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Lipid Biosynthesis in the Southern Lyctus Beetle, Lyctus Planicollis, Leconte (Coleoptera: Lyctidae): Distribution of Carbon From U-(Carbon-14 Labeled)-Glucose in the Principal Lipid Classes and Fatty Acids During Growth and Development.

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LIPID BIOSYNTHESIS IN THE SOUTHERN LYCTUS BEETLE,
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DISTRIBUTION OF CARBON FROM U\(^{14}\)C-GLUCOSE IN THE PRINCIPAL LIPID CLASSES AND FATTY ACIDS DURING GROWTH AND DEVELOPMENT

A Dissertation

Submitted to the Graduate Faculty of the Louisiana State University and Agricultural and Mechanical College in partial fulfillment of the requirements for the degree of Doctor of Philosophy in The Department of Entomology

by

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B.S., Mississippi State University, 1963
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ABSTRACT

By feeding a diet containing glucose-$^{14}$C to larvae of the southern lyctus beetle, *Lyctus planicollis* LeConte, it was demonstrated that palmitic, palmitoleic, stearic, and oleic acids were synthesized from glucose. Of the radioactivity recovered in the fatty acids, 59 to 64.5% was found in oleic acid, 7 to 17.2% in palmitic acid, 7.2 to 15.1% in palmitoleic, and 1.6 to 3.8% in stearic acid. Radioactivity in these four fatty acids correlated closely with the amounts of each acid present in the insect.

The $^{14}$C activity of palmitic acid was highest (17.2%) in larvae which had fed on the diet for 20 days after hatching and decreased to and remained at 7.8% in 40-, 60-, and 75-day larvae. Furthermore, it remained constant in pupae and adults. The amount of palmitic acid (percent distribution) varied in a manner similar to isotope content. Radioactivity recovered in palmitoleic and oleic acids was lowest in young larvae and increased in older larvae, which was also the same for the percent distribution of these two fatty acids during larval development. These results suggest that the
larvae are capable of synthesizing long chain saturated and mono-unsaturated fatty acids from carbohydrate. Oleic acid is the major fatty acid synthesized. It is the most abundant acid in the insect, and incorporates more $^{14}$C radioactivity than the other fatty acids.

The fatty acids synthesized from $^{14}$C-labeled glucose were incorporated into other lipids, mainly triglycerides. Some radioactivity was also present in phosphatidyl ethanolamine and phosphatidyl choline. Other lipid classes did not contain significant radioactivity.

Oleic acid accounted for 60-70% of the total fatty acids throughout all developmental stages. The other major fatty acids found were palmitic (6.1-15.7%), palmitoleic (5.2-13.7%), stearic (1.4-5.2%), and linoleic (2.8-18.9%). Tridecanoic, myristic, myristoleic, pentadecanoic, and occasionally linolenic acids were detected in smaller amounts. Two unidentified components with a carbon chain length shorter than C$_{13}$ were also detected.

The fatty acid content of the larvae did not depend solely on that of the diet. The diet had no palmitoleic, 20.2% oleic and 51.8% linoleic acids. By contrast, larvae had approximately 10% palmitoleic.
60% oleic and 10% linoleic acids.

The major neutral lipid class in all life stages was triglyceride. Other neutral lipid classes detected were monoglycerides, diglycerides, sterols, free fatty acids, sterol esters, hydrocarbons, and an unidentified compound which had a chromatographic behavior slightly less polar than triglyceride but more polar than sterol esters.

The major phospholipid classes found were phosphatidyl ethanolamine and phosphatidyl choline. These two were present in approximately equal quantities in all life stages. Other phospholipid classes detected were lysophosphatidyl choline, sphingomyelin, phosphatidyl serine, phosphatidic acid, and a compound tentatively identified as phosphatidyl inositol.

Lipids are not depleted during embryonic development. This observation indicates that either lipids are not utilized during this developmental period, or, if utilized, the losses are offset by synthesis from non-lipid constituents. However, lipids are utilized about the time the larvae hatch and undergo their first moult at which time a decrease in total fat takes place. Lipids also appear to be utilized during the transition
from larva to pupa and from pupa to adult as shown by a decrease in total lipid during those periods.

Southern lyctus beetle larvae placed in a "low-fat" diet failed to develop normally, and most died within 40 days. All were dead after 60 days on the "low-fat" diet. Therefore, some lipid components are essential dietary constituents for normal growth, development and maturation.
INTRODUCTION

Early lipid research with insects was principally concerned with nutrition. However, within the last decade, the study of insect lipids has expanded to include at least some research in all areas of lipid metabolism. Investigations of synthesis capabilities, distributional patterns in the whole insect and in isolated organs thereof, possible metabolic and structural relationships of the individual lipid classes, and the possible use of fatty acid distribution patterns in insect taxonomy have been performed and reported. New areas, such as the study of the possible role of ether containing phosphoglycerides, are now receiving attention.

The lipid metabolism of the southern lyctus beetle, Lytus planigollis (Coleoptera: Lyctidae) LeConte, has not been studied previously. The purpose of this investigation was to study the capability of this insect to synthesize various lipids, to correlate changes in lipid content and composition with various developmental stages, and to determine whether dietary lipids are required for normal growth and maturation.
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Early lipid research with insects was principally concerned with nutrition. However, within the last decade, the study of insect lipids has expanded to include at least some research in all areas of lipid metabolism. Investigations of synthesis capabilities, distributional patterns in the whole insect and in isolated organs thereof, possible metabolic and structural relationships of the individual lipid classes, and the possible use of fatty acid distribution patterns in insect taxonomy have been performed and reported. New areas, such as the study of the possible role of ether-containing phosphoglycerides, are now receiving attention.

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Terminology

A precise definition of the term lipid has not yet been unreservedly accepted by workers in the field. However, it is generally agreed that those substances which are not soluble or only sparingly so in water and are extractable with, or soluble in, organic solvents such as chloroform, diethyl ether etc., can be termed "lipids" (Gilbert, 1967).

For purposes of discussion in this dissertation the following lipid classification and abbreviations will be used.

TOTAL LIPIDS (TL) - Those compounds which are extracted from tissues by organic solvents, usually chloroform - methanol (2:1, v/v) according to the method of Folch et al. (1957).

NEUTRAL LIPIDS (NL) - Those lipid classes in the TL which can be eluted from a silicic acid chromatographic column with chloroform. If present in the organism, the following classes would be in this group: free fatty acids (FFA), monoglycerides (MG), \( \alpha, \beta \) and \( \gamma \)
-diglycerides (DG), sterols, triglycerides (TG), sterol esters (SE), and hydrocarbons (HC). The NL are also referred to as the non-polar lipids.

PHOSPHOLIPIDS (PL) - Those lipid classes in the TL which are not eluted from a silicic acid column with chloroform, but are eluted with absolute methanol after the NL have been removed from the column. Lipid classes commonly encountered are: lysophosphatidyl choline (LysPC), lysophosphatidyl ethanolamine (LysPE), sphingomyelin (SM), phosphatidyl choline (lecithin) (PC), phosphatidyl inositol (PI), phosphatidyl serine (PS), phosphatidyl ethanolamine (PE), and phosphatidic acid (PA). Ether containing phosphoglycerides are often found in the PL (Lambremont and Wood, 1968). The PL are referred to as the polar lipids.

The NL consist primarily of glycerol esters of fatty acids. The PL are composed in most cases of glycerol-3-phosphate which is esterified with fatty acids on carbon number 1 or 2 (or both) with the various base groups mentioned above on the phosphoric acid on carbon number 3. Choline and ethanolamine are the two most common groups esterified with the phosphoric acid.
Nutrition: Sterols

It is widely accepted that insects require a dietary source of sterol for normal growth and development (Gilmore, 1961, House, 1962, 1965, Dadd, 1963, Clayton, 1964; Fast, 1964; Gilby, 1965, and Gilbert, 1967). This requirement has been verified repeatedly by the failure of insects to synthesize sterols from simple precursors such as acetate and by abnormal development when sterols are omitted from the diet (Clayton, 1964; Vanderzant and Richardson, 1964; Dadd, 1960b; Hobson, 1935a, b; Lambremont et al., 1966; Ritter and Wientjens, 1967; Ito and Horie, 1966; Ito, 1967; Vanderzant, 1968; Robbins et al., 1960; Sridhara and Bhat, 1964, 1965a; Gilby, 1965, and Earle et al., 1965). In most insects, studies concerning their capability to synthesize sterols have revealed that this ability is lacking. Probably the exceptions are due to the synthetic actions of intestinal symbionts (Clayton, 1964) and possibly to a failure to purify the sterols prior to radioassay to remove labeled non-sterolic contaminants. In all studies reported, a dietary sterol was required for normal growth, development, and reproduction. The in-
ability to synthesize cholesterol has been demonstrated in a lobster, *Homarus gammarus* (L.) (Crustacea), a spider, *Avicularia avicularia* (L.) (Arachnida), and a millepede, *Graphidostreptus tumuliporus* (Karsh) (Myriapoda) (Zandee, 1967).

Cholesterol is the primary sterol of insects (Gilby, 1965; Gilbert, 1967). However, there is ample evidence that other sterols may be utilized or converted to cholesterol (Fast, 1964; Gilbert, 1967; Earle et al., 1967a). Of particular interest in this connection are the reports by Bergmann and Levinson (1958) and Levinson (1962) that strictly phytophagous species of Coleoptera, Diptera, Hemiptera, Hymenoptera, Lepidoptera, Orthoptera, a mollusk, and a decaped all contain cholesterol and 7-dehydrocholesterol. It was postulated that the phytosterols (β-sitosterol, stigmasterol, etc.) are converted to cholesterol or 7-dehydrocholesterol. Schaefer et al. (1965) reported that sterols of the host of the Virginia pine sawfly, *Neodiprion pratti* Dyar are predominantly β-sitosterol (92-94%) with smaller quantities of campesterol (6-7%) and cholesterol (1%) present. However, cholesterol was the predominant (73%) sterol in the developing
larvae. $\beta$-sitosterol accounted for 17% and campesterol for 4% of the larval sterols. Gilbert (1967) reviewed several studies, including work from his laboratory on the cecropia moth, *Hyalophora cecropia* L., dealing with the utilization of sterols by insects and concluded that a whole range of sterols can substitute for, or partially spare, cholesterol in the diet. More recently Martin and Carls (1968) stated that phytophagous insects are generally capable of transforming the phytosterols into cholesterol by removal of the ethyl group from carbon number 24.

