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The polyunsaturated fatty acids (PUFA) are known to be required for larval growth and normal wing development in several species of Lepidoptera but most of the basic information necessary for a complete understanding of this nutritional phenomenon has not been gathered. This includes the role of each of the essential fatty acids, the dietary level for normal development, the effect of age, sex, stage of development, and rearing conditions, fatty acid levels of insect tissues under normal and deficiency conditions, the biochemical distribution of the essential nutrients, and the biochemical fate of these critical constituents. The purpose of this research was to collect such information for linoleic acid (18:2) and linolenic acid (18:3) in three Noctuidae.

Trichoplusia ni (Hübner), Autographa californica (Speyer) and Heliothis zea (Boddie) were reared on artificial diets which were supplemented with vegetable oils or individual fatty acids as the only lipid

sources. Pupae were reared to adult eclosion at either 23 or 30°C and relative humidities ranging from 20 to 95%. Nutritional adequacy of diets was assessed by larval growth rates, percentage of pupation, and the degree of wing deformity.

Gas and thin-layer chromatography were used for fatty acid analyses of the total lipids and lipid classes of pupae from the various nutritional and environmental conditions. Radioactive linolenic acid was used to follow the metabolic fate of this essential fatty acid (EFA).

Under standard dietary conditions the PUFA accounted for a greater portion of the fatty acids in the phospholipids (PL) than in the triglycerides (TG) of T. ni larvae, pupae, and adults. There was little change in the component fatty acids when pupae developed at a constant temperature and humidity and no sexual dimorphism in lipid content was detected.

The higher temperature and both humidity extremes acted as stress factors affecting wing condition, apparently at the time of eclosion and wing expansion, in insects reared on marginal levels of dietary EFA. With adequate EFA these stresses were overcome. The 18:3 content of total fatty acids, PL, and TG was not different in T. ni and H. zea reared at the two temperatures.

Linolenate was verified as the sole EFA for normal wings in T. ni, and A. californica was found to have the same qualitative specificity. H. zea was found to utilize either 18:2 or 18:3 for normal wings, although the latter was more than three times as effective.

There was no EFA activity by an EFA analog and several homologous PUFA although they were incorporated into the tissue lipids. Minimal levels of dietary 18:3 for normal wings ranged from 0.05 to 0.25%, depending on the species.

The 18:3 necessary for normal wings could be supplied by feeding larvae an adequate diet one day prior to pupation or it could be depleted by feeding such larvae on 18:3 deficient diet.

The absence of dietary PUFA resulted in abnormally high amounts of monoenoic acids in the tissue lipids. Tissue levels of PUFA increased as their dietary levels increased, resulting in decreased monoene content. The saturated fatty acids were practically unaffected.

A majority of the ^{14}C-18:3 consumed by the larvae was unaltered and was located in the phospholipids and triglycerides. Suboptimal dietary levels or short-term exposure to dietary 18:3 resulted in most of this EFA being incorporated into the phospholipids. Sustained feeding at above optimum levels eventually resulted in a greater quantity of 18:3 in the TG. Phosphatidylcholine and phosphatidylethanolamine contained approximately 65% of the PL 18:3 regardless of the dietary level or feeding period.

Essential Fatty Acids in Noctuid Moths: Their Nutrition and Metabolism

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF PLATES

ESSENTIAL FATTY ACIDS IN NOCTUID MOTHS: THEIR NUTRITION AND METABOLISM

INTRODUCTION

Fatty acids account for a large portion of the lipids found in nature and they impart many of the chemical and physical properties which characterize these lipids. The simple lipids are made up largely of highly insoluble triglycerides which can be rapidly mobilized and degraded to meet the cell's energy demands. The compound lipids, mostly phospholipids, are known to be structurally and metabolically important to the cell. They are essential components of the cell membrane and of subcell structures such as nuclei, mitochondria, and the endoplasmic reticulum, which serve to compartmentalize the cell. They are also important in oxidative phosphorylation and in the transport of materials across biological membranes. Despite the relative importance of lipids in the animal kingdom, our understanding of insect lipids is quite meager. This has been due, in part, to the lack of suitable analytical techniques for studying lipid biochemistry in insects, and to the lack of methods for laboratory rearing of various insect species. In the last twelve years many of these problems have been overcome and progress is now possible. Reviews of the subject usually call attention to the gaps in our knowledge of this subject. Gilbert (1967) concludes his review with the statement, "Although several problems of the function and metabolism of lipid in insects

have been unravelled in recent years, this research area remains ripe for invasion by both the entomologist and biochemist."

When the present research was begun, virtually no information existed on the nutritional requirements for lipids in the Noctuidae, the largest family in the order Lepidoptera. Within this group of moths are found some of the most important agricultural pests. During the course of this study several reports appeared which showed that many Lepidoptera require dietary polyunsaturated fatty acids for normal growth and adult emergence. Very little information was available on fatty acid content and metabolism in these insects, and the role of these nutrients in adult emergence and wing expansion has received only speculation.

The purpose of this study was to establish the nutritional essentiality and metabolic fate of the polyunsaturated fatty acids in three species of noctuid moths. It was hoped to be able to contribute to an understanding of the role of these nutrients in adult emergence and normal wing formation.

HISTORICAL REVIEW

Dietary Lipid Requirements in Insects

Insects exhibit a wide range in their requirements for dietary lipid, the necessity for a sterol being the only requirement in common in species examined thus far. Beyond this rigid requirement, there is no general pattern of need for lipids in development and reproduction. Scoggin and Tauber (1950) have reviewed the early literature regarding such requirements. In the mosquito Theobaldia incidens (Thom.), the termite Zootermopsis angusticollis (Hagen), the mealworm Tenebrio molitor Linnaeus, and a componotus ant, no requirements for lipid other than sterol was demonstrable.

The larvae of Drosophila melanogaster (Meigen), Tribolium confusum Jacquelin duVal, Lucilia sericata (Meig.), and Pseudosarcophaga affinis (Fall.) require a dietary source of lipid for optimal growth but can survive on a lipid-free diet. In addition, beetle larvae of the genera Dermestes, Lasioderma, Sitodrepa, and Ptinus have been grown on fat free diets without any indication that fat would have improved growth (Fraenkel and Blewett, 1946). However, only five percent of the larvae of the related Trogoderma granarium Everts could produce adults when reared on a lipid-free diet (Pant and Pant, 1961).

In the muscid flies there is apparently no requirement for dietary

fatty acids and they may be detrimental (Silverman and Levinson, 1954; Levinson and Bergmann, 1957; Brookes and Fraenkel, 1958). The house-cricket Acheta domesticus (Linnaeus) requires a factor, other than linoleic acid, which is present in wheat germ oil (Ritchot and MacFarlane, 1962). The growth of the parasitic dipteran Agria affinis (Fall.) is stimulated by oleic acid but palmitic, stearic, palmitoleic, linoleic, linolenic, or arachidonic acids have no effect (House and Barlow, 1961).

In his review of insect nutrition, House (1965) states that most insects can probably synthesize all the fatty acids required but a few species are known to require dietary sources of certain unsaturated fatty acids. The first of these discoveries was that of Fraenkel and Blewett (1946) who demonstrated that unless linoleic acid was present in the larval diet of moths belonging to the genus Ephestia, the moths failed to emerge. Suboptimal concentrations of this acid allowed emergence, but there was a loss of wing scales inversely proportional to the amount of linoleic acid in the diet. Linolenic acid had approximately the same effect as linoleic acid, but oleic acid had no effect. The closely related moth Plodia interpunctella (Hübner), which lives on the same food as some species of Ephestia, can grow in the complete absence of fat. These workers subsequently showed that arachidonic acid had no effect on scale formation or adult emergence of Ephestia (Fraenkel and Blewett, 1947). Their evidence also indicated that linoleic acid could not be synthesized by Ephestia kuehniella (Zeller)

whereas Tenebrio molitor could synthesize this acid (or possibly linolenic acid). Since this early work, there have been many reports on the necessity for dietary polyunsaturated fatty acids for normal physiological functions in insects.

Most of these reports have dealt with lepidopterous species but locusts and grasshoppers also were shown to benefit from dietary polyunsaturates. In Schistocerca both linoleic and linolenic acid are equally effective, but without one or the other the insect cannot reach adulthood. In S. gregaria (Forskal) and in Locusta migratoria (Linnaeus) the adults have crumpled wings if dietary linoleic acid is not present in sufficient amounts (Dadd, 1960; 1961). In Melanoplus bivittatus (Say) the effect of a dietary lack of unsaturated fatty acids or linoleic acid was also a crumpling of wings upon adult emergence (Nayar, 1964).

Many phytophagous species among the Lepidoptera have by now been shown to require either linoleic or linolenic acids in their diets. Most vegetable oils contain one or both of these fatty acids as major components. Beck et al. (1949) reported that linoleic acid could replace corn oil in the diet of Pyrausta nubilalis (Hübn.). The rice moth, Corcyra cephalonica (Stainton) requires linoleic acid for larval growth (Uberoi, 1960). Vanderzant, Kerur and Reiser (1957) showed that both linoleic and linolenic acid promoted normal emergence of pink bollworm moths but linolenic acid was about five times more effective than linoleic. Either linoleic or linolenic acid was required for successful

emergence of Galleria mellonella (Linnaeus) (Dadd, 1964), Argyrataenia velutinana (Walker) (Rock, Patton and Glass, 1965), and Carpocapsa pomonella (Linnaeus) (Rock, 1967). Linolenic acid was reported to be an indispensable dietary ingredient for adult emergence in Heliothis zea while linoleic acid supported some emergence and improved maturation (Vanderzant, 1968).

Only three species have been shown to have an absolute requirement for linolenic acid with no substitutive effect by linoleic acid. However, the purity of the fatty acids used in investigations prior to the advent of GLC must be in some doubt, and the need for linolenic acid may be wider than realized. Tamaki (1961) showed that the smaller tea tortrix, Adoxophyes orana (Fischer von Rö̈slerstamm) could emerge from the pupa only when linolenic acid was supplied in the larval diet. Chippendale, Beck and Strong (1964), found similar results with Trichoplusia ni as did Vanderzant (1967) with Estigmene acrea (Drury).

Gordon (1959) reported that gravid females of Blatella germanica (Linnaeus) reared to maturity on diets lacking linoleic acid aborted their egg cases. If this acid was present in the diet during the immature stages but withheld from the mature females, their progeny suffered early deaths. Pepper and Hastings (1943) concluded that females of the sugar beet webworm, Loxostege sticticalis (Linnaeus) required linoleic or linolenic acid for reproduction. Egg production in the boll weevil, Anthonomus grandis (Boheman) was below normal unless fat was

provided in the larval and adult diets (Vanderzant and Richardson, 1964). Earle, Slatten and Burks (1967) concluded that linoleic and linolenic acids supported the highest egg production when in the larval diet of the boll weevil. The adult diet was not as important for egg production.

Thus a wide spectrum of lipid requirements has been demonstrated in different insects but the role of these compounds in development or reproduction is not yet understood.

Fatty Acid Composition and Metabolism in Insects

The fatty acid composition of insects is influenced by their diet but the alteration of body lipids by anabolism and catabolism is also a major factor. In these paragraphs the author will attempt to select from the literature the general patterns of lipid composition and metabolism which have developed and which will be useful as a background to the present report. Detailed accounts of lipid content, fatty acid composition, and metabolism in insects are given in reviews by Fast (1964) and Gilbert (1967).

Fatty Acid Composition

There is no doubt that the major lipid components in insects as in other animals, are the triglycerides--the glycerol esters of long chain fatty acids. The large quantities of free fatty acids

reported in the older literature (cf. Scoggin and Tauber, 1950) are very likely artifacts since precautions were not taken to prevent the hydrolytic cleavage of the glycerides and phosphatides (Gilbert, 1967).

Protoplasmic fatty acids usually vary from C_2 to C_{34} in chain length and are most commonly composed of an even number of carbon atoms. Insect fatty acids consist largely of C_{14} to C_{18} acids. Longer chain acids are found mainly in the lipids of the cuticle (waxes) and account for a very small percentage of the total. Almost all of the studies of fatty acids are based on extraction of whole insects.

Lambremont and Blum (1963) identified 23 fatty acids from the boll weevil, varying in chain length from 6 to 20 carbons. Of these, 14:0[1/], 16:0, 16:1, 17:0, 18:0, 18:1, 18:2, and 18:3 constituted 98 percent of the total, 62 percent containing at least one point of unsaturation. Louloudes et al. (1961) report 17 fatty acids from Periplaneta americana (L.) ranging in chain length from C_8 to C_{18}, with palmitic and the C_{18} series predominating. This is usually the case but exceptions include the aphids which are characterized by their unusually high content of 14:0 (more than 80 percent) and Diptera which have a high proportion of 16:1 (19 to 60 percent). Most other species yielded no more than 2 percent of this acid (Barlow, 1964).

[1/] Fatty acids will be designated by the number of carbons followed by a colon and the number of double bonds.

It is generally believed that the polyunsaturated fatty acids linoleic and linolenic are not synthesized by some insects and their presence in the insect depends on their availability in the diet. The level of these acids varies widely in individuals of the same species, depending on their diets. Schmidt (1964) found that in some insects, the body lipids may contain up to 70 percent polyenoic fatty acids with linoleic and linolenic acids the most common. He found very small amounts of tetraenes and pentaenes. Other reports of insect fatty acids with more than 18 carbons and 3 double bonds are very few. In Formica polyctena Förster females, Schmidt (1963) detected fatty acids with 2 to 5 double bonds. Traces of a tetraenic fatty acid were reported in the larvae of Phalera bucephala (Linnaeus)(Schmidt and Osman, 1962), and Fawzi, Osman and Schmidt (1961) identified a tetraenic fatty acid in the body lipids of Locusta migratoria. Most reports indicate, however, that insects seems to differ markedly from higher animals in their content of long chain polyunsaturated fatty acids where they are often found in considerable quantities.

Fatty Acid Metabolism

The de novo synthesis of long chain fatty acids from carbohydrates and proteins in insects is well established. Ditman (1938) correlated a decrease inglycogen during the prepupal period of Heliothis obsoleta (Fab.) with a simultaneous increase in fat content. Van

Handel and Lum (1961) listed the fatty acids synthesized by the female mosquito from glucose and Levinson and Bergmann (1957) showed that M. vicina (Macq.) can mature normally without lipid or carbohydrate and can produce as much fat from protein as when on a high carbohydrate diet. A. aegypti (Linnaeus) larvae also synthesize fatty acids from amino acids (Kodicek and Levinson, 1960; Wigglesworth, 1942). Utilizing carbon-14, Lambremont and Bennett (1966) showed that glucose, fructose, sucrose and their related glycolytic products, pyruvate and acetate, were readily converted into long-chain fatty acids by larval and adult boll weevils, Anthonomus grandis. Palmitate, stearate, palmitoleate, and oleate made up 86 percent of the radioactive fatty acids. Recently Horie, Nakasone and Ito (1968) reported the synthesis of fatty acids from glucose via pyruvate in Bombyx mori (Linnaeus). The two saturated and two monounsaturated fatty acids reported above for the boll weevil were C-^{14} labeled, but neither linoleic nor linolenic acid was labeled.

