but the sexual dimorphism is greatest in the newly emerged moth. The difference in the quantity of lipid expressed as per cent. fresh weight has been reported to vary from 1% in some Lepidoptera to 50% in the beetle Pachymerus (Niemierko, 1959). There exists some controversy to whether lipid content increases or decreases during metamorphosis, and whether females of any particular species store more lipid than males.

Metemorphosis of holometabolous insects involves
the breakdown of larval tissues and formation of adult
structures. During this stage of development the
animal is dependent upon reserves accumulated during
larval growth for energy and for provision of anabolic
precursors. Lipids and/or carbohydrates may contribute
to the energy expenditure of pupae with the relative
importance of each substrate dependent upon the species
and sex of the animals under investigation (Gilbert, 1967a,
Chen, 1971; Agrell and Lundquist, 1973).