According to Gilby (1965), sterols are present in insects as free sterols or sterol esters. That many insects contain free sterols and fatty acid sterol esters is well documented in the review by Gilbert (1967). This has been further documented in the common house cricket, *Acheta domestica* (L.) (Martin and Carls, 1968); the silverfish, *Lebisma saccharina* L. (Kinsella, 1969); the American cockroach, *Periplaneta americana* (L.), (Kinsella, 1966a); the cereal leaf beetle, *Oulema melanopus* (L.), (Lamb and Monroe, 1968b); and the boll weevil, *Anthonomus grandis* (Boheman), (Lambremont et al., 1966; Bumgarner and Lambremont, 1966).
Several possible functions fulfilled by sterols in insects have been suggested. A structural role in membranes is widely accepted (Fast, 1964; Clayton and Lasser, 1964; Goodfellow and Gilbert (1965). Kiriimura et al. (1962) and Kobayashi et al. (1962) concluded that the brain hormone from the silkworm, *Bombyx mori* (L.) is cholesterol or that cholesterol is a major constituent. This role has been seriously questioned (Goodfellow and Gilbert, 1963, 1965). Another possible function of sterols in insects is suggested by their close relationship to the moulting hormone (ecdysone) (Gilbert, 1967).

**Nutrition: Lipids Other than Sterols**

The dietary requirements for lipids other than sterols are not nearly as well understood as they are for sterols. Fraenkel and Blewett (1946) demonstrated that unless linoleic acid was present in the larval diet of the Mediterranean flour moth, *Ephesia kuehniella* (Zeller), the moths failed to emerge. It was also shown that linoleic acid could not be synthesized by these larvae. Similar results were obtained by Vanderzant et al. (1957) for the pink bollworm, *Pectinophora gossypi.*
Linolenic acid was not as effective as linoleic in promoting adult emergence. The desert locust, *Schistocerca gregaria* (Forskal), requires either linoleic or linolenic acid for adult emergence. Migratory locusts, *Locusta migratoria* (L.), are unable to unfold their wings after the final moult unless there is a dietary source of these fatty acids (Dadd 1960a, 1961). Gordon (1959) reported that German cockroach, *Blatella germanica* (L.), females require linoleic acid for the production of normal offspring. Larval growth of the greater wax moth, *Galleria mellonella* (L.) is not affected by the absence of linoleic acid from its diet. However, the adults cannot successfully emerge from the pupal cuticle unless linoleic acid is added to the larval diet (Dadd, 1964).

Boll weevil larvae are able to develop into normal sized adults without fat in the diet, but egg production is reduced unless fat is present in both the larval and adult diets (Vanderzant and Richardson, 1964). This was confirmed by Earle et al. (1967b) who further demonstrated that egg production is highest when the larval and adult diets of both sexes contain a mixture of triglycerides that include trilinolein or trilinolenin.
silkworm also requires some dietary fatty acids (Ito and Nakasone, 1966; Ito 1967) and linoleic and linolenic are most effective in satisfying this requirement. These two fatty acids can replace wheat germ oil in the larval diet of the Egyptian cotton leafworm, *Prodenia litura* F. (Levinson and Navon, 1969). The lack of wheat germ oil in the larval diet caused slower growth and development and the resulting adults were crippled and had scaleless wings. Linolenic acid is practically as effective as linoleic acid in satisfying the fatty acid requirement of the red banded leaf roller, *Argyrotaenia velutinana* Walker (Rock et al., 1965). Chippendale et al. (1964) demonstrated that methyl linolenate effected both good larval growth and normal wing development in the cabbage looper, *Trichoplusia ni* (Hubner).

From these reports, it is evident that at least some species of Lepidoptera, Orthoptera and Coleoptera do require a dietary source of one or both of the polyunsaturated fatty acids, linoleic and linolenic acids. As with sterols, this requirement of fatty acids has been determined by dietary deletion studies and by the inability of these insects to synthesize such re-
quired constituents from radiolabeled acetate or glucose.

The addition of oleic acid to an insect diet has been shown to be markedly beneficial only in the dipterous parasite Agrius (Pseudosarcophaga) affinis (Fallen) (House and Barlow, 1961).

Some insects such as the larvae of the fruit fly, Drosophila melanogaster (Meigen); the confused flour beetle, Tribolium confusum (Jacquelin duVal); the bottle fly, Lucilia sericata (Meigen); and the dipterous parasite, Pseudosarcophaga (Agrius) affinis (Fallen) are able to survive on a lipid-free diet, but require dietary lipid for optimal growth (Scoggin and Tauber 1950). No dietary fatty acids are required by the muscid flies and, in fact, they may be detrimental (Silverman and Levinson, 1954, Levinson and Bergman, 1957; Brookes and Fraenkel, 1958). In working with another muscid, Musca domestica L., Barlow (1966) reported that he found no evidence that dietary fatty acids accelerated growth or development.

According to House (1965), most insects can probably synthesize all the required fatty acids, but a few species do require dietary sources of the unsaturated fatty acids, linoleic and linolenic.
Lecithin has a growth promoting effect in several insect species, but this is probably because lecithin contains choline, a vitamin essential in the diet of insects (House, 1965).

**Synthesis of Fatty Acids**

Both carbohydrates and proteins may be used by insects to synthesize fatty acids (Fast, 1964; Gilbert, 1967). According to Gilbert (1967) there may be two systems for the biosynthesis of fatty acids. One system is cytoplasmic and involves successive condensation of two-carbon fragments (acetyl Coenzyme A or acetyl CoA) to produce palmitic acid (Wakil, 1961, 1963; Wakil et al., 1964). The second system is essentially a reversal of β-oxidation and is mitochondrial. This system is concerned primarily with elongation of existing fatty acids by two-carbon units (e.g., C16 would be elongated to C18). There is evidence that both these systems occur in insects.

Zebe and McShan (1959) demonstrated *in vitro* that the fat body of the adult southern armyworm, *Prodenia eridania* (Cramer), incorporated acetate into
long chain fatty acids. The cofactors required for maximum incorporation were malonate, ATP, CoA, and a reducing agent such as glutathione or cysteine. The primary fatty acid produced was palmitic acid. Smaller amounts of stearic, oleic, myristic, and lauric acids also were produced. In a similar study Teitz (1961) found that cell free preparations of adult migratory locusts incorporated acetate into fatty acids. Cofactors required for maximum incorporation were ATP, CoA, glutathione, Mg²⁺, NADP, malonate, α-ketoglutarate, and KHCO₃. Again the major fatty acid synthesized was palmitic acid. Tietz-Devir (1963) studied homogenates and sub-cellular fractions of locust fat body. Synthesis of fatty acids was optimal in the presence of supplemental ATP, MgCl₂, glutathione, KHCO₃, and malonate. When the particulate matter was removed by centrifugation, the supernatant was as effective in fatty acid synthesis as the original homogenate. Over 50% of the newly synthesized fatty acid was palmitic acid. These reports show that insects synthesize long chain fatty acids from acetate as do both lower (microorganisms) and higher organisms (vertebrates) (Gilbert, 1967).
Numerous studies concerning the incorporation of radioactive carbon from acetate or glucose into fatty acids have been published. No attempt has been made to include all the reports. Instead, only those reports dealing with fatty acid synthesis in those insects that are known to require the polyunsaturated fatty acids in their diet will be discussed. In these insects, one or both of the 18 carbon polyunsaturated fatty acids have been demonstrated to be essential nutrients by the dietary deletion method.

Horie et al. (1968) injected glucose-U-14C into silkworm larvae. Palmitic and oleic acids were readily synthesized and there was a slight incorporation into palmitoleic and stearic acids. However, there was no incorporation of radioactivity into either linoleic or linolenic acids. This observation confirmed earlier work by Sridhara and Bhat (1964, 1965a, b).

Lambremont et al. (1965, 1966) fed boll weevil larvae a diet containing 14C labeled glucose and its related glycolytic products, pyruvate and acetate. All of these were utilized as fatty acid precursors. Palmitic, palmitoleic, stearic, and oleic acids contained significant radioactivity. However, neither linoleic
nor linolenic acid contained significant radioactivity. Most of the recovered radioactivity was found in palmitic (30%) and oleic (37%) acids.

Nelson and Sukkestad (1968) reared larvae of the cabbage looper on a synthetic diet containing $^{14}$C-acetate. Of the radioactivity incorporated into the fatty acids, 40% was in palmitic and 44% in oleic acids. Palmitoleic and stearic acids were also labeled, but the 18 carbon polyunsaturated acids were not.

In these three insects, the dietary requirement for linoleic or linolenic acid (or both) was confirmed by the inability of the insects to synthesize these compounds from normal precursors. This method of confirming nutritionally essential components is referred to as the synthesis capability method. In situations where no well defined diet is available and a lipid requirement is evident, a study of synthesis capability should indicate which fatty acids are essential, if any. This method has been used to confirm the amino acid requirements of several species of insects. Some insects for which such amino acid work has been done are the green peach aphid, Myzus persicae (Sulzer) (Strong and Sakamoto, 1963), Rhodius
prolixus (Stal) (Pickett and Friend, 1965); and the spotted lady beetle, Coleomegilla maculata DeGeer (Atallah and Killebrew, 1967).

Distribution of Lipids in Insects During Development

Lipid content (TL) of insects varies widely from species to species, with the developmental stage, sex, physiological state, and to a certain degree with the diet (Fast, 1964; Gilbert, 1967). This variation holds true for the amount of the TL accounted for as NL and PL. Further, the distribution of the various lipid classes within the NL and PL fractions has been found to vary considerably as has the percent distribution of fatty acids. In addition, twenty-six reports appearing since these reviews (or not mentioned by them) confirm that variation occurs in one or more of the lipid fractions mentioned above.

In the order Coleoptera the TL composition on a wet weight basis varies from 1.5 to 33.2% (Fast, 1964; Gilbert, 1967). In most instances oleic acid is the predominant fatty acid, and the unsaturated C\textsubscript{18} fatty acids account for a large percentage of the total fatty
acid. For example, results obtained with the adult boll weevil (Lambremont and Bennett 1966) and the cereal leaf beetle (Lamb and Monroe, 1968a) showed that oleic acid was the predominant fatty acid and that the unsaturated C18 fatty acids accounted for over 50% of the total amount of fatty acids.

The predominant NL in insects is the TG (Gilmore, 1961; Gilby, 1965). In some insects high proportions of FFA have been reported, but many workers consider these to be artifacts produced by accidental TG hydrolysis during lipid extraction (Kilby, 1963). TG constitute such a large percentage of the TL that the fatty acid content of the TL very closely approximates that of the TG (Gilby, 1965). The predominance of TG has been well documented in papers appearing since Gilby's review (Martin, 1969; Bumgarner and Lambremont, 1966; Kinsella, 1969 and others).

In the PL, PC is either predominant or roughly equal to PE in all insects except the Diptera and aphids in which PE is predominant (Fast, 1964, 1966; Gilby, 1965). This observation is supported by data obtained from studies on the dipterans Lucilia (D'Costa and Birt, 1966) and the
flesh fly, *Sarcophaga bullata* (Allen and Newburgh, 1965), and in other insects such as the cecropia moth (Thomas and Gilbert, 1967); the cricket, *Gryllus bimaculata* (DeGeer) (Fast, 1967), and the Indian meal moth, *Plodia interpunctella* (Hubner) (Yurkiewicz, 1967).