In vivo studies of the uptake of ^{14}C-acetate in Periplaneta americana by Louloudes et al. (1961) showed that oleate contained the greatest incorporation, accounting for about 45 percent of the radioactivity. About 29 percent was found in the 16-carbon acids, and stearate and linoleate each contained about 10 percent of the radioactivity. With similar studies on another roach, Bade (1964) found that Eurycotis floridana (Walker) shows a labeling pattern consistent with

biosynthesis by the condensation of C_2 units. Oleic acid constituted about half of the newly synthesized fatty acids while palmitate was the most abundant saturated acid. There was only a trace of fatty acids of chain length greater than C_{18} but synthesis of a C_{18} dienoic acid may have occurred at a very low rate. Arachidonic acid was entirely absent. The data of Miura et al. (1965) on Calliphora erythrocephala (Meig.) was in general agreement with these reports.

Both aseptically reared and non-aseptic adult boll weevils synthesize long chain fatty acids from ^{14}C acetate at about the same rate (Lambremont, 1965). Most of the radioactivity was in the C_{16} and C_{18} saturated and monounsaturated fatty acids. This insect appears to be incapable of synthesizing linoleic acid. Lamb and Monroe (1968), in studies with ^{14}C-acetate administered to the cereal leaf beetle, Oulema melanopus (Linnaeus) found 25 percent of the labeled fatty acids to be palmitic acid and 60 percent to be oleic acid. Linoleic and linolenic acids contained an insignificant amount of radioactivity.

Palmitic, stearic, and oleic acids contained the most radioactivity from ^{14}C-acetate incorporation in the silkworm, B. mori. The polyunsaturated fatty acids contained no radioactivity (Sridhara and Bhat, 1964). Larvae of the cabbage looper, Tricholplusia ni, were also unable to synthesize linoleic and linolenic acids from ingested ^{14}C-acetate. Again, nearly all of the label present as fatty acids was contained in palmitate, palmitoleate, stearate, and

oleate (Nelson and Sukkestad, 1968).

Fatty acid oxidation has been the subject of numerous investigations (Fast, 1964; Gilbert, 1967) since in many insects it is from these compounds that energy is directly derived for flight and metamorphosis. No clear-cut pattern emerges which would indicate that some fatty acids are used for energy in preference to others although the acids that can be synthesized from non-lipid precursors would seem to be favored over those that cannot be biosynthesized.

The above, although by no means an inclusive survey, does support the proposition that many insects can synthesize long chain fatty acids from acetate, presumably by the processes found in other organisms. The information is similar to that on vertebrates in that both groups of animals can introduce one double bond into a C_{16} or C_{18} saturated fatty acid but a metabolic block at this point prevents the addition of a second or third double bond. The similarity ends here in that insects appear to be unable to add further C_2 units or double bonds when supplied with 18:2 or 18:3 in the diet. It is of interest that there are no reports of metabolism of ^{14}C polyunsaturated fatty acids in insects.

Temperature-Fatty Acid Interactions

Several reports indicate a relationship between the temperature to which an insect is exposed and the degree of unsaturation of the

endogenous fatty acids. A similar phenomenon appears in other organisms (Pearson and Raper, 1927; Marr and Ingraham, 1962; Lewis, 1962; Knipprath and Mead, 1968). Most studies with insects have involved rather wide temperature differences, e. g. 20 to 30 degrees. The higher temperature was associated with more saturated lipids in Lucilia sericata (Rainey, 1938), Heliothis obsoleta (Ditman, 1938), Calliphora erythrocephala and Phormia terraenovae (R. -D.) (Fraenkel and Hopf, 1940), and P. terraenovae (Cherry, 1959). Munson (1953) found that the level of unsaturation of the fatty acids remained constant when P. americana was reared at any temperature between 17° and 27°C, and was constant but at a much lower value when roaches were reared at 32°C or above. He suggested that the optimum temperature of a key enzyme might be the cause of the observed differences in unsaturation. Recently, Harwood and Takata (1965) found that newly emerged female Culex tarsalis (Coquillett) contain a greater percentage of unsaturated fatty acids when raised at 22°C than at 30°C. The only exception to this pattern in the literature appears to be the report of Van Handel (1966) who examined the de novo synthesis of triglyceride fatty acids in female Aedes sollicitans (Walker) and found no differences in the degree of unsaturation from 10° to 35°C.

The Nutrition and Metabolism of Polyunsaturated Fatty Acids in Other Animals

The metabolic fate of the polyunsaturated fatty acids (PUFA) in vertebrates (mainly the rat) has been the subject of intensive research by many laboratories for several decades. A familiarity with this knowledge was helpful in our studies of insects. A brief review of the current knowledge on this subject is presented here.

Although the role of fat in the diet was investigated earlier, it was Burr and Burr (1930) who demonstrated that the preventative and curative effects of dietary fat were traceable to certain polyunsaturated fatty acids, notably linoleic acid. They also noted the effects of fat deficiency upon reproduction and coined the expression "essential fatty acids." Other symptoms of essential fatty acid (EFA) deficiency in rats include diminished growth, scaly skin, roughened hair coat, necrosis of the tail, diminished skin pigmentation, loss of muscle tone, and a variety of physiological and anatomical changes at the organ and cellular level (Holman, 1968). One of the interesting physiological changes in EFA deficiency is an increased permeability of the skin resulting in an impaired water balance. For this reason the severity of the dermal symptoms of EFA deficiency is affected by changes in humidity. Fatty acid deficiency symptoms have been described for other rodents, and for dogs, swine, ruminants, chickens, fish, monkeys and humans (Holman, 1968).

The results of many years of nutritional studies and lipid analyses have established that certain fatty acids are essential because they cannot be synthesized in the animal body, hence their necessity as a dietary nutrient. Beyond this, however, there is much information indicating that the essential fatty acids undergo a variety of interesting transformations. Moreover, the biosynthesis of other PUFA is now known to occur (Mead, 1968).

Metabolic studies indicate that the PUFA are metabolized by the oxidizing enzymes of the mitochondria (if present as free fatty acids derived from triglycerides). Preservation of the essential fatty acids occurs only after incorporation into certain phospholipids, particularly if they become part of some membrane or other more permanent structure (Mead, 1968). This appears to be the case for arachidonic acid which has been shown to be only slightly oxidized in vitro, being incorporated into phospholipids very rapidly (Coniglio, Davis and Aylward, 1964; Coots, 1965).

The metabolic pathways leading to the synthesis of the various PUFA in the vertebrates were elucidated largely by means of gas chromatographic studies such as that by Stoffel and Ahrens (1960) on the analysis and characterization of menhaden oil. When the positions of the double bond systems of the fatty acids are designated by numbering from the terminal methyl group, it is seen that the 18, 20, and 22-carbon acids fall into three major families: the oleic family

(designated ω 9), the linoleic family (ω 6) and the linolenic family (ω 3). There is also some evidence for a palmitoleic family (ω7). With the evidence available from tracer studies, one can associate the fatty acids of each family with a biosynthetic series starting with the member having the shortest chain and the least unsaturation.

Thus, when linoleic acid (18:2ω 6) is fed, it results in an increase in 20:4ω 6 (arachidonic acid) and 22:5ω 6, which appears to be the last member of the linoleate family (Mohrhauer and Holman, 1963a; Rahm and Holman, 1964 a, b). Linolenic acid (18:3 ω 3) results in the accumulation of 20:5, 22:5 and 22:6, all of the ω 3 family (Edwards and Marion, 1963). Mead (1968) notes that 18:3 itself does not appear to be deposited in the tissue lipids until the dietary level becomes quite high, but its conversion products, particularly 22:6, readily accumulate.

Animals maintained on a fat-free diet accumulate an eicosatrienoic acid (20:3 ω 9). This effect has been noted in the chick, rat, pig and others (Mead, 1968). Fulco and Mead (1959) found that the precursor of the eicosatrienoic acid in fat-deficiency was oleic acid. This indicated clearly that a family of polyunsaturated acids could be formed entirely endogenously from acetate via oleate. Previous knowledge had emphasized that in higher animals, PUFA can be formed only from exogenous di- or trienoic precursors. During the same study, another eicosatrienoic acid was found leading to the

proposal of a fourth family of polyunsaturated acids, in this case derived from palmitoleic acid.

Klenk and Oette (1960) and Klenk and Pflüger (1963) studied the transformations of labeled palmitoleic, oleic, linoleic and linolenic acids in rats and provided evidence that the different families of polyunsaturated acids are not interconvertible. In no case could any evidence be found for transformation of members of these families to those of other families by desaturation or saturation between the terminal methyl and the double bond system.

Various nutritional studies have shown that supplementation of PUFA leads to a decrease of the trienoic acids of the oleic (ω9) and palmitoleic (ω 7) families, and an increase in the polyunsaturated acids resulting from elongation and desaturation of the dietary compounds (Mead, 1968). This has led to studies of competitive inhibition in PUFA conversions. This competitive inhibition is due to the lack of specificity by the enzymes involved in the conversions of the PUFA to longer, more unsaturated, homologues. The work of Holman (1964) and Holman and Mohrhauer (1963) on the quantitative aspects of competitive inhibition among the polyunsaturated fatty acids led to the present theory. They showed that linolenate is a much more efficient inhibitor of linoleate than vice-versa and they related this efficiency to enzyme affinity. From these and other results, Mead (1968) notes that linolenate has about a ten-fold greater affinity for the

enzyme systems concerned than does linoleate, which, in turn, has about a three-fold greater affinity than oleate.

Thus, EFA deficiency has effects at all levels of organization in the organism. The accumulating evidence is that essential fatty acids are required for proper structural elements in membranes which are vital to all cells but it is a testimony to the difficulty of the problem that the tremendous amount of research conducted in this field has not led to the actual biochemical mode of action of the essential fatty acids.

Preliminary Work by the Author

When the cabbage looper, Trichoplusa ni, was first reared on an artificial diet in this laboratory, satisfactory results were obtained if the rearing was conducted at room temperature, (23°C). When we attempted to shorten the life cycle by rearing the immature stages at 30°C, most of the adults had deformed wings and produced few eggs.

The average time required for half of the insects to develop from newly hatched larvae to adults at the lower temperature was 27.1 days compared to 17.8 days at 30°C. At 30°C fewer moths mated (10.5 percent) than at 23°C (90.5 percent) and the mated females averaged fewer eggs (229 vs 796).

Experiments showed that most of the wing deformity resulted

when the pupae were reared at 30°C. When pupae were held at 24°C, most of the adults had normal wings, regardless of the rearing temperature prior to this stage. This showed that the deformity was not due to deterioration of the diet at higher temperature. This was verified when fresh diet or diet containing an anti-oxidant was fed to last instar larvae without improving the wing condition. Further tests pointed to the last half of the pupal stage as the critical time for the temperature effect.

Cabbage loopers reared on bean plants with pupae held at 30°C produced normal adults. A hot ethanol extract of bean leaves incorporated into the larval medium also provided protection against the temperature-dependent wing deformity. Likewise, supplementations with wheatgerm or linseed oil corrected the deformity, linseed oil being more effective.

The author concluded that the unsupplemented medium used in these experiments contained á marginal amount of a dietary factor which, in pupae held at 24°C, was enough to allow most of the adults to emerge with normal wings. However, at 30°C a deficiency of this factor developed. The evidence indicated that this deficiency occurred as a result of temperature-related metabolic or physiological process.

During the course of these experiments reports by Chippendale, Beck and Strong (1965, 1964) identified linolenic acid as the essential fatty acid for normal wings in T. ni. We had noted that the linseed.

oil used in our experiments contained approximately six times more linolenic acid than the wheat germ oil. With this information, the author was able to confirm linolenic acid as the dietary factor responsible for the temperature-dependent wing deformity. Thus, the nutritional requirement for normal wings, when present in threshold levels, could be influenced by the temperature to which the pupae were exposed. These results have been reported (Grau and Terriere, 1967).

Experiments showing a similar temperature effect on wing condition in the alfalfa looper, Autographa californica (Speyer), and the variegated cutworm, Peridroma saucia (Hübner), have been reported (Schafer, Grau and Terriere, 1968).

MATERIALS AND METHODS

Insect Cultures

The noctuid moths used in these experiments were the cabbage looper, Trichoplusia ni (Hübner), the major species, and the alfalfa looper, Autographa californica (Speyer), and the corn earworm, Heliothis zea (Boddie), used for comparative purposes.

The cabbage looper culture originated from a stock obtained from Dr. H. H. Shorey, Riverside, California, in July, 1964. There has been no noticeable loss of vigor during the period of this study. The alfalfa looper and corn earworm cultures were begun from adults and larvae, respectively, collected from the field in Corvallis, Oregon. The moths were kept in one gallon cylindrical cartons fitted with wire-mesh lids at ambient temperature on a 16 hour daylight cycle. Alfalfa and cabbage loopers deposited eggs on waxed paper liners while the earworm eggs were collected on moistened cheesecloth. The two looper species were maintained at ambient humidity. The corn earworms' humidity was increased by placing damp paper towels on top of the cartons in the evening. The insects had access to an eight percent sucrose solution in a bottle fitted with a cellucotton wick (Shorey, 1963a).

Trichoplusia ni is susceptible to a nuclear polyhedrosis virus

which may interfere with mass rearing. In the early studies and when maintaining the stock cultures on the simpler diets, the virus was controlled by fortification of the diet with formalin. However, when the nutrition and metabolism studies were started it was preferable to surface sterilize all eggs used in the experiments by treatment for 30-40 minutes in a 0.03% solution of sodium hypochlorite containing a small amount of Triton X100 as an emulsifier (Chippendale and Beck, 1965). Washes in 70% ethanol and sterile distilled water followed and the eggs were stored in a sterile petri dish on moistened filter paper.

At 30°C the eggs hatched in about two days. A camel's hair brush was used to transfer the newly hatched larvae to the appropriate diet. Larvae for the stock insect cultures were reared in six-ounce waxed paper cups in groups of about 20 (Shorey, 1963b). The experimental larvae were reared singly in vials at 30°C, normally in replicates of ten. A few larvae died in the first instar from handling injury, and a few vials became contaminated with microorganisms. These were discarded.