Lipids are present in all stages of insect development and it is probable that these lipids are metabolically involved. Needham (1931) proposed the theory that all embryos utilize reserve material in the egg yolk in a definite order. According to his theory, although no single substrate is being catabolized at any one time, carbohydrates are generally utilized first, then protein and finally fat. From the review by Babcock and Rutschky (1961) it is evident that data obtained with insect eggs supported Needham's theory. Two coleopterous insects, the Colorado potato beetle, *Leptinotarsa decemlineata* (Say) and the Japanese beetle, *Popillia japonica* Newman, were included in this review. These authors concluded from their own studies that lipids do not play as dominant a role in embryonic development of the large milkweed bug, *Oncopeltus fasciatus* (Dallas) as reported for other insect eggs. There was only a 11.5% decrease in lipids.
during embryonic development. Fast (1964) states that it is generally accepted that the energy required for embryonic development is obtained from fatty acids. Gilbert (1967) demonstrated that in eggs of the Madeira cockroach, *Leucophaea maderae* (F.), the NL fraction decreases and the PL fraction increases during development. It has been speculated that the TG are being used for energy production and the PL may be used for the formation of membranes and organelles of the cell (Gilbert, 1967).

Allais et al. (1964) showed that the TL in the eggs of *Locusta migratoria migratorioides* L. decrease by 31.2% during development of the embryo. The glycerides decreased and the PL increased as they did in the cockroach. The PL of the American cockroach ootheca increased from 6 to 24% of the TL during development (Kinsella, 1966b). Similar results with both the NL and PL were reported for the eggs of the Indian meal moth, *Plodia interpunctella* (Yurkiewicz and Oelsner, 1969). Apparently lipids are involved in energy production in the developing embryo of these insects.

Holometabolous insects tend to accumulate large quantities of fat during the larval stage (Fast, 1964).
The specific fatty acids accumulated vary with the insect species and to a certain extent with the diet. Young silkworm larvae contained more stearic and linoleic acids and less palmitic and oleic acids than older larvae (Nakasone and Ito, 1967). In *S. bullata* most of the lipid was deposited during the last few larval instars (Sun and Brookes, 1968).

As Gilby (1965) noted, the variations in the changes in PL content during the life cycle of insects make it impossible to generalize.

TG, which are abundant in insect tissues, seem to function primarily as an energy store. Such a function during embryonic development has already been discussed. A similar role for TG has been demonstrated in the diapause of the boll weevil (Lambremont et al., 1964), and in the khapra beetle, *Trogoderma Granarium* Everts, (Karnavar and Nair, 1969). Fatty acid oxidation has also been shown to supply much of the energy necessary for flight, particularly in locust and moths (Gilbert, 1967). TG are probably also involved in production of energy for metamorphosis (Pearincott, 1960; Villeneuve and Lémonde, 1963).
METHODS AND MATERIALS

Rearing

A laboratory culture of southern lyctus beetles was maintained at approximately 27°C and 50% relative humidity on dried and debarked pecan wood. All the eggs analyzed, as well as those that were held to obtain newly hatched larvae, were collected from adults from this culture. Larvae used in these experiments were reared on an artificial diet which consisted of the following substances:

- Whole wheat flour 500 g
- Corn meal (Masa Harina)\(^R\) 400 g
- Bacto yeast extract 96 g
- Methyl-p-hydroxy benzoate 2 g
- Ascorbic acid 2 g

This diet plus approximately 240 microcuries (\(\mu\text{Ci}\)) of glucose-\(\text{U}^{14}\text{C}\) per 1000 g diet was mixed overnight in a ball mill. Southern lyctus beetles have been reared through four generations utilizing this diet with no apparent abnormalities.
Chemicals

The glucose-$U^{-14}$C was certified and stated to be 99% pure by the manufacturer (California Biochemical Company) and was not further purified before use. All solvents were suitable for chromatography and were used without further purification. The boiling point range for the petroleum ether was 20-40°C. Lipid standards were purchased from the Hormel Institute (Austin, Minn.); Applied Science Laboratories (State College, Pa.) and Sigma Chemical Company (St. Louis, Mo.), and standard methyl esters of fatty acids were supplied by the Metabolism Study section, National Institutes of Health, Bethesda, Maryland, through the courtesy of Dr. William H. Coldwater.

Collection of Insect Tissue

Using the following procedure, 20,000 eggs were collected from specially prepared corks for subsequent analysis for each of the 8 days (0 through 7) before hatching. Corks, one inch long, with a nail driven through the center long axis and a pencil mark down one
side were cut into 1/8 in. thick discs, soaked in a sugar, starch, and water solution (15 : 15 : 270, w/w/v) for 2 hr, then dried at room temperature. As the corks were needed, they were realigned by the pencil mark and placed inside an inverted wide-mouth quart jar along with 500 to 1000 adult beetles. The females inserted their ovipositor into the slits of the cork and deposited their eggs. After 24 hr the corks were removed and held at approximately 27°C until the eggs were collected.

Zero (0)-day eggs were collected immediately after the adults were removed from the corks by placing the individual discs under a binocular microscope (10x magnification) and transferring the eggs to a chloroform - methanol solution (2 : 1, v/v). All samples were stored at -20°C in this solution. From 1 to 7 days after removal of adults from the corks, collections were made for the 1-day, 2-day, etc. eggs in similar manner. Twenty thousand first instar larvae (8 days after removal of adults from the corks) were collected in a similar manner. Two replicates of each age group of eggs and first instar larvae were collected for lipid analysis. The wet weight of 125 eggs was taken for each age by using an electrobalance.
First stage larvae were similarly weighed.

Thirty first instar larvae were collected as described above, but were placed in a 1 oz plastic cup along with approximately 22 g of glucose-\textsuperscript{14}C labeled artificial diet. Approximately 45 such cups were prepared as a group on 22 occasions. Twelve groups (45 cups each) were examined and the larvae removed on 30 and 40 mesh sieves after the larvae had fed on the diet for 20 days. The larvae from 6 of these groups were considered as one replicate and those from the remaining 6 groups were considered as another replicate. As the larvae were collected they were weighed and stored in chloroform - methanol at -20° C. These larvae are referred to as 20-day larvae. Three groups were removed on a 20 mesh sieve after the larvae had fed on the diet for 40 days. In this instance each group comprised a replicate. The larvae were weighed and stored as above and are referred to a 40-day larvae.

Three groups were examined 60-61 days after the larvae were placed in the diet. Approximately 45% of the insects collected at this time were pupae; the remainder were larvae. As with the 40-day larvae, each group was considered as a replicate. Each replicate of the larvae and pupae
were weighed and stored as above. These are referred to as 60-day pupae and 60-day larvae. Three more groups were held until several adults were evident in the cups (approximately 75 days) before examination. Enough adults and larvae were collected from each group to constitute three replicates of each. These were collected and stored as above and are referred to as 75-day adults and 75-day larvae.

One group (45 cups) was examined after the larvae had fed for 20 days, but the larvae were returned to the diet after an additional quantity (approximately 500 μCi) of glucose-\(^{14}\)C was added to the diet. These larvae were then removed on a 20 mesh sieve after feeding a total of 40 days on the diet (including the first 20 day period).

Five g samples of two different preparations of diet were removed for lipid analysis.

Lipid Extraction and Analysis

Each insect sample was removed from the freezer and homogenized in the 2 : 1 chloroform – methanol solution in which it had been stored. Chloroform – methanol solution was added to the diet samples and the various samples were
individually homogenized 3 times in a ground glass tissue homogenizer. Preparation of the TL extract followed a slight modification of the methods of Folch et al. (1957) and Lambremont et al. (1966). The water soluble materials were removed from the chloroform - methanol extract and phase separated with double-distilled water instead of an aqueous 0.03% magnesium chloride solution. The final TL extract was stored in 25 ml of chloroform rather than in n-hexane. Triplicate 25 μl aliquots were pipetted into tared aluminum electrobalance cups, the chloroform was evaporated in a vacuum dessicator, and the weights were recorded on a Cahn R electrobalance. Those weighed samples from radioactive TL extracts were saved for radioassay. One ml of the radioactive TL extracts was also used for radioassay.

Known amounts of TL were separated into NL and PL according to the method used by Wood and Snyder (1968). This method involves a small column of silicic acid (Biorad -325 mesh) from which NL are eluted with chloroform and PL with methanol. Both the NL and PL were brought to a final volume of 2 ml in chloroform, and aliquots were removed for weighing and subsequent radio-
assay as were the TL. Since the amount of radioactivity in the PL was extremely low, 0.5 ml aliquots of the 2 ml fractions were removed for radioassay.

The lipid classes present in the NL and PL fractions were determined by thin layer chromatography (TLC) according to the method utilized by Lambremont and Graves (1969) except that the second solvent system for NL was petroleum ether-diethyl ether-glacial acetic acid (84:15:1, v/v/v) and that for the PL was chloroform-methanol-glacial acetic acid-saline (50:25:8:4, v/v/v/v). As described by these authors, the lipid spots were made visible in an iodine chamber, and the areas were marked on one of two lanes developed for each radioactive sample and these marked areas were subsequently scraped into scintillation counting vials for radioassay. Appropriate known NL and PL standards were developed along with each thin layer chromatogram. Identification of the nitrogen-containing PL was confirmed by spraying with a ninhydrin solution (Skidmore and Entenman, 1962). The choline-containing PL were further identified by spraying with Dragendorf reagent (Skidmore and Entenman, 1962). For identification and documentation purposes the thin layer chromatograms of
nonradioactive samples and the unscraped lanes of the radioactive samples were sprayed with concentrated sulfuric acid and charred in an oven at 160°C. All TLC were documented by Polaroid photography.

Known quantities of the TL samples were saponified according to the method of James (1960) and methylated by a modification of the procedure of Lambremont and Blum (1963). The fatty acids liberated by saponification and subsequent acidification were methylated by heating them at 65°C for 2 hr in a mixture of 10 ml methanol, 1 ml 2,2-dimethoxypropane, and 1 ml concentrated sulfuric acid. The fatty acid methyl esters were extracted, dried and stored in n-hexane until analysis by gas liquid chromatography (GLC).

The fatty acid methyl esters were separated and analyzed by GLC. The conditions for GLC were similar to those used by Lambremont et al. (1965). The 16 ft. x ¼ in. stainless steel column was packed with 20% diethylene glycol adipate and 2% phosphoric acid coated on acid washed chromosorb-W (60-80 mesh). The temperatures were 245°C for the injector, 195°C for the column and 225°C for the detector. Helium at a flow rate of 75 ml
per hr was the carrier gas and a hydrogen flame ionization detector was used. A stream splitter inserted before the detector was used to divert approximately 90% of the fatty acids around the hydrogen flame to an outlet where glass capillary tubes were used to collect the fatty acid methyl esters and the eluate between fatty acid peaks. The capillaries containing the GLC fractions were immediately placed in liquid scintillation counting vials containing scintillation solution.

The percentage distribution of the fatty acids was determined by the triangulation method. Identification of the fatty acids was by comparison of retention times with those of authentic standards. These identifications were confirmed by plotting the log of the retention time versus the carbon number (James, 1959). Identification of the unsaturated fatty acids was further supported by analyzing samples after catalytic hydrogenation. This method involved the addition of platinum oxide to a hexane solution of the methyl esters, bubbling hydrogen through the mixture for about 15 min and then allowing the mixture to remain under hydrogen for at least 2 hr (Lambremont 1969, personal communication).
All the radioassays were made with a Beckman LS-250 liquid scintillation spectrometer. For all counts except those involving TLC scrapings the scintillation mixture consisted of 15 ml of a solution containing 3 g of 2,5-diphenyloxazole and 100 mg of 1,5-bis-2(5-phenyloxazolyl)-benzene per 1000 ml of toluene. For TLC scrapings, the scintillator was the BBOT-naphthalene-ethylene glycol monomethylether-toluene-water mixture described by Snyder (1968).

All counts were taken for sufficient time to keep the error below ±3%. Appropriate corrections were made for background radiation and counting efficiency by counting blanks and carefully calibrated $^{14}$C-benzoic acid standards in scintillation solution. The automatic quench correction mechanism was used during all counting to correct counting efficiency variations due to quenching.
RESULTS

Total Lipid Content During Embryonic Development

Data concerning changes in TL content of the developing embryo are presented in Tables I and II. During the first 3 days of embryonic development there was a decrease in TL content of the eggs. As a percentage this decrease was from 22.0 to 19.8% (Table I) and on a ug/egg basis the decrease was from 2.44 ug to 2.08 ug/egg (Table II). This represents a 14.8% decrease in TL. However, it appears that there was an increase in TL to higher levels in the later embryonic stages as indicated by the higher lipid content (24.9% or 2.56 ug/egg) in the 7-day eggs than in the 0-day eggs (22.0% or 2.44 ug/egg). The high percentages of TL in the 4-day and 6-day larvae are questionable. In both instances results from one replicate were comparable to results from the 7-day eggs (24.9% TL) and TL content in the other replicate was considerable higher (34.8% for 4-day and 34.6% for the 6-day eggs). In the 5-day eggs, TL content of one replicate was comparable (24.1%) to that of the 7-day eggs and the other replicate had a low TL content (17.5%).
The data (Tables I and II) show that there is a decrease in TL about the time the larvae hatch and undergo their first moult. This decrease is from 24.9% to 17.9% TL and from 2.56 ug to 2.18 ug TL per egg and first instar larva respectively, and represents a 14.8% decrease in TL/egg.

Neutral Lipid and Phospholipid Content During Embryonic Development

The distribution of the NL and PL is also presented in Tables I and II. As with the TL, the NL and PL decreased through the first 3 days of embryonic development. This is shown by the decrease in NL and PL from 2.35 ug and 0.30 ug/egg respectively in the 0-day eggs to 2.03 ug and 0.17 ug/egg respectively in the 3 day eggs. This represents a loss of 0.32 ug of NL and 0.13 ug of PL. On a percent basis it is evident that the NL content decreased very little (i.e. from 21.2 to 20.5) during the entire embryonic period (Table I). On a weight basis this NL decrease was from 2.35 ug/0-day egg to 2.11 ug/7-day egg (Table II) and this represents a 10.2% decrease.
The PL increased on a percent basis from 2.7 to 3.2% (Table I) and on a weight basis from 0.30 ug/0-day egg to 0.33 ug/7-day egg (Table II). This 0.03 ug/egg increase in PL represents a 10% increase.

As stated earlier, there seemed to be an increase on a percent basis in the TL during embryonic development. However, when the amounts of TL recovered in the NL and PL are summed (2.65 ug/0-day egg and 2.44 ug/7-day egg) there appeared to be a slight (7.9%) decrease in the TL content. Therefore, no consistent changes in TL, NL and PL were apparent during embryonic development.

The decrease in TL during hatching and the first larval moult is reflected in a decrease in both the NL from 2.11 ug to 1.28 ug per egg and first instar larva respectively and the PL from 0.33 ug to 0.15 ug per egg and first instar larva respectively (Table II). This decrease may not be as large as these weights indicate, since only 66.3% of the TL was recovered in the NL and PL of the first instar larvae.

The results of TLC analysis of NL during embryonic development are illustrated in Figure 1. The predominant NL class throughout embryonic development as well as the
entire life cycle was the TG (Figure 1, e). From the intensity and area of the charred spots on the thin layer chromatograms (See Figure 1), it is estimated that TG account for at least 85% of the NL fraction. Other classes present in the NL were MG (Figure 1, b) DG (b and c), FFA (d), SE (g), HC (h), sterols (c), and an unidentified compound (f).

This unidentified spot is located immediately above the TG in the TLC system used. Since glyceryl ether diesters are known to run in this position in the TLC system used, the NL fraction was examined for the presence or absence of an ether bond according to the method of Wood and Snyder (1968). This method involved hydrogenolysis of carboxylate and phosphate esters in the NL sample with lithium aluminum hydride (LiAlH₄) and subsequent TLC of the glyceryl ether (Lambremont and Wood, 1968). No compounds containing ether bonds were detected in the NL.

Methyl esters of fatty acids are also known to have a chromatographic position just above TG in the TLC system used. Therefore, 2 TLC plates were streaked with about 4 mg NL each and developed. The unknown spot was made visible in an iodine chamber, marked and held at room tempera-
ture until all the iodine color had disappeared. The unknown was then scraped into a beaker, and extracted from the silica gel with chloroform - methanol (2 : 1). The chloroform - methanol was evaporated and the unknown was examined by GLC. No GLC peak was obtained. Therefore, it is probable that the unknown spot is not a fatty acid methyl ester and it therefore remains unidentified.

The TLC of the PL is illustrated in Figure 2. The predominant PL classes in the eggs were PE (Figure 2, g) and PC (d). Other PL classes present were LysPC (b), SM (c), PI (e), PS (f) and PA (h). Based on the intensity and area of the spots, it appears that PE and PC are the largest components and are about equal in content.

Fatty Acid Distribution During Embryonic Development

The elution sequence and separation of the fatty acid methyl esters from a lipid sample from 40 day larvae and an authentic mixture are illustrated in Figure 3. The fatty acids present as well as their percent distribution in the developing embryo are shown in Table III. The major fatty acids detected were palmitic, palmitoleic,
stearic, oleic, and linoleic acids. Other fatty acids identified were tridecanoic (C\textsubscript{13}), myristic, myristoleic?, and pentadecanoic acids. The C\textsubscript{18} unsaturated fatty acids accounted for approximately 67% of the total fatty acids. Stearic acid accounted for approximately 2.8% of the total fatty acids. Palmitic and Palmitoleic acids each accounted for about 8.9% of the total fatty acids.

Confirmation of the unsaturated fatty acids, palmitoleic and linoleic, by the hydrogenation procedure is illustrated in Table IV. The identification of myristoleic acid (C\textsubscript{14}:1) must remain questionable, but there was a consistent decrease in this peak and an increase in the myristic acid (C\textsubscript{14}) peak after hydrogenation. Also there was a consistent decrease in the tentatively identified pentadecanoic acid (C\textsubscript{15}) peak after hydrogenation which indicates the presence of some unsaturated fatty acid. Other than these discrepancies, the unsaturated components were quantitatively converted to their saturated counterparts. That is, the percent distribution of palmitic acid (C\textsubscript{16}) increased by the same amount that palmitoleic (C\textsubscript{16}:1) decreased, and the increase in the percent distribution of stearic (C\textsubscript{18}) is equal to the
sum of the decreases in oleic ($C_{18:1}$) and linoleic acids (Table III). Two small peaks ($?-1, ?-2$ in Figure 5B) of carbon chain length shorter than $C_{13}$ remain unidentified.

There was relatively little change in the percent distribution of fatty acids during embryonic development (Table III). In the 5-day eggs, results from one replicate were very similar to that of the 4- and 6-day eggs, but the other replicate was low in oleic acid and high in short chain ($C_{14}$) components. Apparently this replicate was treated improperly at some stage of analysis, and some of the fatty acids decomposed to the short chain components.

An interesting point about the data in Table III is the high content of palmitic and stearic acids in the 7-day eggs. About 40% of the eggs had hatched at this time and it appears that fatty acid synthesis had begun. It appears that palmitic and stearic acids in the 7-day eggs were converted to oleic acid during the 24 hr before the first instar larvae were collected.
Total Lipid Content During Development from Larvae to Adults

A summary of the TL content of larvae, pupae and adults is presented on a percent basis in Table V and on a weight basis in Table VI.

On a percent basis (Table V) it would appear that the larvae utilize some lipids during the first 20 days of growth. However, on a weight basis (Table VI), it is clearly evident that TL are increasing during this time. Apparently some other component is increasing more rapidly than lipids during this time and as a result the percent TL content appears to decrease.

The increase in TL during larval development is evident from the increase from 17.9% in first instar larvae to 18.9% in the 75-day larvae (Table V). However, the magnitude of this increase is best shown by the increase from 2.2 µg to 665.0 µg TL per larvae during development (Table VI).

Since the magnitude of the increase in TL content of the southern lyctus beetle larvae was so large, an attempt was made to determine whether these larvae
followed the "heterogonic" of "heterauxetic" growth pattern reported for 2 other species of Coleoptera, the yellow mealworm, *Tenebrio molitor* (L.) (Finkel, 1948) and the Japanese beetle (Battista, 1954) and for several other species of insects (Fast, 1964). Heterauxetic growth is based on the parabola $y = bx^k$, in which $y$ represents the magnitude of the component part (lipids in this study), $x$ represents the total organism (wet weight in this study), and $b$ and $k$ are constants. In logarithmic form this equation becomes $\log y = \log b + k \log x$. If, when the data are plotted on double log paper, the points fall along a reasonably straight line, growth is said to be heterauxetic (Finkel, 1948). The value of $k$ is obtained from the slope of the line and, therefore, indicates whether the ratio of the rate of increase of the component part (TL) is greater than ($k > 1.00$), equal to ($k = 1.00$), or less than ($k < 1.00$) that of the whole (wet weight). The constant $b$ gives the elevation of the line. The heterauxetic constant means that an increase of 1% in $x$ (wet weight) is associated with an increase of $k\%$ in $y$ (TL).