Pupae for stock cultures were collected two to three days prior to adult emergence and placed on moist vermiculate in the one gallon cartons. When the wing condition was to be recorded, pupae were placed in pint jars containing vermiculite. The jars were equipped with screened lids and contained paper strips on which the moths

could climb and expand their wings.

Description of Diets

Three artificial diets were used in the study. A diet based on dry beans (Shorey, 1963b), containing additional antimicrobial compounds (Shorey and Hale, 1965), was used for rearing the stock cultures and in some of the early experiments. The meridic diet described by Chippendale and Beck (1965), for T. ni was also used. This is a modification of the rearing medium described for the bollworm, H. zea (Vanderzant, Richardson and Fort, 1962). These diets will be referred to as the bean medium and wheat-germ medium, respectively. When it became necessary to study the effect of individual fatty acids, an essentially lipid-free basal medium was used. This was very similar to the modified wheat-germ diet of Chippendale, Beck and Strong (1964). The diet, referred to hereafter as the wheat-germ residue medium, was prepared by extracting whole wheat-germ with four times its weight of chloroform-methanol (2:1 v/v) in a Waring blender for two minutes, then removing the solvent by filtration under vacuum. This step was repeated with one-fourth less solvent. The residue was then refluxed for one hour with five times its weight of the solvent. After the wheat-germ residue was filtered free of solvent it was washed three additional times with excess solvent before being oven-dried.

The composition of the wheat-germ residue medium is shown in Table 1. The dietary components, except for individual fatty acids, were obtained from Nutritional Biochemicals Corporation, Cleveland, Ohio, unless otherwise specified. When the fatty acid source was omitted, the medium was practically free of lipids except for the added sterol.

Table 1. Composition of the wheat-germ residue diet.

Component[1]	% Composition
Wheat-germ residue, lipid free	4.50
Casein, vitamin free	3.50
Dextrose	3.50
Cellulose (Alphacel)	3.00
Salt mixture (Wesson's)	1.00
Cholesterol	0.30
Choline chloride solution[2]	1.00
Vitamin solution[3]	10.00
Ascorbic acid	0.50
Sodium alginate[4]	0.50
Sorbic acid	0.15
Agar (Difco Bacto)	2.50
Fatty acid source[5]	0.75
Water	68.70

[1] Purchased from Nutritional Biochemical Corporation unless otherwise specified.
[2] An aqueous solution containing 10 g. choline chloride/100 ml.
[3] See Chippendale and Beck, 1965
[4] Matheson, Coleman and Bell
[5] When present, based on amount estimated in the unextracted wheat germ medium.

When the nutrition and metabolism of individual fatty acids was studied, either methyl esters or triglycerides of high purity fatty acids were used. These were obtained from The Hormel Institute, Austin, Minnesota, and were >99% pure by the supplier's analyses. Occasional checks by gas liquid chromatography (GLC) confirmed this purity.

The linolenic acid-1-^{14}C used was obtained from Nuclear Chicago Corporation, Des Planes, Illinois, and had a specific activity of 42.3 mc/mM. The supplier claimed a radiopurity of 99%, but our analysis by thin-layer chromatography (TLC) using silver nitrate impregnated silica-gel G and hexane-ethyl ether-acetic acid (75:25:1), (Morris, 1966) showed approximately 96% radioactive 18:3 at the time of use.

The dwarf bush bean, P. vulgaris L, was used occasionally as a natural diet for the T. ni larvae.

Preparation of Diets

The bean diet was prepared according to Shorey (1963b), poured into the cups, and allowed to solidify before securing the cardboard lids. All components of the wheat-germ diet except ascorbic acid were blended together and then sterilized by autoclaving at 121°C and 15 pounds pressure for 30 minutes. After the diet had cooled below 65°C the ascorbic acid was added in a solution of sterilized distilled

water and the mixture was again blended. This diet was dispensed in sterilized 23 x 85 mm. shell vials which were stoppered with sterile cotton plugs. Approximately five grams, enough to rear one larva to pupation, were placed in each vial.

To guard against batch to batch variation in the wheat-germ residue diet, large batches were prepared and subdivided before the addition of the fatty acids under study. After autoclaving and re-blending, aliquots (usually the amount necessary to make up 100g) were weighed into beakers and held at about 65°C. The fatty acids to be added were dispersed in a solution containing one ml. of 95% ethanol, approximately 33 mg. of alpha-tocopherol (antioxidant), and five ml. of sterile water containing Tween 80[2/] (1 drop Tween 80/10 ml. water) in screw-capped test tubes. The tubes were shaken and heated to approximately 65°C. The final mixing of the individual batches of experimental diets in the beakers was accomplished with a motordriven stirrer. During stirring, the contents of the tubes containing the emulsified fatty acids were shaken and added. A final addition was the ascorbic acid solution.

This diet was dispensed into the shell vials with plastic squeeze bottles. Sterile cotton was used to stopper the vials after the diet solidified, and larvae were placed singly in vials within 36 hours of diet preparation.

[2/] Polyoxyethylene sorbitan monooleate; purchased from Nutritional Biochemicals Corporation.

Bioassay of Diets

The nutritional adequacy of the diets was measured by the percentage of larvae which pupated, the larval growth rates, and the degree of wing deformity in the test groups.

Larval growth rates were measured in terms of days required for 50 and 90% pupation. Each larva was observed daily, beginning the ninth day after hatching, to record the time of pupation.

Wing deformity was rated upon completion of emergence and wing expansion. The moths were classified in four categories: (A) perfectly formed wings; (B) wings with minor deformities (fore wings perfect or slightly twisted at the extremities, hind wings with some scales missing, and/or not fully expanded, moths fully able to fly); (C) severely deformed wings (fore and hind wings with few to most scales missing and never fully expanded, moths unable to fly). If the moths were fully formed within the pupa, but did not emerge, or if any part of the pupal case remained attached to the adult, i.e. incomplete emergence, they were classified as " D" .

Temperature and Humidity Control

Initially, in studies of temperature effects, the pupae were held in a convection-type laboratory incubator at a temperature of $30^{\circ} \pm 2^{\circ}C$. The room temperature experiments were in a room with

circulating air and temperature controlled to 23° ±3°C. The relative humidity (RH) at these temperatures averaged 95 and 45%, respectively. In later studies, growth chambers with a circulating air supply and temperature control to ± 2°C were used for all experiments. The normal RH in these chambers ranged from 45 to 65%.

Regulation of RH for pupae held in the growth chambers was achieved by placing several emergence jars in larger partially covered jars inside the chambers. These jars could be held at 95% RH (approximately) with moist towels or 20% RH with $CaSO_4$. Humidity was measured with a Abbeon Relative Humidity and Temperature Indicator, Model No. M2A4.

Measurement of Total Lipids

Insect tissue, fresh or frozen at -20°C, was weighed and blended with chloroform-methanol (2:1 v/v) in a Servall Omni-Mixer for two minutes at 90 volts. Samples consisted of one male and one female specimen and experiments were replicated a minimum of three times unless otherwise noted. Extraction was by the method of Bligh and Dyer (1959), modified by washing the extracts with a dilute saline solution prior to solvent removal (Folch, Lees, and Sloane-Stanley, 1957). The completeness of extraction was checked by the method of Folch, Lees, and Sloane-Stanley (1957). This showed >99% extraction of the lipids. Extracts were taken to dryness in a 50°C water bath under nitrogen. Oxidation losses were avoided by minimum exposure of the samples to air.

The total lipid extract was diluted to a known volume with solvent and two or three equal aliquots were pipetted into separate, preweighed, aluminum dishes. After evaporation of the solvent, the dishes were weighed to 0.01 mg., the weights averaged, and the percent lipid calculated.

Separation of Lipid Classes

Separation of the total lipid into the main lipid classes was accomplished by the TLC method of Freeman and West (1966), in which a single thin-layer plate is developed with successive polar and nonpolar solvent systems. Application of the lipid to the plate, and the development was performed in a nitrogen atmosphere.

The developed plates were sprayed with a solution of 0.2% 2', 7'-dichlorofluorescein in 95% ethanol and the lipids were visualized under ultraviolet light. The method was standardized with lipids of > 99% purity obtained from the Hormel Institute, Austin, Minnesota.

Preparation of Methyl Esters

Fatty acids were converted to their methyl esters prior to GLC analysis. The conditions suggested by Lowry[3/] regarding the type and quantity of catalyst and the time and temperature required to methylate

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[3/] Robert R. Lowry, Department of Agricultural Chemistry, Oregon State University, personal communication.

the different classes of lipids were followed. Table 2 shows these conditions. Since most fatty acids are in the form of various esters in their biological environments, the methyl esters can be easily formed by transesterification using an excess of five percent HCl in anhydrous methanol. This procedure was used for the free fatty acid and phospholipid classes. The latter class requires the longest time period for the transesterification, therefore, samples of total lipids were also prepared under these conditions. When esters of only the glyceride or steryl ester classes were to be prepared, methylation was accomplished by the less time consuming saponification with KOH.

Table 2. Conditions for preparing methyl esters of fatty acids.

Lipid Material	Catalyst[1]	Amount of Catalyst[2] (ml./0-100mg sample)	Bath (0^{o} C)	Time (min.)
Total lipids	5% HCl	3	80	90
Mixed phospholipids	5% HCl	3	80	90
Free Fatty acids	5% HCl	3	50	4
Cholesterol esters	0.4M KOH	2	50	30
Triglycerides, Diglycerides, and Monoglycerides	0.1M KOH	2	50	5

[1] Catalyst is dissolved in methanol in all cases. The HCl requires anhydrous (super-dry) methanol.

[2] Equal volumes of catalyst and diethyl ether are used.

Total lipid samples were placed in screw cap culture tubes with Teflon-lined caps, and the solvent was removed under nitrogen. Thin-layer chromatography plates on which the lipid classes had been separated were scraped in the appropriate regions and the scrapings placed in the tubes. After diethyl ether was added to solubilize the lipids, the proper catalyst was added and the capped tubes were heated in a water bath for the required period.

After cooling, the mixtures in the tubes were brought to a favorable pH for extraction by adding two ml. of 0.15 N HCl when KOH had been used, or two ml. of water when HCl was the hydrolyzing agent. Extraction was accomplished by vigorously shaking the mixture with two ml. of re-distilled hexane. The tubes were briefly centrifuged to facilitate the formation of a two-phase system. The upper hexane layer, containing the methyl esters, was removed with a syringe and combined with a second two ml. hexane extraction of the mixture in a clean tube.

The esters were stored in hexane at -20^{o}C under nitrogen until concentrated for analysis. The steroids and small amounts of hydrocarbons that are present in the final sample of esters do not usually interfere with the GLC analysis (Holman and Rahm, 1966). Occasionally, however, the extract required purification prior to GLC. This was done by passing the extract through a column of silicic acid (100-200 mesh) which had been washed with three percent diethyl

ether in hexane. The methyl esters were eluted with additional amounts of this solvent.

Separation and Identification of Fatty Acids

Except for a portion of the work with radioactive fatty acids, all fatty acid analyses were by GLC. The instrument was an Aerograph model 204B equipped with a hydrogen flame detector. The six foot by 1/8 inch aluminum column was packed with a polar liquid phase of EGS or DEGS on acid washed Chromsorb P (60/80 mesh). Nitrogen was used as a carrier gas and normal operating temperatures were 225°, 185°, and 210°C for the injector, column, and detector ovens, respectively.

Mixtures of high purity fatty acid esters, prepared according to National Heart Institute fatty acid standards, were purchased from Applied Science Laboratories, Inc. The four test procedures outlined by Horning, et al. (1964) were conducted with these standard mixtures. These procedures define the load limits of the column and detector, separately; the linear range of the instrument; the response of the instrument to homologs of differing weight and double-bond structure; and the separation efficiency of a given column. These parameters must be defined for quantitative work on the specific problem under study. Quantitative results with these standards (KA, KB, KC, KD) agreed with the

stated composition data with a relative error of less than five percent for major components (>ten percent of total mixture), and less than ten percent for minor components (<ten percent of total mixture).

Samples to be analyzed were taken to a concentration which, with one to two microliters, would insure optimum GLC conditions. The retention time of the recorded fatty acid peaks were compared to those of standards for identification. Quantitation was generally accomplished by determining the relative area percent of a fatty acid. Area was calculated as the product of peak height and retention time. Absolute amounts of the fatty acids were determined by using a known amount of methyl heptadecanoate as an internal standard. The detector responses relative to 17:0 were used to adjust the peak areas of the fatty acids so that the weight percent of each component could be calculated (Holman and Rahm, 1966).

Separation and Assay of Radioactive Lipids

The lipid classes containing ^{14}C-labeled fatty acids were scraped from the plates into scintillation vials containing 10 ml. of a scintillation solution (6 g. 2, 5-diphenyloxazole and 100 mg. 1, 4-bis [2-[5-phenyloxazolyl]] benzene per liter of reagent grade toluene). The vials were cooled and held in the dark at least 15 minutes before counting. Radioactivity was measured in a Nuclear-Chicago scintillation spectrometer. Samples were generally counted to within two

percent error. The observed counts were converted to dpm (disintegrations per minute) by using the method of channels ratio quench correction described by the manufacturer.

Methyl esters of the total lipid or of a lipid class were separated by TLC on silver nitrate-impregnated silica gel G with hexane-diethyl ether (90:10 v/v) as solvent (Malins, 1966). The fatty acid spots were located by spraying the plates with 2', 7'-dichlorofluorescein and viewing under ultraviolet light. Some plates were visualized by means of radioautography using the developed film to locate spots for quantitative ^{14}C assay.

Some mixtures of radioactive methyl esters were separated by GLC, and trapped for additional radioassay. An effluent splitter was used to divert about 70% of the gas stream to the collector, a cigarette filter (Hammarstrand, 1968). The 16 mm. silliconized filters were impaled on a needle fitted to the gas outlet. Collections were made during the elution of the compound as indicated by the hydrogen flame detector. The filter was placed directly into a counting vial and assayed by liquid scintillation.

The phospholipid class was scraped from the TLC plate into 20 ml. of chloroform-methanol-water (65:40:5 v/v/v) after separtion from the neutral lipid. This slurry was transferred to a sintered-glass funnel and washed with several portions of the same solvents. The filtrate was taken to dryness and the residue dissolved in a small

amount of chloroform. The component phospholipids were separated by TLC on plates of silica gel G using chloroform-methanol-water (65:25:4 v/v/v) (Privett et al., 1965). The PL were visualized under ultraviolet light after spraying with Rhodamine 6 G. Radioassay was by radioautography or scintillation counting.