A coordinate plot of the TL content per larva
against the wet weight per larvae is shown in Figure 4. A
parabolic curve was obtained. When the same data were
plotted on a double log grid the results were as shown in
Figure 5. A reasonably straight line was obtained, and
therefore, it is concluded that these larvae follow the
heterauxetic growth pattern. The value of \( k \) calculated
from this line was found to be 1.41 which indicates that
the TL accumulate more rapidly than wet weight.

As evidenced by the decrease in TL content from
mature larvae (18.9%) to the pupal stage (15.1%) (Table
V), there appeared to be some lipid utilization during
this metamorphosis. This decrease is even more evident
when it is shown that TL decreased from 665.0 \( \mu g \) per
mature larva to 482 \( \mu g \) per pupa (Table VI). This de-
crease could be due in part to lipid utilization during
the pupal stage, since there was a cross section of all
pupal ages represented in these pupal samples.

There was a further decrease in TL content as the
pupae reached the adult stage. On a percent basis this
decrease was from 15.1% in the pupae to 13.8% in the
adults (Table V). On a weight basis this decrease was
from 482.0 \( \mu g \) per pupa to 253.0 \( \mu g \) per adult. Evident-
ly lipids were utilized in this pupal to adult metamor-
phosis. This decrease in lipid content could be due in
part to utilization by the young adults since some of the
adults remained in the cups for a few days before being
collected.

In order to determine whether southern lyctus beetle
larvae could develop normally on a "low-fat" diet, 1000 g
of the diet were extracted 3 times in chloroform - methan-
ol (2 : 1, v/v) in a WaringR blender and then dried at
room temperature. First instar larvae were placed on this
diet as outlined earlier for the normal diet. After 40
days on this "low-fat" diet, only six extremely small lar-
vae out of 120 were recovered. Using normal diet, after
40 days 50.2% of the larvae were recovered and they weigh-
ed 1.5 mg each. After 60 days on the "low-fat" diet, out
of 120 larvae placed on the diet none were recovered.
Normally after 60 days pupae are abundant. Therefore, it
is assumed that some lipids are required for normal devel-
opment of this insect.
Neutral Lipid and Phospholipid Content During Development from Larvae to Adults

The percent content of NL and PL in larvae, pupae and adults is shown in Table V and the ug NL and PL per insect is presented in Table VI. On a percent basis it appears that the NL increased and the PL decreased during larval development (Table V). However, on a weight basis, (Table VI) it is evident that the content of both the NL and PL increased, but that the NL increased more rapidly than did the PL. The NL increased by a factor of 477.8 and the PL by a factor of 176.0.

The NL decreased, as did the TL, during the metamorphosis from mature larvae to pupae. On a percent basis this decrease does not seem very significant (i.e. from 17.6% in the mature larvae to 13.3% in the pupae) (Table V). However, the decrease was substantial on a weight basis (i.e. from 621.1 ug per larva to 425.6 ug per pupa) (Table VI). During this metamorphosis the PL content remained rather constant both on a percent basis (Table V) and on a weight basis (Table VI). The NL appear to be utilized during the larval to pupal
transformation, while the PL are conserved. As mentioned earlier for the TL, the decrease in the NL and the increase in PL during this period could be due to pupal metabolism.

During the pupal to adult metamorphosis there was a further decrease in the NL and in this instance, a decrease in the PL also (Table VI). This decrease could be due to lipid utilization during the metamorphosis and during the first few days of the adult stage (or both) as mentioned earlier for the TL.

TLC of the larval, pupal, and adult NL are illustrated in Figure 1. As previously stated, the TG are the predominant NL class in all life stages. Other NL classes present in the larvae and adults were the MG, FFA, SE, HC, sterols, and an unidentified compound. These same lipid classes were present in the pupae except that the unidentified compound was absent (see lane 9 of Figure 1).

TLC of the larval, pupal, and adult PL are illustrated in Figure 2. As in the eggs, the predominant PL were PE and PC. Other PL classes present were LysPC, SM, PS, PI, and PA.

The NL and PL classes present seemed to be rather constant in all life stages. Even though the individual
lipid classes were not quantitated, it was evident from the TLC results that there was no obvious qualitative changes in any one lipid class as compared to the others. One exception was the absence of the unknown compound in the NL of the pupae.

Fatty Acid Content in the Diet and During Development from Larvae to Adults.

The percent distribution of fatty acids in the larvae, pupae, adults, and the artificial diet is presented in Table VII. Nine fatty acids were detected in the diet, namely tridecanoic (C\(_{13}\)), myristic (C\(_{14}\)), myristoleic (C\(_{14:1}\)), pentadecanoic (C\(_{15}\)), palmitic (C\(_{16}\)), palmitoleic (C\(_{16:1}\)), stearic (C\(_{18}\)), oleic (C\(_{18:1}\)), and linoleic (C\(_{18:2}\)) acids. The two unidentified peaks were also present in the diet. Linoleic was the predominant fatty acid, accounting for 51.8% of the total. Oleic acid accounted for 29.2% and palmitic acid for 12.3% of the total. Stearic acid comprised only 1.7% of the total and there was only a trace of palmitoleic acid.

The same fatty acids found in the diet were also detected in the larvae, but the percent fatty acid distribu-
tion was quite different (Table VII). Oleic was the predominant fatty acid in the larvae, comprising approximately 61% of the total. Approximate distribution of the other major fatty acids in the larvae was: linoleic acid, 10.4%, palmitoleic acid, 10.3%, palmitic acid, 9.0% and stearic acid, 2.5%. Occasionally a trace of linolenic acid was detected in the larval, pupal, and adult samples. Apparently the fatty acid content of the larvae is not solely dependent on that of the diet. This diet is low in fat content (2.4% TL) and these results agree with those reported by Bumgarner and Lambremont (1966) for boll weevils. These workers showed that boll weevils feeding on a high fat diet had a fatty acid composition dependent on that of the diet. When a low fat diet was given to the boll weevils, their fatty acid composition was dependent on the fatty acids synthesized by these insects.

During the first 20 days on the diet, there was a decrease in the percent of oleic and palmitoleic acids, and an increase in the percent of palmitic and linoleic in the larvae (Table VII). As mentioned earlier, the TL increases substantially during this period and it is probable that all these fatty acids are increasing on a
weight basis, with palmitic and linoleic acids increasing more rapidly than oleic and palmitoleic acid.

After the first 20 days on the diet, there was a gradual decrease in linoleic acid and a gradual increase in palmitoleic acid in the larvae (Table VII). Oleic acid also increased, but not gradually. Instead, the percent of oleic acid increased to about 61% during the second 20 days on the diet and stayed at this level throughout the remainder of the larval development.

The fatty acids present in the pupae and adults were the same as those identified earlier for the eggs, diet and larvae. The percent fatty acid distribution was essentially the same in pupae and adults as that for the larvae (Table VII).

As stated earlier, there was a significant decrease in TL during the metamorphosis from mature larvae to pupae, and a further decrease during the metamorphosis from pupae to adults. Therefore, it seems that the fatty acids are utilized without discrimination as to chain length or unsaturation, since their percent distribution did not change in any consistent or significant manner during these changes.
Incorporation of Glucose-$^{14}$C into Total Lipid, Neutral Lipid and Phospholipid of Larvae, Pupae and Adults

The specific activities in disintegration per minute (or dpm) per mg TL for the lipid samples of larvae, pupae and adults and the distribution of the $^{14}$C activity to the NL and PL are presented in Table VIII. These TL specific activities are probably not very meaningful, since it is not known how much of the glucose-$^{14}$C was ingested by the larvae. However, it is interesting to note that the percent of the TL radioactivity found in the NL and PL (Table VIII) closely approximates that of the TL weight distributed to the NL and PL (Table V). For example, the NL were lower on a percent of weight basis in the 20-day larvae (8.2%) and also lower on a percent of radioactivity basis (83.7%) than in the other larvae, pupae and adults. Just as the larval NL increased on a percent basis (Table V) and on a weight (Table VI) basis, the percent of TL radioactivity in the NL also increased. The percent of the TL radioactivity and weight found in the PL decreased during larval development. As stated earlier, both the NL and
PL increase during larval development, but the NL increase more rapidly than the PL which makes it appear that the radioactivity in the PL is decreasing.

The specific activities of the NL and PL during development from larvae to adults are compared with the percent (on a weight basis) of the TL accounted for by these 2 fractions in Table IX. As with the TL, these specific activities are probably not very meaningful. However, it is interesting to note that only in the pupal stage did the specific activity of the PL approach that of the NL. This high specific activity of the pupal PL is associated with an increase in PL content on a percent basis (Table IX) and on a weight basis (Table VI). These results indicate that the predominant lipid synthesized in the pupae is PL which is in agreement with results obtained with the bollworm Heliothis zea (Boddie) by Lambremont and Graves (1969). The specific activity of the PL decreased substantially in the young adults as it did in the bollworm.

The distribution of NL $^{14}$C activity into the NL classes as determined by TLC zonal analysis is shown in Table X. Practically all (88 to 91%) of the NL radio-
activity was located in the TG in the larvae, pupae, and adults. Lambremont et al. (1966) found that newly emerged adult boll weevils reared on a larval diet containing $^{14}C$-acetate had 60.5% of the radioactivity in the FFA, only 20.8% in the TG, 11.3% in the SE, 4.4% in the HC, and 2.2% in the PL. No significant radioactivity was found in any NL class of the southern lyctus beetle other than in the TG (Table 8). Slightly more radioactivity was found in the HC than in the other classes, but this radioactivity was very low. The small amount of radioactivity in the nonsaponifiable fraction (Table XI) verifies this low activity in the HC. Based on charred TLC plates (see Figure 1), HC appear to be the second only to TG as the most abundant NL class.

The apparent failure of the southern lyctus beetle to esterify labeled fatty acids with sterol is in agreement with results found for the American cockroach (Vroman et al., 1965). The boll weevil has been shown to incorporate labeled fatty acids into several lipid classes including SE (Lambremont et al., 1966). It should be pointed out that the SE comprised a very small portion of the NL. Therefore, the amount of radioactivity present,
if esterification did occur, would be small and difficult to detect.

No glucose-$^{14}$C was incorporated into the sterols, which indicates that the southern lyctus beetle cannot synthesize sterols. This result agrees with those from most other studies on sterol synthesis by insects.

There was a low amount of radioactivity present in the small amount (50-90 ug) of PL which could be satisfactorily separated by TLC. Therefore, an accurate assessment of the distribution of PL radioactivity was not possible. However, data provided by the 40-day larvae which had eaten the highly radioactive diet did clarify these results somewhat. In this instance 36.6% of the radioactivity recovered from the TLC plate was found in the PE, 26.2% in PC, 9.0% in PS, 4.1% in LysPC, 2.8% in SM, 2.8% at the origin, and 18.6% in the PA fraction. Although the radioassay data for the other PL TLC scrapings were variable, it was evident that PE, PC and PA were the major PL classes into which labeled fatty acids were incorporated. Usually PE contained slightly more label than did PC.
Incorporation of Glucose-$^{14}$C into Fatty Acids of the Larvae, Pupae and Adults

A summary of the $^{14}$C detected in the various fatty acids of the larvae, pupae and adults is presented in Table XII. The small fraction of the radioactivity in fatty acids with a chain length less than 16 carbons was combined for this table. Significant radioactivity was incorporated into palmitic, palmitoleic, stearic, and oleic acids. Linoleic acid appeared to be labeled in some instances. However, this is due to oleate tailing beyond the sensitivity of the GLC mass detector (Lambremont et al., 1965), and as a result, oleic acid $^{14}$C activity was collected with the linoleic acid fraction. Linolenate (C$_{18}$-3) was included in this table since traces of this fatty acid did appear in a few samples. However, in most instances the radioactivity in this fraction (Table XII) merely reflects a collection of activity where linolenic would have been, if it had been present.

Palmitic and stearic acids contained the highest percent of the radioactivity recovered in the fatty
acids after 20 days on the diet, then decreased and remained low throughout larval development. As mentioned earlier, the same was true for the percent distribution of these fatty acids. Palmitoleic and oleic acids contained the lowest percent of the radioactivity after 20 days of the diet, then increased and remained high throughout larval development. The percent distribution of these fatty acids fluctuated in a similar manner.

Oleic was the only fatty acid in which the percent of the recovered radioactivity decreased during the larval to pupal metamorphosis (Table XII). This decrease is probably not significant, especially when it is considered that in the adults this percent of oleic acid is comparable to that of 75-day larvae. All other fatty acids remained constant.

The percent distribution of the recovered radioactivity in the adult fatty acids was the same as that in the larvae.
Table I- Changes in the percent (by wet weight) of total lipid (TL), neutral lipid (NL), and phospholipid (PL) content during embryonic development.

The average recovery of the TL from silica acid column chromatography calculated as the sum of NL and PL was 94.7%.

<table>
<thead>
<tr>
<th>Eggs age in days</th>
<th>Larvae</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>TL</td>
<td>22.0</td>
</tr>
<tr>
<td>NL</td>
<td>21.2</td>
</tr>
<tr>
<td>PL</td>
<td>2.7</td>
</tr>
</tbody>
</table>
Table II - Weights (µg per egg) of the total lipid (TL), neutral lipid (NL), and phospholipid (PL) during embryonic development.

<table>
<thead>
<tr>
<th></th>
<th>Eggs (age in days)</th>
<th>Larvae</th>
<th>1st Instar</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>TL</td>
<td>2.44</td>
<td>2.46</td>
<td>2.23</td>
</tr>
<tr>
<td>NL</td>
<td>2.35</td>
<td>2.20</td>
<td>1.83</td>
</tr>
<tr>
<td>PL</td>
<td>0.30</td>
<td>0.19</td>
<td>0.19</td>
</tr>
</tbody>
</table>
Table III - Percent distribution of fatty acids during embryonic development.

<table>
<thead>
<tr>
<th>Age (days)</th>
<th>C_{14}</th>
<th>C_{14-15}</th>
<th>C_{15+}</th>
<th>C_{16}</th>
<th>C_{16-1}</th>
<th>C_{18}</th>
<th>C_{18-1}</th>
<th>C_{18-2}</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>6.5</td>
<td>2.0</td>
<td>1.8</td>
<td>2.3</td>
<td>8.4</td>
<td>10.0</td>
<td>2.1</td>
<td>63.2</td>
</tr>
<tr>
<td>1</td>
<td>4.9</td>
<td>1.6</td>
<td>2.2</td>
<td>3.2</td>
<td>9.1</td>
<td>9.8</td>
<td>2.1</td>
<td>62.9</td>
</tr>
<tr>
<td>2</td>
<td>4.5</td>
<td>1.6</td>
<td>2.2</td>
<td>2.9</td>
<td>8.2</td>
<td>10.1</td>
<td>2.0</td>
<td>64.6</td>
</tr>
<tr>
<td>3</td>
<td>4.1</td>
<td>1.2</td>
<td>1.8</td>
<td>2.0</td>
<td>9.0</td>
<td>10.3</td>
<td>2.5</td>
<td>65.8</td>
</tr>
<tr>
<td>4</td>
<td>4.0</td>
<td>1.4</td>
<td>1.8</td>
<td>2.2</td>
<td>8.6</td>
<td>9.4</td>
<td>2.5</td>
<td>65.0</td>
</tr>
<tr>
<td>5</td>
<td>10.6</td>
<td>1.6</td>
<td>2.5</td>
<td>2.6</td>
<td>9.1</td>
<td>8.8</td>
<td>2.6</td>
<td>55.1</td>
</tr>
<tr>
<td>6</td>
<td>5.1</td>
<td>1.5</td>
<td>2.2</td>
<td>2.2</td>
<td>8.8</td>
<td>9.7</td>
<td>2.5</td>
<td>65.2</td>
</tr>
<tr>
<td>7</td>
<td>4.3</td>
<td>2.0</td>
<td>1.4</td>
<td>1.4</td>
<td>15.7</td>
<td>7.9</td>
<td>5.2</td>
<td>59.0</td>
</tr>
<tr>
<td>8a</td>
<td>1.2</td>
<td>0.4</td>
<td>0.5</td>
<td>0.5</td>
<td>8.9</td>
<td>8.0</td>
<td>3.3</td>
<td>71.2</td>
</tr>
</tbody>
</table>

a First instar larvae.
Table IV- Effect of hydrogenation on the percent distribution of the major fatty acids from eggs (E), larvae (L), and pupae (P).

U=Unhydrogenated, H=Hydrogenated

<table>
<thead>
<tr>
<th>Sample</th>
<th>C₁₄</th>
<th>C₁₅</th>
<th>C₁₆</th>
<th>C₁₆₁</th>
<th>C₁₈</th>
<th>C₁₈₁</th>
<th>C₁₈₂</th>
<th>C₁₈₃</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 Day E-U</td>
<td>1.5</td>
<td>2.6</td>
<td>3.6</td>
<td>8.1</td>
<td>8.9</td>
<td>2.1</td>
<td>59.6</td>
<td>4.8</td>
</tr>
<tr>
<td>0 Day E-H</td>
<td>1.6</td>
<td>1.6</td>
<td>3.0</td>
<td>17.1</td>
<td>Ta</td>
<td>66.9</td>
<td>3.8</td>
<td>T</td>
</tr>
<tr>
<td>Net change</td>
<td>+0.1</td>
<td>-1.0</td>
<td>0.6</td>
<td>+9.0</td>
<td>-8.9</td>
<td>-64.8</td>
<td>-55.8</td>
<td>-4.8</td>
</tr>
<tr>
<td>40 Day L-U</td>
<td>0.8</td>
<td>1.1</td>
<td>1.1</td>
<td>7.6</td>
<td>12.3</td>
<td>2.3</td>
<td>60.7</td>
<td>11.8</td>
</tr>
<tr>
<td>40 Day L-H</td>
<td>0.8</td>
<td>0.7</td>
<td>0.9</td>
<td>20.2</td>
<td>Ta</td>
<td>76.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Net change</td>
<td>0.0</td>
<td>0.4</td>
<td>-0.2</td>
<td>+12.6</td>
<td>-12.3</td>
<td>+74.0</td>
<td>60.7</td>
<td>-11.8</td>
</tr>
<tr>
<td>60 Day P-U</td>
<td>0.5</td>
<td>0.5</td>
<td>0.4</td>
<td>7.5</td>
<td>14.2</td>
<td>2.0</td>
<td>63.4</td>
<td>11.3</td>
</tr>
<tr>
<td>60 Day P-H</td>
<td>0.7</td>
<td>T</td>
<td>T</td>
<td>22.6</td>
<td>0.0</td>
<td>76.7</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Net change</td>
<td>+0.2</td>
<td>-0.5</td>
<td>0.4</td>
<td>+15.1</td>
<td>-14.2</td>
<td>-74.7</td>
<td>63.4</td>
<td>-11.3</td>
</tr>
</tbody>
</table>

Ta=trace
Table V - Changes in the percent (by wet weight) of total lipid (TL), neutral lipid (NL), and phospholipid (PL) content during post-embryonic development. The average recovery of the TL from silicic acid column chromatography calculated as the sum of NL and PL was 99.7%.

<table>
<thead>
<tr>
<th></th>
<th>Larvae</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th>Pupae</th>
<th></th>
<th></th>
<th></th>
<th>Adults</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1st instar</td>
<td>20 day</td>
<td>40 day</td>
<td>60 day</td>
<td>75 day</td>
<td>60 day</td>
<td>75 day</td>
<td>60 day</td>
<td>75 day</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TL</td>
<td>17.9</td>
<td>10.8</td>
<td>12.6</td>
<td>14.4</td>
<td>18.9</td>
<td>15.1</td>
<td>13.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NL</td>
<td>10.5</td>
<td>8.2</td>
<td>12.0</td>
<td>13.3</td>
<td>17.6</td>
<td>13.3</td>
<td>12.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PL</td>
<td>1.2</td>
<td>2.0</td>
<td>1.4</td>
<td>1.0</td>
<td>1.0</td>
<td>1.1</td>
<td>1.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>


Table VI- Weights (μg per larva, pupa or adult) of the total lipid (TL), neutral lipid (NL) and phospholipid (PL) during post-embryonic development from first instar larvae to adults

<table>
<thead>
<tr>
<th></th>
<th>Larvae</th>
<th>Pupae</th>
<th>Adults</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1st instar</td>
<td>20 day</td>
<td>40 day</td>
</tr>
<tr>
<td>TL</td>
<td>2.2</td>
<td>17.0</td>
<td>182.0</td>
</tr>
<tr>
<td>NL</td>
<td>1.3</td>
<td>13.2</td>
<td>173.8</td>
</tr>
<tr>
<td>PL</td>
<td>0.2</td>
<td>3.3</td>
<td>19.3</td>
</tr>
</tbody>
</table>
TABLE VII- Percent fatty acid distribution in larvae, pupae, adults and larval diet.