RESULTS AND DISCUSSION

It has been established that most Lepidoptera require a dietary polyunsaturated fatty acid and that the needs of Trichoplusia ni are met only with linolenic acid. Beyond this there are many facets of the overall problem to be explored. The fatty acid content (especially linolenate) of insects from environmental conditions which produce normal and deformed moths, the deposition pattern of linolenic acid within the insect lipids, and the point in the life cycle at which 18:3 must be present to assure normal wings, are examples of the facts necessary for understanding how the PUFA might effect normal eclosion and wing expansion. Thus there is a need for a more thorough understanding of the metabolic fate of EFA in the affected insects. The experiments reported here were designed to meet this need.

Experiments with Bean and Wheat-germ Media

Fatty Acid Composition of Experimental Diets

The fatty acid content of the experimental diets was determined for comparison with the fatty acid patterns in the insects reared on these diets. Table 3 shows the GLC analysis of the bean plant and the two artificial diets used in the initial studies. The six major

fatty acids were present in significantly different proportions in these three diets, the most important differences being the virtual absence of oleate and the high proportion of linolenate in the bean plant. As a result of the high percentage of linolenate, the other fatty acids occurred in relatively low amounts compared to their levels in the artificial diets. No fatty acids of greater chain length or more unsaturation than 18:3 were present in the bean plant extracts.

Table 3. Fatty acid content of larval diets.

Diet	Relative Percent of Total Fatty Acids					
	16:0	16:1	18:0	18:1	18:2	18:3
Bean leaves	12.8	2.2	3.0	tr[1/]	8.4	73.6
Bean medium	31.9	5.0	6.3	7.2	39.4	10.2
Wheat-germ medium	21.5	.4	1.7	19.1	51.7	5.6

[1/] Tr = < 0.2%

The bean medium contained much less 18:3 and although the other unsaturated fatty acids were present in higher amounts than in the bean plant, there was 2.4 times more saturated fatty acids in this diet. In the wheat-germ medium palmitoleate was present in very low amounts, while oleate and linoleate contributed more to the total fatty acids than in the other diets. Linolenic acid was the longest and most unsaturated fatty acid in the bean medium but a trace of 20:0 was present in the wheat-germ medium. The sources of lipid in the wheat-germ diet are whole wheat-germ and wheat-germ

oil. Therefore, variations in these products may result in variations in the percentages of the component fatty acids in the diet. Additionally, wheat-germ which has been stored several months may undergo alterations in its fatty acid composition. Some recent results (Schaefer, 1968) indicate that the polyunsaturated fatty acids are most rapidly affected by storage and the 18:2 and 18:3 may decrease more than 70% during a six-month period.

Fatty Acids and Total Lipids of *Trichoplusia ni*

The Total Fatty Acids of Pupae. The total fatty acid content of pupae from larvae reared on the three diets discussed above is shown in Table 4. The large amount of 18:3 in the plant-reared insects compared to those reared on the laboratory prepared diets is a reflection of the amount of this fatty acid in the respective food sources. These insects are unable to synthesize 18:2 and 18:3 (Nelson and Sukkestad, 1968). The other fatty acids can be synthesized by *T. ni* as is evidenced by the 16:1 in wheat-germ-reared insects and the 18:1 in plant-reared insects compared to their levels in the respective diets. The two major fatty acids in the cabbage looper, except where 18:3 is the major dietary fatty acid, are palmitate and oleate. Thus, except for the PUFA, *T. ni* can significantly alter its fatty acids both qualitatively and quantitatively from those available in the diet.

Table 4. Pupal fatty acids of Trichoplusia ni reared on three larval diets. [1]

Diet	Relative Percent of Total Fatty Acids					
	16:0	16:1	18:0	18:1	18:2	18:3
Bean leaves [2]	21.1	1.1	5.5	9.5	6.1	56.7
Bean medium [3]	36.4	10.9	3.7	31.6	13.9	3.5
Wheat-germ medium [3]	33.1	9.5	1.8	36.3	17.5	1.8

[1] All 2-3 day old pupae reared at 25°C.

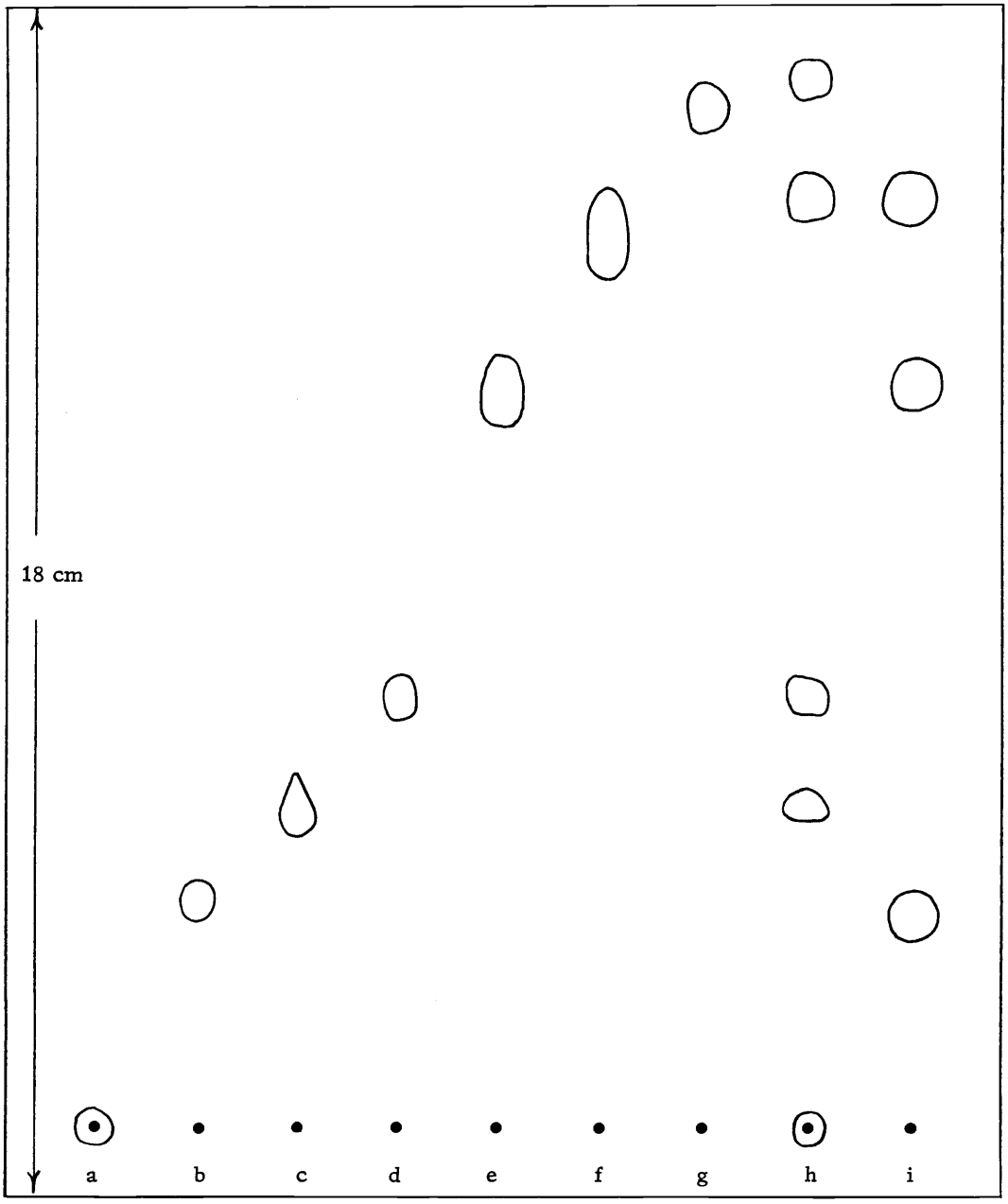
[2] Equal numbers of female and male pupae.

[3] Female pupae.

Phospholipid and Triglyceride Fatty Acids During Life Stages. The foregoing discussion has dealt with the distribution of fatty acids in all lipids of T. ni. General relationships between dietary and tissue fatty acids can be studied with this approach but important changes in the fatty acids within a particular class of lipids may be masked. This is particularly true of the lipid classes which constitute only a small part of the total lipids. Phospholipids (PL) often make up only a fraction of the total lipids compared to the triglycerides (TG) (cf. Gilby, 1965). Since members of the former class are vital components of the structural and biochemically active membranes, their fatty acid content is of considerable interest.

A more definitive study was begun by separating the total lipids of whole insects into lipid classes as described under "Methods." Plate I shows a typical lipid class separation. When the fatty acid

Plate I



Drawing of a TLC separation of the lipid classes. a) phosphatidylcholine; b) monoolein; c) linoleic acid; d) cholesterol; e) diolein; f) triolein; g) cholesteryl linolenate; h) mixture of phosphatidylcholine, oleic acid, cholesterol, tristearin, cholesteryl linoleate; i) mixture of monoolein, diolein, triolein. Load: a-g, 50 mg; h, 25 mg each; i, 40 mg each. Solid support: silica gel G; developing solvent: diethyl ether-benzene-ethanol-acetic acid 40:50:2:0.2 followed by diethyl ether-hexane 6:94.

esters of each class were analyzed by GLC the TG was found to be the lipid class with the greatest quantity of fatty acids. The order of the other classes, in quantity of fatty acids, was phospholipids $\geq$ diglycerides > free fatty acids >> steryl esters >> monoglycerides.

The small amount of monoglyceride did not permit quantitation of fatty acids. The amount of fatty acids in the steryl esters was generally adequate for quantitation of the major fatty acids. Oleate was the predominant fatty acid and palmitate was second in abundance. Linoleate or linolenate usually accounted for only a few percent of the total fatty acids and were often undetectable. The free fatty acids contained as much as 25 to 35% PUFA with 18:1 and 16:0 accounting for most of the remainder. Diglycerides contained two to eight times more PUFA than the triglycerides with 16:0, 18:1, 18:2, and 18:3 usually constituting more than 85% of the fatty acids.

The fatty acid content of PL and TG classes were compared as T. ni developed from larvae to adult. The triglycerides were included because they represent the depot lipids. Larvae, prepupae, early, mid, and late pupae, and the two day old, unfed adults were the stages analyzed. These insects were reared on the standard wheat-germ medium at 30° and 95% RH. Figure 1, showing the amounts of the six major fatty acids, summarizes the results of these experiments.

The acids tended to predominate in one class more than the

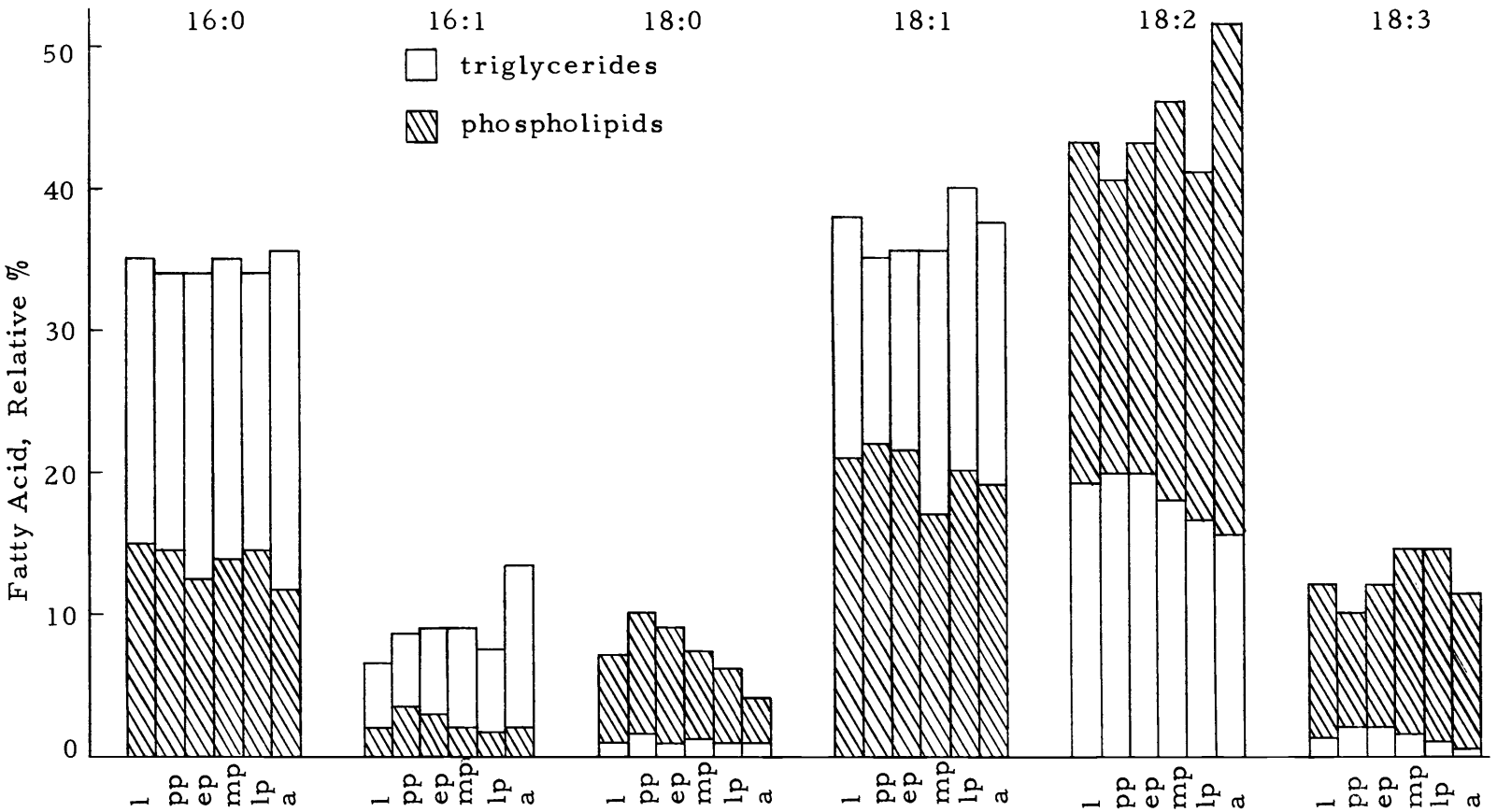


Figure 1. Fatty acid distribution in phospholipids and triglycerides of T. ni life stages. Larvae reared at 30°C, 95% RH, on the wheat-germ medium. Life stages designated as: l, larvae; pp, prepupae; ep, early pupae (0-1 days); mp, mid pupae (3-4 days); lp, late pupae (6-7 days); a, adults (2 days).

other in all the life stages. Palmitate, palmitoleate and oleate were relatively more concentrated in the TG fraction, while 18:0, 18:2 and 18:3 accounted for a greater portion of the PL acids. There were no major shifts in distribution of a fatty acid during the life stages. The distribution of fatty acids in the TG of the pupae was essentially the same as found by total fatty acid analyses (see Table 2). This probably results from the greater contribution of this class compared to the other classes of lipids.