<table>
<thead>
<tr>
<th>Sample</th>
<th>C14</th>
<th>C14</th>
<th>C14</th>
<th>C15</th>
<th>C16</th>
<th>C16</th>
<th>C18</th>
<th>C18</th>
<th>C18</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diet</td>
<td>3.4</td>
<td>0.2</td>
<td>0.5</td>
<td>0.6</td>
<td>12.6</td>
<td>Tb</td>
<td>1.7</td>
<td>29.2</td>
<td>51.8</td>
</tr>
<tr>
<td>1st Instar&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.7</td>
<td>0.4</td>
<td>0.5</td>
<td>0.5</td>
<td>6.9</td>
<td>8.0</td>
<td>3.3</td>
<td>71.2</td>
<td>5.4</td>
</tr>
<tr>
<td>20 Day Larvae</td>
<td>9.5</td>
<td>1.5</td>
<td>1.6</td>
<td>1.8</td>
<td>13.2</td>
<td>5.2</td>
<td>3.7</td>
<td>46.4</td>
<td>18.9</td>
</tr>
<tr>
<td>40 Day Larvae</td>
<td>3.1</td>
<td>0.8</td>
<td>1.2</td>
<td>1.2</td>
<td>8.0</td>
<td>1.9</td>
<td>2.2</td>
<td>61.1</td>
<td>10.4</td>
</tr>
<tr>
<td>60 Day Larvae</td>
<td>0.9</td>
<td>0.6</td>
<td>0.9</td>
<td>0.6</td>
<td>7.7</td>
<td>13.7</td>
<td>1.8</td>
<td>64.8</td>
<td>9.0</td>
</tr>
<tr>
<td>75 Day Larvae</td>
<td>3.9</td>
<td>1.1</td>
<td>1.8</td>
<td>1.7</td>
<td>7.3</td>
<td>12.8</td>
<td>1.4</td>
<td>61.8</td>
<td>8.2</td>
</tr>
<tr>
<td>60 Day Pupae</td>
<td>2.1</td>
<td>0.7</td>
<td>0.8</td>
<td>0.8</td>
<td>6.1</td>
<td>10.8</td>
<td>1.8</td>
<td>66.2</td>
<td>10.6</td>
</tr>
<tr>
<td>75 Day Adults</td>
<td>2.6</td>
<td>1.2</td>
<td>1.6</td>
<td>1.5</td>
<td>7.9</td>
<td>10.5</td>
<td>2.0</td>
<td>61.0</td>
<td>12.3</td>
</tr>
</tbody>
</table>

<sup>a</sup> These larvae had not yet fed on the diet.

<sup>b</sup> T = trace.
Table VIII. $^{14}$C (dpm) per mg of total lipid (TL) recovered from a silicic acid column in neutral lipid (NL) and phospholipid (PL). The average recovery of TL radioactivity was 99% when the activity of NL and PL was summed.

<table>
<thead>
<tr>
<th>Developmental stage</th>
<th>$^{14}$C Recovered as NL (dmp/mg TL)</th>
<th>%</th>
<th>$^{14}$C Recovered as PL</th>
</tr>
</thead>
<tbody>
<tr>
<td>20 Day Larvae</td>
<td>742</td>
<td>617</td>
<td>83.2</td>
</tr>
<tr>
<td>40 Day Larvae</td>
<td>595</td>
<td>578</td>
<td>97.2</td>
</tr>
<tr>
<td>60 Day Larvae</td>
<td>658</td>
<td>634</td>
<td>96.3</td>
</tr>
<tr>
<td>75 Day Larvae</td>
<td>493</td>
<td>487</td>
<td>98.7</td>
</tr>
<tr>
<td>60 Day Pupae</td>
<td>697</td>
<td>625</td>
<td>89.6</td>
</tr>
<tr>
<td>75 Day Adults</td>
<td>599</td>
<td>54</td>
<td>90.3</td>
</tr>
</tbody>
</table>
Table IX- Specific activity (dpm/mg) of the neutral lipid (NL) and phospho lipid (PL) compared with the percent of the total lipid (TL) accounted for by these fractions

<table>
<thead>
<tr>
<th>Developmental stage</th>
<th>dpm/mg NL</th>
<th>%NL by weight</th>
<th>dpm/mg PL</th>
<th>% PL by weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>20 Day Larvae</td>
<td>798</td>
<td>77.9</td>
<td>453</td>
<td>19.6</td>
</tr>
<tr>
<td>40 Day Larvae</td>
<td>620</td>
<td>95.5</td>
<td>363</td>
<td>10.7</td>
</tr>
<tr>
<td>60 Day Larvae</td>
<td>697</td>
<td>92.5</td>
<td>502</td>
<td>6.9</td>
</tr>
<tr>
<td>75 Day Larvae</td>
<td>528</td>
<td>93.4</td>
<td>382</td>
<td>5.3</td>
</tr>
<tr>
<td>60 Day Pupae</td>
<td>720</td>
<td>88.3</td>
<td>639</td>
<td>7.5</td>
</tr>
<tr>
<td>75 Day Adults</td>
<td>584</td>
<td>92.6</td>
<td>370</td>
<td>10.4</td>
</tr>
</tbody>
</table>
Table X - Percent of the neutral lipid (NL) radioactivity (dpm) recovered by thin layer chromatography in the various NL classes during post embryonic development.  C=Origin  DG=Diglyceride  TG=Triglycerides  S=Sterol SE=Sterol Ester  HC=HydroCarbon  Average recovery of the radioactivity applied to the thin layer chromatograms was 98% with a range of 90 to 112%.

<table>
<thead>
<tr>
<th>Developmental stage</th>
<th>C</th>
<th>a</th>
<th>DG/S</th>
<th>DG</th>
<th>FFA</th>
<th>TG</th>
<th>b</th>
<th>SE</th>
<th>HC</th>
</tr>
</thead>
<tbody>
<tr>
<td>20 Day Larvae</td>
<td>1.7</td>
<td>0.8</td>
<td>0.8</td>
<td>1.9</td>
<td>1.7</td>
<td>88.8</td>
<td>0.8</td>
<td>0.6</td>
<td>1.2</td>
</tr>
<tr>
<td>40 Day Larvae</td>
<td>2.1</td>
<td>1.3</td>
<td>0.9</td>
<td>0.4</td>
<td>2.6</td>
<td>88.0</td>
<td>1.3</td>
<td>1.3</td>
<td>1.2</td>
</tr>
<tr>
<td>60 Day Larvae</td>
<td>1.0</td>
<td>1.5</td>
<td>1.5</td>
<td>0.4</td>
<td>1.5</td>
<td>88.4</td>
<td>1.5</td>
<td>1.5</td>
<td>2.0</td>
</tr>
<tr>
<td>75 Day Larvae</td>
<td>1.3</td>
<td>0.7</td>
<td>2.0</td>
<td>1.3</td>
<td>0.7</td>
<td>91.3</td>
<td>0.7</td>
<td>0.7</td>
<td>1.3</td>
</tr>
<tr>
<td>60 Day Pupae</td>
<td>0.9</td>
<td>1.3</td>
<td>1.3</td>
<td>0.9</td>
<td>0.9</td>
<td>91.2</td>
<td>1.3</td>
<td>0.9</td>
<td>1.3</td>
</tr>
<tr>
<td>75 Day Adults</td>
<td>1.2</td>
<td>1.2</td>
<td>0.6</td>
<td>1.2</td>
<td>1.2</td>
<td>89.0</td>
<td>1.2</td>
<td>1.2</td>
<td>2.5</td>
</tr>
</tbody>
</table>

a Blank area between the origin and the first spot, the area between C and d on Figure 1.

b Unidentified spot, area f on Figure 1.
Table XI - Recovery of total lipid radioactivity (dpm) in the saponifiable (S) and non-saponifiable (NS) fractions

<table>
<thead>
<tr>
<th>Developmental stage</th>
<th>Total dpm recovered</th>
<th>dpm recovered in S</th>
<th>%</th>
<th>dpm recovered in NS</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>20 Day Larvae</td>
<td>7518</td>
<td>7314</td>
<td>97.3</td>
<td>203</td>
<td>2.7</td>
</tr>
<tr>
<td>40 Day Larvae</td>
<td>13,412</td>
<td>13,283</td>
<td>99.0</td>
<td>129</td>
<td>1.0</td>
</tr>
<tr>
<td>60 Day Larvae</td>
<td>10,792</td>
<td>10,572</td>
<td>98.5</td>
<td>217</td>
<td>1.5</td>
</tr>
<tr>
<td>75 Day Larvae</td>
<td>5277</td>
<td>5139</td>
<td>97.4</td>
<td>138</td>
<td>2.6</td>
</tr>
<tr>
<td>60 Day Pupae</td>
<td>9425</td>
<td>9196</td>
<td>97.6</td>
<td>229</td>
<td>2.4</td>
</tr>
<tr>
<td>75 Day Adults</td>
<td>6472</td>
<td>6261</td>
<td>96.7</td>
<td>211</td>
<td>3.3</td>
</tr>
</tbody>
</table>


Table XII: Percent radioactivity (dpm) recovered by GLC in the various fatty acids

The larvae were continuously fed glucose $^{14}$C in their diet. The average recovery of radioactivity was 92%. The GLC collection between adjacent peaks is signified by a dash.

### Fatty acid carbon number double bonds

<table>
<thead>
<tr>
<th>Stage</th>
<th>$^{&lt;}$C$_{16}$</th>
<th>C$_{16}$</th>
<th>$^{&lt;}$C$_{18}$</th>
<th>C$_{18}$</th>
<th>$^{&lt;}$C$_{18}$</th>
<th>C$_{18}$</th>
<th>C$_{18}$</th>
<th>C$_{18}$</th>
<th>C$_{18}$</th>
<th>C$_{18}$</th>
<th>No Peak</th>
</tr>
</thead>
<tbody>
<tr>
<td>20 Day Larvae</td>
<td>2.0</td>
<td>17.2</td>
<td>7.2</td>
<td>2.2</td>
<td>3.8</td>
<td>59.0</td>
<td>1.0</td>
<td>0.7</td>
<td>1.1</td>
<td>0.4</td>
<td>5.0</td>
</tr>
<tr>
<td>40 Day Larvae</td>
<td>1.2</td>
<td>7.8</td>
<td>12.9</td>
<td>1.4</td>
<td>2.5</td>
<td>61.2</td>
<td>1.6</td>
<td>4.4</td>
<td>1.6</td>
<td>1.2</td>
<td>4.3</td>
</tr>
<tr>
<td>60 Day Larvae</td>
<td>0.8</td>
<td>7.0</td>
<td>15.1</td>
<td>1.0</td>
<td>2.4</td>
<td>62.8</td>
<td>2.8</td>
<td>2.7</td>
<td>0.9</td>
<td>0.7</td>
<td>3.8</td>
</tr>
<tr>
<td>75 Day Larvae</td>
<td>1.1</td>
<td>7.1</td>
<td>13.6</td>
<td>0.6</td>
<td>1.6</td>
<td>65.1</td>
<td>2.0</td>
<td>1.9</td>
<td>0.9</td>
<td>1.3</td>
<td>4.7</td>
</tr>
<tr>
<td>60 Day Pupae</td>
<td>1.2</td>
<td>7.6</td>
<td>14.2</td>
<td>1.3</td>
<td>2.4</td>
<td>57.6</td>
<td>3.0</td>
<td>2.7</td>
<td>1.7</td>
<td>1.4</td>
<td>6.5</td>
</tr>
<tr>
<td>75 Day Adults</td>
<td>1.4</td>
<td>8.0</td>
<td>11.8</td>
<td>0.4</td>
<td>2.3</td>
<td>64.5</td>
<td>2.8</td>
<td>1.9</td>
<td>1.1</td>
<td>1.3</td>
<td>4.6</td>
</tr>
</tbody>
</table>

- The $^{14}$C activity in this fraction was a result of tricetate tailing into the linoleate component; see Lambrecht et al 1965.

- GLC fractions were collected for 40 to 45 minutes past C$_{13}$:3 at 5 minute intervals.
Figure 1. Thin layer chromatography of neutral lipids (NL). Approximately 300 µg of the biological samples were applied at the origin (a). Lane 1 = 0-day eggs, 3 = 7-day eggs, 4 = 1st stage larvae, 6 = 20-day larvae, 7 = 40-day larvae, 9 = 60-day pupae, 10 = 75-day adults. The standards (lanes 2, 5, and 8) from bottom (c) to top (h) consisted of the following pure lipids: cholesterol, oleic acid, trilinolein, cholesteryl oleate and n-heptacosane. Using another standard, b was identified as diglycerides.
Figure 2. Thin layer chromatography of phospholipids (PL). Approximately 60 μg of the biological samples were applied at the origin (a). Lanes 1 and 2 = 0-day eggs, 4 = 7-day eggs, 5 = 20-day larvae, 6 = 40-day larvae, 7 = 60-day pupae, 9 and 10 = adults. The standards (lanes 3 and 8) from bottom (b) to top (h) (excluding e) consisted of the following pure lipids: lysophosphatidyl choline, spingomyelin, ω-γ-dipalmitoyl-DL-α-phosphatidyl choline, ω-γ-dipalmitoyl-DL-α-phosphatidyl serine, ω-γ-dipalmitoyl-DL-α-phosphatidyl ethanolamine and phosphatidic acid. Area e was tentatively identified as phosphatidyl inositol.
Figure 3.  

A. Elution sequence and separation by GLC of a standard mixture of fatty acid methyl esters.

B. Elution sequence and separation by GLC of methyl esters of fatty acids from 40-day larvae.
Figure 4. A plot of the increase in total lipid content versus the increase in wet weight of the larvae.
Figure 5. A double logarithmic plot of the increase in total lipid content versus the increase in wet weight of the larvae.
$K = 1.41$
DISCUSSION

Lipids During Embryonic Development

The failure of the southern lyctus beetle to deplete its lipid reserves during embryonic development is different from results of similar studies on many other insects (Babcock and Rutschky, 1961; Fast, 1964). The rather constant TL content during development of the embryo could indicate synthesis of lipid from non-lipid components or the utilization primarily of non-lipid constituents for energy purposes or both.

The decrease in TL as well as both NL and PL during the last few hours of embryonic development and the first few hours of larval development could possibly agree with Needham's (1931) theory that lipids are utilized last in developing embryos. However, this decrease could also be due to lipid utilization during the first moult and in subsequent development of the first instar larvae.

The lipid classes present in both the NL and PL are essentially the same as those reported for most other insects (Fast, 1964). The predominance of TG in the NL, and PE and PC in the PL also agrees with results reported
for other insects (Fast, 1964; Gilbert, 1967). The approximately equal quantities of PE and PC in the PL also agrees with results presented in earlier reports for insects other than Diptera and aphids as discussed in the literature review.

The fatty acids present and their percent distribution are also largely the same as given in published reports on insects other than Diptera and some Homoptera in which fatty acids with a chain length of C\textsubscript{16} or less predominate (Fast, 1964). Possibly the percent of the total fatty acids accounted for by palmitoleic acid is slightly higher than that reported in insects other than Diptera and some Homoptera.

Lipids During Development from Larvae to Adults

The rapid increase in TL content of the larvae is comparable to what occurs in many other insects (Finkel, 1948; Fast, 1964). Finkel (1948) calculated a k value of 1.14 for the yellow mealworm, and that of the southern lyctus larvae was 1.41. Therefore, the ratio of the increase in total lipid to the increase in wet weight of the southern lyctus larvae is greater than
that in the yellow mealworm.

Apparently the large store of lipids in mature larvae is utilized during metamorphosis from the larval to the pupal, and from the pupal to the adult stages. A similar utilization of lipids has been reported in other insects (Fast, 1964; Gilbert, 1967).

Both the NL and PL reflect the increase in TL in the larval stage and, as shown by the incorporation of glucose \(^{14}\text{C}\), the primary lipid classes synthesized were the TG, PE, PC, and PA. The other lipid classes also increased in amount as evidenced by their consistent appearance on the TLC plates, but these increases are probably due to absorption from the diet since they contained very little radioactivity.

The apparent failure of the southern lyctus beetle larvae to synthesize significant amounts of HC from \(^{14}\text{C}\)-glucose is interesting. HC are thought to function mainly as a barrier against water loss in the insect integument and these lyctus larvae can develop normally in wood with a moisture content as low as 8%. Possibly these HC are absorbed from the diet.

Lambremont et al. (1966) showed that the boll
weevil incorporates labeled fatty acids which were synthesized from acetate into SE and FFA. This ability was not demonstrated in these lyctus larvae. Evidently the synthesized fatty acids are esterified to form TG, PE and PC and do not remain as FFA.

No sterol synthesis was evident in these larvae and, if a sterol is needed for normal development and reproduction, it must be present in the diet.

The NL and PL classes present in larvae, pupae, and adults were the same as those reported for other insects (Fast, 1964; Gilbert, 1967).

As revealed by GLC analysis and radioassay of the TL fatty acids, the rapid increase in larval TL is due primarily to synthesis of palmitic, palmitoleic, stearic, and oleic acids. Linoleic acid also contributed to the increase in larval TL, but this was due to uptake from the diet since this fatty acid was not labeled with $^{14}C_{0}$.

The fatty acids present in the larvae, pupae, and adults were the same as those found in the eggs. However, as pointed out in the results, there were some changes in the percent distribution of these fatty acids. The low levels of palmitoleic and oleic acids in the
20-day larvae apparently increased in later stages at the expense of palmitic and stearic acids. This at least suggests that these larvae are capable of desaturating palmitic (C₁₆) and stearic (C₁₈) acids to their monoenoic analogs, palmitoleic and oleic acids. This ability has been demonstrated in the boll weevil (Lambremont et al., 1965).

Oleic acid contained most (about 60%) of the ¹⁴C activity recovered in the fatty acids of the larvae, pupae, and adults. Palmitoleic acid contained about 12.5%, palmitic acid about 9.8%, and stearic acid about 2.5% of the total fatty acid activity. These results indicate that the major fatty acid synthesized is oleic. The absence of ¹⁴C activity from linoleic acid in all life stages examined indicates that this fatty acid is possibly an essential dietary component, if needed for normal development and reproduction in the southern lysus beetle.

The larvae were unable to develop on a diet which had been extracted 3 times with chloroform - methanol (2:1). Since most of the lipids were extracted, it is not possible to be sure which lipid class or fatty acid(s) are essential. However, since sterols are essential in all insects involved in similar studies, and
these larvae cannot synthesize them, it is probable that sterols are essential nutrients. Also, linoleic acid is essential in the diet of several insects and it was not synthesized by these lytus larvae. Since linoleic acid was a major constituent in all developmental stages, it is probable that this fatty acid is also an essential nutrient.
CONCLUSION

1. The major neutral lipid class throughout development of the southern lyctus beetle is that of triglyceride. The major phospholipids are phosphatidyl cholines and phosphatidyl ethanolamines. These phospholipids were approximately equal in quantity.

2. Larvae are capable of synthesizing palmitic, palmitoleic, stearic, and oleic acids. Linoleic acid is not synthesized from glucose and, if needed by this insect, it must be added to the diet. Most (approximately 60%) of the total 14C activity in the fatty acids was found in oleic acid which indicates that this fatty acid is the principal product of fatty acid synthesis in this insect.

3. The synthesized fatty acids are esterified to form mainly triglycerides with smaller amounts being esterified to form the two principal phospholipids, namely, phosphatidyl ethanolamine and phosphatidyl choline.

4. The principal fatty acids present throughout all developmental stages are palmitic, palmitoleic, stearic, oleic, and linoleic acids. Smaller amounts of tride-
cianoic, myristoleic, and pentadecanoic acids were detected. Oleic acid accounted for approximately 60-70% of the fatty acids.

5. The percent distribution of fatty acids in larvae does not depend solely on that of the larval diet, but is a combination of fatty acids taken in the food and those it can synthesize from other metabolic intermediates.

6. Larvae cannot synthesize sterols and, if sterols are needed for growth and development, they must be added to the diet.

7. The lipids do not decrease significantly during the embryonic stages indicating that they probably do not supply the energy necessary during this time. However, lipids are utilized about the time the eggs hatch and the larvae make their first moult.

8. Based on a decrease in total lipid on a percent basis and on a weight basis, it is evident that lipids are utilized during the metamorphosis from mature larvae to pupae and from pupae to adults.

9. Some lipids are necessary in the diet for normal larval development as evidenced by their failure to develop past 40 days on a low fat diet.


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Vandervant, E. S., D. Kerur, and R. Reiser. 1957. The role of dietary fatty acids in the development of the pink bollworm. J. Econ. Entomol. 50:606-608.


VITA

Joe Kennon Mauldin was born March 7, 1941, at Laurel, Mississippi. He was graduated from Shady Grove High School in Jones County, Mississippi in May, 1959. He received a Bachelor of Science degree in Entomology from the Mississippi State University in Starkville, Mississippi in May, 1963. He received a Master of Science with a major in Entomology and a minor in Biochemistry from Mississippi State University in January, 1965.

He was married to Sylvia La June Moss of Laurel, Mississippi, on August 25, 1963. A son, Todd, was born on August 29, 1967.

Presently he is a candidate for the Doctor of Philosophy degree in Entomology, with a minor in Biochemistry, at the Louisiana State University.
EXAMINATION AND THESIS REPORT

Candidate: Joe Kennon Mauldin

Major Field: Entomology

Title of Thesis: Lipid Biosynthesis in the Southern Lyctus Beetle, Lyctus planicollis LeConte (Coleoptera: Lycidae): Distribution of Carbon from U-14C Glucose in the Principal Lipid Classes and Fatty Acids During Growth and Development  

Approved:

[Signature]
Major Professor and Chairman

[Signature]
Dean of the Graduate School

EXAMINING COMMITTEE

[Signature]

[Signature]

[Signature]

Date of Examination:

January 13, 1970