More than three-fourths of the PL acids were unsaturated during all stages, with the adults containing up to 85% of these acids. The TG averaged approximately 65% unsaturated fatty acids from larva to adult. Palmitate accounted for almost all of the saturated acids in this class, about 35%, and roughly two-thirds of the saturates in the PL, averaging 13.4%. Stearate made up five to ten times more of the PL fatty acids than those of the TG (Figure 1).

Considering the extent of unsaturation in the component acids, the two monoenes accounted for a greater percentage of the TG fatty acids than the two saturated or two PUFA. The PUFA were selectively deposited in the PL as they made up at least half, and as much as 64%, of the acids in this class, but no more than 22% of those in the TG. The PL/TG ratio for each fatty acid (Figure 1) was calculated for each stage. This ratio is not an indication of total quantities present, but does indicate the relative importance of a fatty acid

to the lipid class. Beyond the early pupal stage, linolenate had the highest ratio, indicating that this acid had a greater tendency to deposit in the PL. Another indication of the preferential accumulation of PL 18:3 is that during most stages, it made up more than twice the percentage of PL fatty acids than of dietary fatty acids. Palmitoleate and stearate had this relationship also, but these acids can be biosynthesized whereas linolenate cannot.

Sexual Dimorphism in Lipid Level. Gilbert (1967) notes that in most insects the female contains more lipid than the male, but that the reverse may be true for the Lepidoptera. Indeed, he presents evidence that this is true in the American silkmoth Hyalophora cercropia where sexual dimorphism first becomes apparent at pupation. The male pupa contains 50% more lipid than the female per gram fresh weight. The report does not indicate whether the relative fatty acid composition of the lipid is altered. If this phenomenon occurs in the cabbage looper, unequal sex ratios of pupae in a sample could lead to erroneous conclusions.

An experiment was conducted to determine if sexual dimorphism occurred in the total lipid or relative fatty acid content of prepupae and pupae. All insects were reared on the same batch of wheat-germ medium at 30°C and approximately 90% RH. Table 5 lists the lipid and fatty acid composition of the prepupae and pupae. The total lipid content increased about two percent, on a fresh weight basis,

Table 5. Total lipid and fatty acid composition of male and female T. ni pupae.[1/]

Life Stage	Sex	Total Lipid (% wet wt.)	Relative Percent of Total Fatty Acids[2/]					
			16:0	16:1	18:0	18:1	18:2	18:3
Early prepupa	Female	9.2 (.06)[3/]	33.8	8.2	1.4	41.0	14.0	1.6
	Male	8.8 (.76)	34.6	8.5	1.4	40.5	13.4	1.6
Late prepupa	Female	11.4 (1.76)	34.4	9.3	1.3	41.2	12.1	1.7
	Male	10.5 (.79)	34.3	10.8	1.2	39.6	12.6	1.5
Pupa (8 hrs.)	Female	11.3 (1.45)	34.2	8.1	1.4	41.9	12.9	1.5
	Male	10.5 (.41)	34.3	10.2	1.4	39.6	13.1	1.4
Pupa (near eclosion)	Female	7.4 (4.29)	34.0	7.0	1.2	43.4	13.3	1.1
	Male	7.7 (.93)	35.2	8.2	1.4	41.0	13.1	1.1

[1/] Reared on wheat-germ medium at 30°C, 90% R.H.

[2/] Each value is an average of 2 replications of 2 insects each, duplicate analyses.

[3/] Range.

from the early prepupal stage to the late prepupal stage. The newly formed pupae contained the same amount of lipids as the late prepupae but those near eclosion contained about three and one-half percent less. The most important fact is that differences in lipid content between males and females of a given life stage were insignificant, as were the relative amounts of the individual fatty acids.

The only difference was a slightly higher amount of 16:1 in late male prepupae and pupae. This difference is reflected by the small decrease of 18:1 in these samples. The fatty acids of the PL were analyzed separately, but no differences could be seen between males and females at the same stage of development. This was also true when the fatty acid content of the TG was examined.

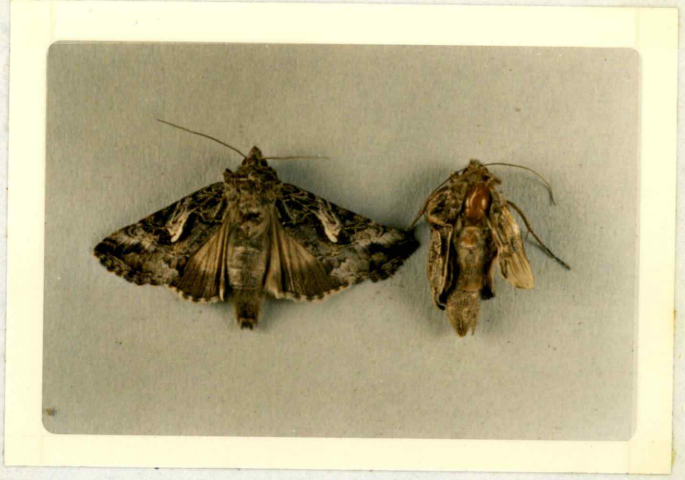
Environmental Effects on the Wing Deformity

Pupal Fatty Acids of Normal and Deformed Moths. Grau and Terriere (1967) reported that T. ni pupae from a larval diet containing marginal amounts of 18:3 produced a greater number of deformed moths at 30°C than those reared at 24°C. Plate II-a contrasts moths reared from these conditions. This led to the hypothesis that the linolenic acid, required for normal wings, was being destroyed or used for other purposes when rearing was carried out at 30°C, thus creating a deficiency of this fatty acid.

In a test of this hypothesis pupae reared at 24 and 30°C were

PLATE II

a. Trichoplusia ni from pupae reared at 30°C (left, Class C) and 24°C (right, Class A) when the larvae developed on the bean medium.



b. Autographa californica from pupae reared at 30°C with 0.25% linseed oil in the larval bean medium (left, Class A) and without linseed oil (right, Class C).

analyzed for fatty acids throughout the pupal stage. Early experiments indicated the necessity of using uniform diets since one source of variation in pupal lipid content was the differences in fatty acid composition of the bean or wheat-germ diet. As shown earlier pupal fatty acid composition partially reflects the lipid composition of the larval diet. Thus, it was necessary to rear the larvae on the same or similar batches of diet.

With larvae reared on bean medium there were no changes in fatty acid composition which could be attributed to differences in pupal rearing temperature. Each fatty acid remained at approximately the same level throughout this period. Pupae which were within 24 hours of adult emergence (8-9 days for 24° and 5-6 days for 30°) contained slightly more overall unsaturation in the fatty acids when reared at 24°C. This was a result of more di- and triene and less monene at this temperature. The level of linolenic acid did not differ significantly between 24 and 30°. Moths emerging from pupae held at these temperatures were shown previously to have approximately 96 and 16% "normal wings" respectively (Grau and Terriere, 1967).

The results of fatty acid analyses of T. ni. pupae reared on wheat-germ medium are given in Table 6. Again, there was very little change in fatty acid content during pupal development at either temperature, the only apparent change being the decrease in 18:3

content from first- to last-day pupae. However, 18:3 reached the same level in both experiments by the last day of development.

Table 6. Effect of rearing temperature and age of Trichoplusia ni pupae on fatty acid distribution.

Pupal Temp.	Pupal Age (Days)	Relative Percent of Total Fatty Acids[2/]					
		16:0	16:1	18:0	18:1	18:2	18:3
24°	1	33.9	8.7	2.1	36.0	17.5	1.8
"	2	33.4	9.4	2.0	36.6	16.9	1.7
"	3	34.2	8.9	2.1	35.8	17.3	1.7
"	4	32.6	8.7	1.9	37.2	18.0	1.6
"	5	33.4	9.0	2.3	36.7	17.2	1.4
"	6	32.5	7.9	2.0	38.3	17.7	1.6
"	7	33.3	7.8	2.0	37.7	17.9	1.3
"	8	33.5	9.1	2.0	37.4	16.7	1.3
30°	1	34.0	9.0	1.9	36.6	16.8	1.7
"	2	33.6	8.8	2.1	37.2	16.6	1.7
"	3	32.9	8.4	2.3	37.1	17.6	1.7
"	4	34.6	8.6	2.2	36.0	17.0	1.6
"	5	33.4	10.8	1.9	37.8	14.9	1.2
"	6	34.6	9.9	2.1	36.7	15.4	1.3

[1/] Larvae reared on wheat-germ medium.

[2/] Values for males and females averaged.

A further insight into this problem was attempted by examining the PL and TG fatty acids in pupae from the two temperatures. Groups of ten pupae of each sex were analyzed. The results from males and females from the same temperature were averaged (Table 7). The

TG fatty acids were the same at 24 and 30°C except for a slight increase in 18:2 at the lower temperature. The PL acids showed modest differences between the temperatures, the 30° PL containing more 18:1 and less PUFA. The difference in linolenate between the two temperatures was barely significant, and considering the difference in wing response, was much smaller than expected.

Table 7. Temperature influence on phospholipid and triglyceride fatty acids in T. ni pupae[1/].

Lipid Class	Pupal Temp. (°C)	Relative Percent of Total Fatty Acids					
		16:0	16:1	18:0	18:1	18:2	18:3
Phospholipids	24	12.2	1.6	5.8	17.5	49.0	13.9
"	30	13.4	1.8	6.3	20.5	45.1	12.9
Triglycerides	24	35.4	7.5	1.5	36.0	18.0	1.6
"	30	36.6	7.9	1.4	36.2	16.5	1.4

[1/] Reared on wheat-germ medium; pupae within one day of eclosion.

When the total fatty acids of Autographa californica and Heliothis zea were analyzed similar results were obtained, i.e., no significant differences in EFA were noted between temperatures. The former species reacted to the higher pupal rearing temperature much the same as T. ni but H. zea showed no sign of the wing deformity at either temperature when reared on the bean or wheat-germ medium.

Thus, a study of the relative levels of the component fatty acids, especially linolenic acid, in the pupae did not indicate why

the moths of T. ni and A. californica emerging from pupae reared at 24° had normal wings while those emerging from pupae reared at 30°C had a high incidence of deformed wings.

If the higher temperature resulted in a decline in all of the fatty acids and no change in their relative amounts, a reduction in 18:3 might become critical. However, a calculation of the actual amount of this acid in the T. ni pupae showed that the levels were not significantly different in insects reared at the two temperatures.

When linseed oil was used as a source of linolenic acid in the larval diet, normal moths of T. ni were obtained from pupae held at 30° C. (Grau and Terriere, 1967) as were those of A. californica (Schafer, Grau, and Terriere, 1968). Plate IIb shows adults of the alfalfa looper when pupae were reared at 30°C with and without linseed oil in the larvel bean medium. Table 8 shows the influence of 0.25% linseed oil in the bean medium on the pupal fatty acid content of the cabbage looper, alfalfa looper, and corn earworm. These species responded similarly to the modified diet and contained about ten percent of their fatty acids as linolenate compared to about four percent on the unsupplemented diet.

Table 8. Pupal fatty acids of three species reared on bean medium containing 0.25% linseed oil.[1/]

Species	Relative Percent of Total Fatty Acids					
	16:0	16:1	18:0	18:1	18:2	18:3
T. ni	33.0	6.8	4.2	30.2	14.7	11.1
A. californica	34.2	4.8	5.5	28.8	16.3	10.4
H. zea	34.5	5.9	3.6	29.9	16.8	9.3

[1/] All pupae from 30°C and within 1 day of eclosion.

The Role of Temperature and Humidity. During the months following the experiments described above, periodic checks were made of the response of the stock insect culture to rearing at 30°C. A varying but definite change in the wing deformity response was found to coincide with the use of new incubators. The humidity conditions in the new equipment differed from those of previous experiments and this factor played a more important role in the expression of the linolenate deficiency than had been thought. In the earlier experiments the humidity was maintained uniformly high and was thought to have little effect during the relatively hardy pupal stage since severe deformities were observed. Such humidities are known to obscure fatty acid deficiency symptoms in mammals.

Table 9 lists the wing condition of adult T. ni from pupae reared at two temperatures and several humidities. Direct comparisons of results at one RH and two temperatures are not entirely valid since

there is more water in the air at the higher temperature. If the effect is related to the absolute amount rather than the relative amount of moisture, comparisons should be made at only one temperature.

Table 9. Wing condition of T. ni reared at different temperatures and humidities.

Larval Diet	Pupal Rearing Conditions	Number of Specimens	Wing Condition, % 1/		
			A	B	C+D
Wheat-germ medium	30°, 95% RH	22	0	0	100
"	30°, 65% RH	21	72	14	14
"	30°, 20% RH	29	14	3	83
"	23°, 50% RH	43	42	28	30
Bean Medium	30°, 95% RH	199	1	4	95
"	30°, 65% RH	76	45	24	31
"	30°, 45% RH	111	49	19	32
"	30°, 20% RH	21	5	10	85
"	23°, 95% RH	126	13	13	74
"	23°, 45% RH	112	60	11	29
Bean Medium with Linseed Oil	30°, 95% RH	26	42	50	8
"	30°, 45% RH	25	68	28	4
"	23°, 95% RH	27	70	19	11
"	23°, 45% RH	27	81	0	19

1/ Wing classification described in methods.

The first three conditions at 30°C, with pupae from wheat-germ medium, show almost as large a range in wing response from

humidity changes as was previously credited to temperature change. Considering only the 30° conditions from the two larval diets, it can be seen that the relative humidities of 95% and 20% gave nearly the same response, a high incidence of severe wing deformity. Less deformity occurred when the RH was held at a more moderate level. The results at the lower temperature were not as clear, but it is seen from the bean medium data that the incidence of the deformity was slightly less at 95% RH.

The experiments in which linseed oil was added to the bean medium as a linolenate source show that this supplement largely overcame the adverse effect of temperature and humidity. The most dramatic reversal of the deformity occurred at 95% RH. Even here separate temperature and humidity effects were evident, for the lower of the two temperatures at the same humidity and the lower of the two humidities at the same temperature, resulted in a greater number of perfect wings than the reverse situation.

In additional experiments, pupae from bean medium were taken from 30°C and 65% RH approximately eight hours prior to eclosion and reared at 30°C in either 95% or 45% RH. Of 39 moths from the 95% RH chamber and 44 moths from the 45% RH chamber, the percent of "normal wings" was 3 and 57, respectively, indicating that the humidity influence on the wing condition occurs at the time of emergence and wing expansion. These data indicate that both temperature and humidity are important factors in the wing deformity

with adverse humidity being more critical than high temperature.

To more accurately assess the temperature influence, the larvae were reared on the more easily controlled wheat-germ residue diet, described under "Methods." The larval diet contained 0.050, 0.125 and 0.250% methyl linolenate and the pupae were maintained at 95% RH and either 23° or 30°C. Table 10 shows that the temperature effect was most obvious at the 0.125% level of 18:3, the threshold amount for normal wings. Linolenate at 0.050% is not enough to allow normal wings under the most ideal conditions and 0.250% is near the amount where wings develop normally at either temperature.

Table 10. Effect of linolenate concentration on wing deformity on T. ni pupae reared at two temperatures.[1/]

Dietary 18:3 Concentration (%)	% Normal Wings[2/]	
	23°C	30°C
0.05	0	0
0.125	83	54
0.250	100	90

[1/] 95% RH maintained at each temperature.

[2/] Wing deformity classes A and B combined.

To further explore the effect of environmental conditions on the expression of the nutritional deficiency, larvae were reared on both diets and pupae were held at 23° or 30°C and either 20 or 95% RH for measurements of total lipid, relative amounts of the fatty

acid, and percent of actual linolenate. Samples were taken at the mid-point and at the last day of the pupal stage. Table 11 lists the total lipid content of pupae from each diet. The only significant differences in total lipid occurred in last-day pupae from the 95% RH condition at either temperature and either diet. These two conditions were previously shown to result in the most severe wing deformity. An increase in water content or in non-lipid constituents at 95% RH would produce such a result since the calculations were on a fresh weight basis. However, the water content of pupae from the four environmental conditions was found to remain unchanged during the pupal stage. Pupal weight decreased approximately 25% in the pupae reared at 23°C and 20 or 95% RH. These results prove that an actual decrease in total lipid did occur at the high relative humidity with either temperature.

The total fatty acid composition was essentially the same for all four combinations of temperature and humidity. As in previous analyses, there was no indication that the relative amount of 18:3 was significantly different.

The actual concentration of linolenic acid is shown in Table 11. In every instance the level of 18:3 was less at the end of the pupal stage than at the beginning. Since the two conditions resulting in the least 18:3 were those resulting in the least total lipid (95% RH at either 23 or 30°C), it is understandable that these two conditions

Table 11. Effect of temperature and humidity on total lipids and linolenate content in T. ni pupae.

Pupal Rearing Conditions	Pupal Age (Days)	Total Lipid[1] (% of Wet Wt.) Bean Medium	Total Lipid[1] (% of Wet Wt.) Wheat-germ Medium	Linolenic Acid[2] (% of Wet Wt.) Bean Medium	Linolenic Acid[2] (% of Wet Wt.) Wheat-germ Medium
30°, 95% RH	0	4.7 a[3]	9.7 c	.230	.138
30°, 95% RH	3	4.3 a	9.1c	.219	.112
"	6	2.3 b	8.3 d	.148	.081
30°, 20% RH	3	4.6 a	10.9 c	.242	.117
"	6	3.8 a	9.8 c	.204	.102
23°, 95% RH	5	3.8 a	10.1 c	.193	.123
"	9	2.8 b	7.8 d	.165	.086
23°, 20% RH	5	4.4 a	10.8 c	.248	.148
"	9	4.6 a	10.2 c	.221	.119

[1] Mean of 3 replicates.

[2] Mean of 2 replicates.

[3] Means followed by same letter are not significantly different at the 5% level.

would result in more wing deformity. Evidently the higher humidity causes the insects to expend more lipid during the pupal stage. Temperature is of less importance in this regard. These data do show a temperature factor, however, since at each humidity level, with one exception, the higher temperature resulted in less linolenic acid by the end of the pupal stage.

The difference in total lipid and linolenate levels in the pupae reared on the two diets is of interest. Pupae resulting from rearing on the wheat germ medium contain nearly twice as much total lipid as those on the bean medium, but the reverse is true of their linolenate content. The latter fact reflects the level of 18:3 in the bean medium (Table 3). A perplexing aspect of these analyses is that, even though the bean medium furnishes more 18:3, it is not significantly better than the wheat germ diet in preventing wing deformity (Table 9). One explanation for this is that none of the 18:3 levels shown in Table 11 are adequate for normal wings under the conditions examined. Another possibility is that the form of the 18:3 resulting from growth on the bean medium is less satisfactory than that resulting from the wheat-germ medium. In other words, the nature of the compound lipid containing the linolenic acid may have a bearing on the adequacy of the diet.

These experiments clearly show that with marginal levels of dietary 18:3, temperature and humidity become stress factors

resulting in an increased expression of the deficiency. If the levels of 18:3 are adequate, these stresses are overcome.

It is clear that workers studying the nutrition of essential fatty acids in insects must define their temperature and humidity conditions.

Experiments With Controlled Diets

The problem of variable levels of PUFA in the previous diets was overcome by an exhaustive chloroform-methanol extraction of the wheat germ (see "Methods"). This assured a basal diet virtually free of lipids. Dietary fatty acids could be added and studied for their effects on time to pupation, wing deformity, and level of pupal fatty acids. Rearing during these experiments was done at 30^{o}C and 95% RH, in a growth chamber.

The extracted and unsupplemented wheat-germ diet used as the negative control still contained about 46 mg. of lipid per 100 grams of diet. This amount may have resisted extraction or it may have been present in other dietary components. The PUFA content of these lipids was such that total consumption of 18:3 by a larva eating 2.5 g of medium, an intake above normal, would amount to only 45 micrograms. The error due to these unextracted fatty acids is thought to be small.

Effect of Lipids on Larval Development

The effect of several plant oils and fatty acids on pupation in the cabbage looper and the alfalfa looper is shown in Table 12. Two criteria were used to evaluate the larval nutrition of these diets: the percentage of larvae reaching pupation, and the number of days required for 50 and 90% pupation. With no added lipid in the wheat-germ residue diet, only four percent of the T. ni larvae pupated and 19 days were required for half of these larvae to reach pupation. Wheat-germ oil added to the diet at a level of 0.75% was the amount calculated to supply the level of fatty acids found in the standard wheat-germ medium. Nearly all larvae reared on this control diet and on the other vegetable oil supplemented diets became pupae, but linseed oil was superior for larval development.

Individual fatty acids were fed in some experiments to avoid the possibility of interactions between the fatty acids present in the vegetable oils. The saturated fatty acids, palmitic and stearic, did not promote larval growth in T. ni. These acids appeared to have a toxic action since no larvae developed beyond the third instar and many died in the first instar. In contrast to this the unsupplemented medium permitted survival to the fourth or fifth instar and some larvae were able to pupate. The response to these two fatty acids was the same whether alone or in combination, as the free acid, or as

Table 12. Effect of supplementation with plant oils and fatty acids on the larval development of T. ni and A. californica reared on the wheat-germ residue diet.

Supplement	Amount (mg/100g)	Initial No. Larvae	% Pupated	Mean Days to Pupation 50%	Mean Days to Pupation 90%
		T. ni			
None	---	220	4	19.0	23.0
Wheat-germ oil[1]	750	98	98	12.9	15.1
Safflower oil[2]	750	20	100	14.5	16.1
Cottonseed oil[3]	750	20	95	14.5	15.7
Linseed oil[4]	750	187	94	11.8	13.1
Methyl palmitate	250	40	0[7]	----	----
Methyl stearate	250	40	0[7]	----	----
Methyl oleate	250	20	35	17.2	18.8
Methyl linoleate	100	20	90	15.9	18.7
" "	150	20	80	15.5	17.9
" "	250	40	85	15.8	18.7
" "	500	20	60	18.7	21.6
Trilinolein	150	20	90	14.8	16.9
"	460[5]	20	85	15.0	18.2
Methyl linolenate	50	60	83	15.1	17.2
" "	100	104	87	14.2	16.3
" "	150	85	94	13.2	16.0
" "	250	121	92	13.7	16.3
" "	430[6]	20	60	17.0	21.8
" "	500	60	58	16.5	19.6
Trilinolenin	150	20	95	13.5	15.1
"	430	20	100	12.8	14.1
Methyl linoleate	250	20	80	16.0	21.7
Methyl linolenate	250				

Table 12. (continued)

Supplement	Amount (mg/100g)	Initial No. Larvae	% Pupated	Mean Days to Pupation 50%	Mean Days to Pupation 90%
		A. californica			
None	---	20	10	21.5	21.8
Wheat-germ oil	750	10	100	11.0	11.8
Linseed oil	750	10	100	11.0	12.0
Methyl linoleate	250	10	100	13.5	17.0
" "	500	10	100	12.7	17.0
Methyl linolenate	50	20	45	16.0	18.5
" "	100	40	80	13.8	16.1
" "	150	39	93	12.7	15.0
" "	250	10	100	11.8	16.0
" "	500	10	100	12.3	13.0

1/ Contains 6.6% 18:3.

2/ Contains < .50% 18:3.

3/ Contains no detectable 18:3.

4/ Contains 53-56% 18:3.

5/ Equivalent to 18:2 in 750 mg wheat-germ oil.

6/ Equivalent to 18:3 in 750 mg linseed oil.

7/ Larvae died within three instars.

the methyl ester.

Methyl oleate had a positive effect on larval growth compared to the lipid-free diet, but did not support optimal growth. Only 35% of the larvae pupated and 17.2 days were required for 50% pupation. In H. zea reared on a diet containing only oleate (Vanderzant, 1968) 96% of the larvae reached the pupal stage. Rock, Patton, and Glass (1965) also showed that this fatty acid was nearly comparable to 18:2 and 18:3 in supporting larval development in the red-banded leaf roller. Either these species differ from T. ni in their response to 18:1, or the diet used was contaminated with PUFA.

The growth of T. ni larvae on the diets containing 100 to 250 mgs of methyl linoleate was essentially the same, averaging 85% pupation with a mean 50% pupation time of 15.7 days. A level of 500 mg resulted in less pupation and a longer larval period. When linoleic acid was supplied as the triglyceride, pupation and development were better than with the same amount of the methyl ester.

The mean number of days to 50% pupation for T. ni was approximately two days less on methyl linolenate. Growth rate improved as the level of this ester increased from 50 to 150 mg per 100 g of diet. As with 18:2, 500 mg of methyl linolenate caused a large decrease in pupation and an increase in the time to pupation. Results with trilinolenin or methyl linolenate at 0.15% in the diet were the same, but at 0.43% the triglyceride was superior to the ester.

A combination of methyl linoleate and methyl linolenate, each at 250 mg/100 g of diet, resulted in 80% pupation. This was better than the 58 (18:3) or 60 (18:2) percent occurring at the 500 mg level of these methyl esters but poorer than the results with the individual fatty acids at 250 mg. Apparently there is no interaction between these two acids.

Autographa californica, like T. ni, exhibited low survival and poor growth on the lipid-free diet. The alfalfa looper developed slightly faster on diets containing wheat-germ or linseed oil but the more rapid development seen in T. ni on linseed oil was not evident. As methyl linolenate in the diet of A. californica was increased from 0.05%, the percent pupation increased and the number of days to pupation decreased in contrast to results with T. ni (Table 12). The alfalfa loopers developed more rapidly than the cabbage loopers at a comparable level of methyl linoleate or methyl linolenate.

Although data were not recorded for H. zea, it was observed that approximately 50% of the larvae pupated when fed the lipid-free medium. The development period was longer than that of control groups but this is considerably better than results with the two looper species. It was also noted that H. zea responded linearly in pupation, like A. californica, to increases in dietary methyl linoleate or methyl linolenate from 50 mg to 500 mg per 100 g of diet.

Thus, in summary, the two loopers require a source of dietary

fatty acid for larval development. Even with H. zea development is abnormal without fatty acids. The most favorable development occurs with plant oils. When the component fatty acids were assayed individually with T. ni, only those with some degree of unsaturation promoted larval development and pupation. Of these, linolenate had greater activity than linoleate which, in turn, was more active than oleate.

Most of the fatty acids in plant oils are in the triglyceride form. When these acids were assayed as triglycerides, the results were better than with the methyl esters. This may be due to easier assimilation from the gut or to an absence of deleterious side reactions with these more neutral compounds.

Pupal weights of T. ni from the above experiments showed that the heaviest pupae resulted when linseed oil was the lipid source or when linolenate was used at 150 mg/100 g diet. There was no significant difference in the weights of pupae when the methyl ester or triglyceride of a fatty acid were compared.

Effect of Lipids on Wing Deformity

Trichoplusia ni. The ultimate measure of EFA nutrition in these moths is the wing deformity. Table 13 shows the extent of this deficiency in T. ni reared on various sources of fatty acids. Only a few larvae reached the pupal stage on the lipid-free diet and,

Table 13. Effect of supplementation with plant oils and fatty acids on wing condition of T. ni reared on the wheat-germ residue diet.

Supplement	Amount (mg/100g)	No. of Specimens	Wing Condition, Percent			
			A	B	C	D
None	---	7	0	0	0	100
Wheat-germ oil	500	17	6	0	88	6
"	750	48	6	19	71	4
"	1000	29	14	45	38	3
Safflower oil	750	14	0	0	29	71
Cottonseed oil	750	14	0	0	0	100
Linseed oil	750	117	85	11	4	0
Methyl linoleate	50	8	0	0	0	100
" "	100	7	0	0	0	100
" "	150	15	0	0	7	93
" "	250	21	0	0	5	95
" "	500	22	0	0	4	96
Methyl linolenate	50	62	0	0	28	72
" "	100	95	8	14	75	3
" "	150	82	56	18	23	3
" "	250	119	75	19	6	0
" "	430	19	79	11	10	0
" "	500	32	75	19	3	3
Trilinolenin	430	18	100	0	0	0
Methyl linoleate + Methyl linolenate	250 + 250	22	59	27	14	0

as expected, none of the adults could split open the pupal cuticle to commence emergence. There was a linear relationship between the number of A + B wings and the level of dietary wheat-germ oil, but even at the highest rate these accounted for only 59% of the adults. This agrees with the earlier experiments which showed that the wheat-germ medium containing wheat-germ oil as the lipid was an inadequate source of linolenic acid for T. ni reared at 30°C and 95% humidity.

These results differ from those of Chippendale, Beck and Strong (1965) who reported that 92% of T. ni had perfect wings when wheat-germ oil was used at approximately 0.715% of diet. These workers cite evidence that wheat-germ oil contains 5.2% linolenic acid (Nelson, Glass and Geddes, 1963) but the data provided by their source of the oil indicate a level of 3.5 percent.[4/] These specifications would indicate that linolenic acid at either 39 or 26 mg per 105 g of diet, depending on the values used, was adequate for normal wings. Another report by these authors indicates that 100 mg of methyl linolenate per 100 g of diet is near the threshold level (Chippendale, Beck and Strong, 1964). These results suggest that the requirement is less when linolenic acid is supplied as a plant oil than when it is the only lipid.

[4/] In a letter from Viobin Corporation, Monticello, Illinois, on May 25, 1966.

From the previously cited data of Chippendale et al., we would expect wheat-germ oil to be three to four times better than methyl linolenate as a source of 18:3 in the looper diet. The wheat-germ oil used in the present study contained 6.6% linolenic acid according to our analyses. The amount of 18:3 present in the three levels of wheat-germ oil tested (33-66 mgs, Table 13) was about half as much as was required for similar results when supplied as the methyl ester (50-150 mg). The same comparison when linolenate was supplied in linseed oil shows that the fatty acid ester is fully equivalent to the oil. This anomaly may be due to the fact that the level of 18:3 in wheat-germ oil is approximately one-eighth that in linseed oil, and thus the tests were in a range of greater biological variation. It may also reflect a difference in the type of glycerides in the two oils.

The possibility that less linolenic acid was required when other fatty acids were present prompted an experiment in which the yield of normal moths was compared from diets containing 18:3 alone and in combination with other fatty acids. Methyl linolenate was assayed at 50, 100, 150, and 250 mg/100 g of diet with and without cottonseed oil at 750 mg/100 g of diet. The cottonseed oil contained approximately 33, 38 and 17% of 16:0, 18:1 and 18:2, respectively, and no measurable 18:3. Thus, it was a good source of fatty acids other than the one required for normal wings. Significantly better wing conditions were obtained when linolenate was combined with this

oil (data not shown). Although no normal moths were obtained at the lowest rate of 18:3 and some were caught inside the pupal case, all of the moths escaped the case when this diet included cottonseed oil. On the control diets containing only cottonseed oil there was no adult emergence. The sparing effect of the cottonseed oil was most evident at the intermediate levels of 18:3. At 100 mg of linolenate/100 g of diet, the percentage of normal wings was improved by a factor of 2.6, while at 150 mg the improvement was 1.2. At the 250 mg level, cottonseed oil had no effect.

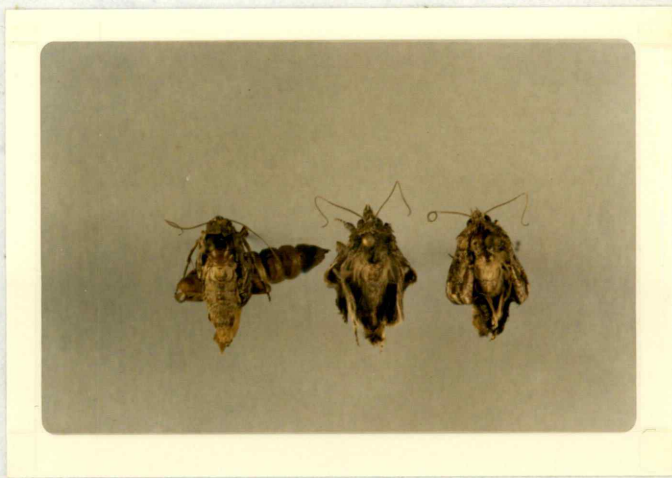
Table 13 lists the results obtained with other plant oils commonly used as sources of fatty acids in artificial diets. The safflower oil used in these experiments contained less than 0.5% linolenic acid, allowing only 29% of the adults to escape the pupal case, and these were severely deformed. The results with cottonseed oil have already been discussed. The linseed oil used throughout this study contained 53-56% linolenic acid. Nearly all the adults from larvae reared on 75 mg of linseed oil/100 g of diet were capable of normal flight and 85% had perfectly formed wings (Plate IIIa)..

Plates III-b – V-a show the wing condition of T. ni from this diet supplemented with several levels of linolenate.

According to the data in Table 13, the optimum or adequate level for 18:3 was reached at 0.25% and further supplementation to 0.50% did not improve the wing condition. At these quantities all but

PLATE III

a. Trichoplusia ni with perfectly developed (A) wings from wheat-germ residue diet containing 0.75% linseed oil and a pupal rearing temperature of 30°C.



b. Trichoplusia ni from larval diets containing 0.05% (left, Class D) and 0.10% (middle and right, Class C) linolenate. Pupae were reared at 30°C and 95% RH.

PLATE IV

a. Trichoplusia ni reared from larvae on diets containing 0.10% 18:3. The wings of the moth on the left were spread to aid in depicting the condition classified as a severe deformity (Class C).

b. Trichoplusia ni (Class A) from larval diet containing 0.25% linolenate. Pupa reared at 30°C and 95% RH.

PLATE V

a. Trichoplusia ni (Class A) from larval diet containing 0.43% trilinolenin. Pupa reared at 30°C and 95% RH.

b. Trichoplusia ni from larval diets containing 0.05% 18:3. Examples of incomplete adult emergence which results in assigning these moths to Class D.

6 to 10% of the adults could fly and about 75% had wings in category "A" (Plate IV-b). The results with methyl linoleate confirm the fact that T. ni cannot utilize this fatty acid for adult eclosion.

When trilinolenin was used at 0.43% in the diet (the amount of 18:3 supplied in the linseed oil control) the results were much better than with the same amount of the methyl ester, with all moths classed as perfect (V-a). Methyl linolenate at 0.25% combined with an equal amount of linoleate resulted in fewer normal moths than when assayed alone at this rate. This is not necessarily a contradiction to the previous finding that other fatty acids can spare linolenic acid. The polyunsaturated fatty acids may compete for the same site within a critical class of lipids, in which case 18:3 would not occupy as many of these sites when an equal amount of 18:2 is available. The sparing fatty acids could be the ones usually associated with the depot lipids which, when present, permit more of the PUFA to be used elsewhere.

Several reasons can be listed for the differences that have been found between the results of the present study and those of other workers. The significant role that environmental conditions play in the expression of the deficiency symptom has been adequately discussed above. Chippendale, Beck and Strong (1964, 1965) do not cite the temperature and relative humidity conditions for pupal rearing in their experiments. Failure to control these parameters

could lead to results different from the writer's. Another source of variation is the laboratory culture used. The purity of the fatty acids is not a likely source of disagreement since both the present study and the one mentioned above used fatty acids of equal purity from the same supplier.

A definite possibility for the different response is the "lipid-free" basal diet which contained extracted wheat-germ. The wheat-germ residue used by Chippendale, Beck and Strong (1964, 1965) was prepared by extracting whole wheat-germ twice in a Waring blendor and then drying.[5/] The author found considerable lipid in wheat-germ extracted in this manner and took the additional steps described in a previous section to assure a more fully extracted product. Analysis of the fatty acids was performed regularly as an additional control. Apparently the above workers did not measure the fatty acid content of their extracted residue.

The subjective nature of the wing deformity classification is another source of error when comparing work from different laboratories. Rather late in the bioassay experiments, the writer realized that he was probably being too critical in differentiating the perfect (A) and slightly deformed (B) moths. The moths classed as having a minor deformity very often had perfect fore wings and were capable of near-normal flight.

Chippendale, Beck and Strong (1965) state that moths they

[5/] Personal communication from Dr. S. D. Beck.

classified as having a minor wing deformity were capable of only limited flight. In the present work these were classed as severely deformed. Because of these differences, the A and B classes of the author can generally be considered to contain normal moths. The writer considered moths that remained partially within the pupal case to not be fully emerged and assigned them to class D (Plate V-b).

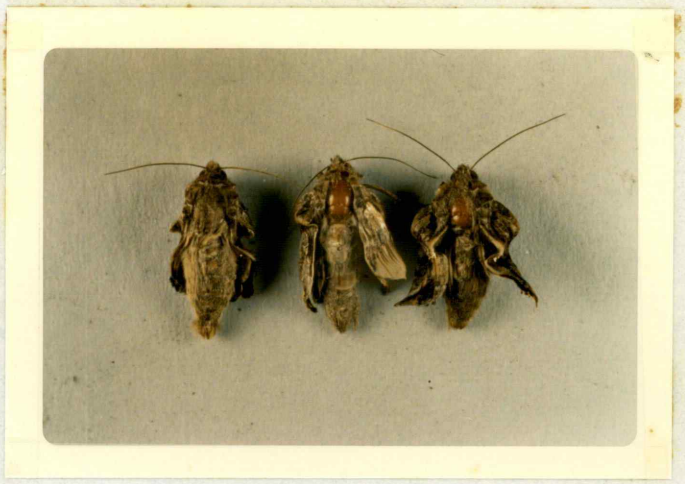
Autographa californica. There is no information in the literature associating an EFA with wing deformities in the alfalfa looper, A. californica. The effect of two plant oils and 18:2 and 18:3 on wing deformity in this species is shown in Table 14. In general, the results show that the alfalfa looper has the same qualitative requirement as the cabbage looper in that only linolenic acid allows the moths to emerge with normal wings. The quantitative requirement appears to be different, however. All moths that emerge from wheat-germ oil supplementation are severely deformed (Plate VI-a) whereas in tests with the cabbage looper 25% were normal. Even linseed oil resulted in fewer perfect wings.

The tests with methyl linoleate confirm its lack of activity. Experiments with graded increases of methyl linolenate confirmed the lesser response of this species than with T. ni, as there was an average of 14% more severely deformed alfalfa loopers at levels from 100 to 500 mg of linolenate/100 g of diet. Trilinolenin was not

Table 14. Effect of supplementation with plant oils and fatty acids on wing condition of A. californica and H. zea reared on the wheat-germ residue diet.

Supplement	Amount (mg./100g)	No. of Specimens	Wing Condition, Percent			
			A	B	C	D
		A. californica				
None	---	2	0	0	0	100
Wheat-germ oil	750	37	0	0	89	11
Linseed oil	750	24	71	17	8	4
Methyl linoleate	250	12	0	0	0	100
" "	500	14	0	0	0	100
Methyl linolenate	50	8	0	0	88	12
" "	100	28	0	4	96	0
" "	150	32	34	25	41	0
" "	250	38	55	29	16	0
" "	500	36	75	6	11	8
		H. zea				
None	---	11	0	0	0	100
Wheat-germ oil	750	8	100	0	0	0
Linseed oil	750	7	100	0	0	0
Methyl linoleate	50	12	0	0	8	92
" "	100	18	0	0	61	39
" "	250	34	9	18	62	11
" "	500	9	67	22	11	0
Methyl linolenate	25	12	17	0	83	0
" "	50	20	90	5	5	0
" "	100	10	70	20	0	10
" "	250	26	77	12	8	3

PLATE VI



a. Autographa californica (Class C) from diets containing 0.75% wheat-germ oil. Pupae were reared at 30°C and 95% RH.

b. Heliothis zea from larval diets containing 0.05% (left, Class D), 0.10% (middle, Class C) and 0.25% (right Class C) linoleate. Pupae were reared at 30°C and 95% RH.

included in these experiments.

Heliothis zea. These data are also reported in Table 14. After the completion of these experiments, a report by Vanderzant (1968) described the effects of 18:2 and 18:3 on the wing condition of H. zea. The author had previously presented his results on this subject to the Entomological Society of America.[6/] Vanderzant (1968) showed approximately 13% emergence of H. zea from larvae reared without added dietary lipids, although the moths were deformed. This indicated that some contaminant PUFA was present although the diet did not contain any known source of fatty acids. Indeed, Vanderzant's analyses of these pupae showed small amounts of 18:2 and 18:3.

The striking difference between this species and the two species of loopers is that both 18:2 and 18:3 promote emergence and normal moths. Very few adults emerged with 0.05% linoleate in the diet but emergence improved with increased 18:2 until all emerged at 0.5%.

Plates VI-b and VII-a show the wing condition of earworms reared on diets containing linoleate. At 0.25% linoleate, the author found only 9 percent of the moths with perfect wings but 0.50% produced 67% of the moths in this class. These results contrast with those of Vanderzant (1968) who reported a maximum of 22.6% perfect

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[6/] National meeting, New York City, December 1967.

PLATE VII

a. Heliothis zea from a larval diet containing 0.25% 18:2 showing the variation in Class C wing deformity obtained at this near adequate level. Pupae were reared at 30°C and 95% RH.

b. Heliothis zea (Class A) from larval diets containing 0.05% linolenate. Pupae were reared at 30°C and 95% RH.

wings at 0.20% dietary methyl linoleate and no improvement with additional amounts. This is inconsistent with the fact that the control diet used by Vanderzant contained corn oil which supplied approximately 285 mg of 18:2/100 g of diet. Her results with the control diet cannot be attributed to 18:3 in the corn oil since this amount of oil supplied much less linolenate than was found to be required for normal wings.

Only small amounts of linolenic acid were required for a high percentage of normal moths in the present study with H. zea. Methyl linolenate at 25 mg/100 g of diet resulted in complete emergence although 83% of the moths were severely deformed. Plate VII-b shows the typical wing condition of H. zea reared on 0.05% 18:3. At levels of 50, 100 and 250 mg, the sums of the perfect and slightly deformed moths were 95, 90 and 89%, respectively. Vanderzant (1968) reported 86.8% moths in these categories with 0.25% linolenate, but increasing the amount had no further effect. Comparing the two polyenes at 0.25% in the diet, the writer found methyl linolenate to be 3.3 times more effective than methyl linoleate for the production of moths capable of flight.

Although the three species studied required PUFA for development of normal adults, they differed in the level and specificity of this requirement. The cabbage looper and the alfalfa looper responded to linolenic acid and not to linoleic acid, but the corn

earworm was like most other moths thus far studied in that either fatty acid allowed normal wings.

Studies with higher animals have shown that different families of PUFA are not interconvertible (Klenk and Pflüger, 1963). Likewise since H. zea analyses, to be reported below, gave no evidence of tissue 18:3 from dietary 18:2, it is not likely that 18:2 is being converted to 18:3 by the earworm. The greater effectiveness of 18:3 may be due to its stronger affinity for enzymes at a site that influences emergence in these insects. As noted above, linolenic acid is known to have a greater enzyme affinity than linoleic acid in mammalian systems (Mead, 1968).

Effect of Lipids on Total Fatty Acids of Pupae

Trichoplusia ni. The influence of dietary oils and fatty acids on the total fatty acids of T. ni pupae is shown in Table 15. Wheat-germ oil added to the lipid-free diet resulted in nearly the same tissue fatty acid distribution as the standard wheat-germ medium from which it was derived. Linseed oil in the diet increased the 18:3 content to approximately 14.0% and, virtually eliminated the wing deformity as shown previously. The inadequacy of safflower and cottonseed oil is readily apparent from the low level of 18:3 in the pupal tissue. The major differences in pupal fatty acids from loopers fed these dietary oils occurred in the polyenes--the fatty acids which

Table 15. Effect of supplementation with plant oils and fatty acids on the pupal fatty acids of T. ni reared on the wheat-germ residue diet. [1/]

Supplement	Amount (mg/100g)	No. Samples [2/]	Relative Percent of Total Fatty Acids					
			16:0	16:1	18:0	18:1	18:2	18:3
None	---	3	33.3	19.5	1.8	44.7	Tr [3/]	0
Wheat-germ oil	750	3	32.2	10.3	1.7	37.6	16.4	1.8
Linseed oil	750	4	32.3	7.5	2.4	39.2	4.5	14.1
Safflower oil	750	1	30.8	7.7	2.1	35.5	23.5	0.4
Cottonseed oil	750	1	33.2	10.4	2.2	37.5	16.7	0
Methyl oleate	100	1	32.5	22.4	1.6	43.5	0	0
Methyl linoleate	50	1	31.8	18.2	2.1	44.7	3.2	0
" "	100	3	33.5	16.9	1.4	41.9	6.3	0
" "	250	4	34.3	12.6	1.9	39.1	12.1	0
" "	500	2	34.4	7.8	2.0	31.9	23.9	0
Methyl linolenate	50	4	32.2	17.9	2.0	45.6	Tr	1.6
" "	100	6	33.2	17.2	2.0	44.1	Tr	2.8
" "	150	5	33.6	16.5	1.9	43.1	Tr	4.5
" "	250	10	34.8	14.5	2.0	41.5	Tr	6.9
" "	500	3	34.8	10.9	1.9	36.5	Tr	15.0
" "	750	1	33.0	6.2	2.4	34.2	Tr	24.0
Methyl linolenate + Methyl linolenate	250 + 250	2	34.0	11.4	1.9	34.6	11.6	6.5

[1/] Pupae within 1 day of adult eclosion.

[2/] Samples consisted of 2-4 pupae analyzed 2-3 times each.

[3/] Tr = < 0.2%.

T. ni is unable to synthesize.

Analyses of pupae from the lipid-free diet revealed that the absence of PUFA in the tissue lipids resulted in abnormally high amounts of monoene, particularly 16:1. It is of interest that the saturated fatty acids occurred in approximately the same amounts from normal and abnormal diets. Dietary oleate did not affect tissue levels of 16:0, 18:0, or 18:1 but 16:1 was slightly higher.

When dietary PUFA were supplied, major changes occurred in the pupal fatty acid patterns. As linoleate was increased, the pupae contained correspondingly higher amounts of this fatty acid. This increase was reflected in a decrease of the monoenoic fatty acids and no change in the saturated acids. At 500 mg/100 g of diet the total fatty acid content of 16:1 had decreased 60.0% and that of 18:1, 28.6% from the lipid-free condition.

In a study of high purity dietary fatty acids upon fatty acid composition, Keith (1967) noted that dietary 18:2 caused a marked decrease in 16:1 and 18:1 in D. melanogaster. In rats, dietary 18:2 caused a moderate decrease in 16:1 and 18:1 in the liver (Mohrhauer and Holman, 1963b), but chickens showed a response to 18:2 much like the insects. In the depot fat of this organism 16:1 and 18:1 decreased sharply (Marco et al., 1961).

The pupal fatty acids were similarly affected by increases of dietary linolenate except that this acid was incorporated into the lipids

to a lesser extent. As a consequence, the reduction of 16:1 and 18:1 in the pupae was not as great in insects fed linolenate as in those fed linoleate.

Five hundred mg methyl linolenate/100 g of diet resulted in nearly the same amount of tissue 18:3 as the linseed oil control diet which contained the equivalent of approximately 400 mg of 18:3. This is consistent with the observations made earlier, that linolenic acid is utilized more efficiently in the presence of other dietary fatty acids, and the triglyceride form, which predominates in the oil, is more effective than the ester. When linoleate and linolenate were supplied together, each at 0.25% in the diet, the acids were incorporated in the same amounts as when they were given singly.

As with the dietary oils, a constant saturated to unsaturated ratio of pupal fatty acids was maintained as greater levels of 18:2 or 18:3 were fed. However, the total unsaturation increased as the di- or triene replaced the monoene.

Autographa californica. Table 16 shows the fatty acid content of alfalfa looper pupae reared on the wheat-germ residue diets. Results from the lipid-free diet followed the general pattern found in the cabbage looper, i.e., a greatly increased monoene content, compared to diets containing the oils, but they also revealed a slight decrease in palmitate. When methyl linoleate was fed at 250 and 500 mg/100 g of larval diet, A. californica pupae contained the same amount of 18:2

Table 16. Effect of supplementation with plant oils and fatty acids on the pupal fatty acids of *A. californica* and *H. zea* reared on the wheat-germ residue diet. [1/]

Supplement	Amount (mg/100g)	No. Samples [2/]	Relative Percent of Total Fatty Acids					
			16:0	16:1	18:0	18:1	18:2	18:3
		A. californica						
None	---	1	24.5	24.1	1.8	49.3	Tr [3/]	0
Wheat-germ oil	750	1	28.5	6.6	1.9	44.1	16.8	2.1
Linseed oil	750	1	27.2	6.4	2.5	43.2	4.8	15.9
Methyl linoleate	250	1	26.5	13.1	1.9	46.6	11.9	0
" "	500	1	28.1	7.8	1.9	38.9	23.3	0
Methyl linolenate	50	1	26.2	17.7	1.7	50.5	Tr	3.3
" "	100	1	29.6	12.1	3.3	49.3	0	5.7
" "	250	1	31.5	11.0	1.8	45.1	0	10.6
" "	500	1	29.3	8.0	2.0	39.8	0	20.9
		H. zea						
None	---	2	29.0	17.5	1.7	51.4	Tr	0
Wheat-germ oil	750	1	31.0	5.4	2.5	32.7	25.0	3.4
Linseed oil	750	3	30.3	5.0	3.2	37.1	6.0	18.4
Safflower oil	750	1	30.9	4.3	2.5	33.9	28.3	Tr
Cottonseed oil	750	1	34.8	5.9	2.6	35.3	21.4	0
Corn oil	750	1	30.2	4.5	1.5	33.6	30.2	0
Methyl linoleate	50	1	34.2	16.8	2.3	43.8	2.9	0
" "	100	1	34.5	18.2	3.4	38.8	5.1	0
" "	250	2	36.9	10.8	1.5	37.0	13.8	0
Methyl linolenate	25	2	32.0	22.2	1.4	42.6	Tr	1.4
" "	50	2	32.7	21.9	1.3	41.7	Tr	2.0
" "	100	2	35.4	19.6	1.8	39.5	Tr	3.4
" "	250	2	37.8	8.7	1.7	42.7	Tr	8.9

1/ *A. californica* pupae with 1 day of adult eclosion, *H. zea* pupae about midway through development.
2/ Samples of 2-4 pupae analyzed 2-3 times each. 3/ Tr = < 0.2%.

as did T. ni on these dietary levels. However, when equal dietary rates of methyl linolenate are compared, e.g. 0.05%, it is seen that A. californica pupae had 2.3 times more 18.3 in their tissues. The difference factor decreased as dietary linolenate was increased, but at 0.50% there was still 1.4 times more 18:3 in the alfalfa loopers. It was previously shown (Tables 13 and 14) that more A. californica adults were deformed than those of T. ni when comparisons were made at the same levels of linolenate in the diet. This, coupled with the above observation indicates that alfalfa loopers have a greater requirement for dietary 18:3. As in T. ni, the monoenoic fatty acids of A. californica decreased as either of the PUFA increased. Palmitoleate was the more responsive of the two monoenes.

Heliothis zea. Table 16 also lists the fatty acids of the earworm pupae from these larval diets. Comparisons of the data from diets containing vegetable oils showed that H. zea incorporated somewhat larger amounts of 18:2 and 18:3 into the tissue lipids than the two loopers. As a result, the earworm pupae contained less 16:1 and 18:1 than the other species. Vanderzant (1968) reported that with corn oil or linseed oil as lipid sources, H. zea contained about 42% 16:0. This is much more than was found in the present study with these oils.

Again in this species, the important difference between insects reared on a lipid-free diet and those reared on the fortified diets was

in their content of monoenoic acids. When the individual PUFA were supplied alone, the much larger accumulation of these fatty acids observed in experiments with diets containing the oils was not evident. The pupal content of 18:2 from diets with 0.25% linoleate was only slightly higher than in the other two species but this level of linolenate in the diet resulted in 18:3 deposits intermediate between T. ni and A. californica. Although 16:0 varied to a greater extent in H. zea than in the loopers, the general pattern of monoene replacement by polyenes prevailed.

Fatty acid analyses of pupae from similar experiments by Vanderzant (1968) differed from those of the present study. In her experiments, where either linoleic or linolenic acids were used as the only dietary lipid, palmitic acid varied from 31.3 to 42.6%, and with one exception, made up a greater portion of the fatty acids than in the work described here. In addition, palmitoleic levels in the Vanderzant report were consistently lower with the same dietary rates of 18:2 or 18:3 than the levels found in the present work, and this fatty acid did not decrease with every increase in dietary PUFA. By contrast, oleic acid occurred at a higher amount than in the present study with each experimental condition. These differences may be due to analytical error since an inspection of Vanderzant's results shows that her analyses of duplicate samples varied much more than those of this study.

General Comments. These three species of moths demonstrated fatty acid dietary interactions differing in several respects from those found in vertebrates. A major difference was the absence of a plateau in tissue levels of PUFA as the dietary content was increased. Trichoplusia ni reared on bean plants deposited over 60% of its fatty acids as 18:2 and 18:3 (Table 4). Similar results have been noted in other insects (Schmidt, 1964). Thus, these insects tend to accumulate the PUFA rather than transform them to the longer, more unsaturated compounds commonly found in studies with rats and fish.

Data from this and other laboratories shows that insects cannot convert 18:2 or 18:3 into such products. Thus the essentiality of these acids for growth and adult eclosion must not be due to their role as precursors to other fatty acids. This contrast with the rat where arachidonic acid, a product of 18:2 metabolism, is actually the fatty acid utilized for correction of the EFA deficiency.

Fatty Acid Distribution in the Lipid Classes

The type of lipid into which the various fatty acids were incorporated was determined in an effort to gain a more detailed account of EFA metabolism in cabbage looper pupae. The insects used in these experiments were reared on the wheat-germ residue diet which was supplemented, as required, with fatty acids or linseed oil. Initially the methyl esters of the phospholipids (PL), free fatty acids

(FFA), monoglycerides (MG), diglycerides (DG), triglycerides (TG) and steryl esters (SE) were measured by GLC after TLC isolation. However, the analysis of monoglycerides was soon discontinued as this class had a fatty acid content too low for accurate quantitation.

Special attention was given to the steryl esters, even though they constituted only a small portion of the total lipids, since these compounds often have hormone-like properties or act as precursors to certain hormones. The fatty acid content of these esters in T. ni pupae was examined under conditions of adequate and deficient linolenic acid, but there was no evidence that this class was involved, other than in a passive way, with EFA distribution. Linolenic acid was often undetectable in these compounds even when it was the only dietary fatty acid. Similarly, analyses of FFA and DG, from experiments involving various levels of dietary PUFA, did not provide any insight into the action of linolenic acid as the EFA for normal wings. Therefore, only the PL and TG classes were studied in further comparison of fatty acid contents.

Table 17 shows the fatty acids of the PL and TG classes in pupae of T. ni reared on diets containing linseed oil or PUFA. With each dietary situation, 18:0, 18:2 and 18:3 made up a greater portion of PL than TG, while 16:0, 16:1 and 18:1 occurred to a greater relative extent in the TG. The linseed oil used in the study contained approximately 15 and 53% of 18:2 and 18:3, respectively. Despite this

Table 17. Pupal fatty acids of the phospholipid and triglyceride classes in T. ni reared on linseed oil or PUFA supplemented wheat-germ residue diets. [1/]

Supplement	Amount (mg/100g)	Lipid Class	Relative Percent of Fatty Acids					
			16:0	16:1	18:0	18:1	18:2	18:3
Linseed oil	750	PL	13.2	1.2	7.0	21.8	13.0	43.8
"	"	TG	33.9	9.0	1.2	41.5	3.4	11.0
Methyl linoleate	150	PL	13.2	5.3	7.0	35.3	37.0	2.2
"	"	TG	37.5	13.2	0.4	43.9	5.0	0
Trilinolein	150	PL	12.6	4.8	6.5	35.5	38.0	2.6
"	"	TG	37.6	11.4	0.7	45.3	5.0	0
"	460	PL	12.8	2.6	7.6	20.7	54.5	1.8
"	"	TG	37.0	9.5	0.7	39.4	13.4	0
Methyl linolenate	150	PL	13.9	4.7	5.8	40.2	2.1	33.3
"	"	TG	35.6	13.9	0.8	46.6	0.2	2.9
Trilinolenin	150	PL	13.6	3.5	6.7	35.1	2.4	38.7
"	"	TG	35.7	13.0	0.5	47.6	0.2	3.0
Methyl linolenate	430	PL	15.3	2.5	6.6	27.3	3.2	45.1
"	"	TG	36.3	10.3	0.9	40.3	0.7	11.5
Trilinolenin	430	PL	13.6	1.7	7.3	24.2	1.8	51.4
"	"	TG	35.3	9.6	0.9	43.1	0.2	10.9

[1/] All pupae from 30°C, 95% RH and within 1 day of eclosion.

large difference, the PL/TG ratios for the two fatty acids were essentially the same, indicating that, at these dietary levels, there was no selective deposition of either acid.

There was no difference in deposition of 18:2 with dietary methyl linoleate or trilinolein at 150 mg/100 g of diet. As dietary trilinolein increased from 0.15% to 0.46%, linoleate increased 1.43 times in the PL and 2.68 times in the TG. This suggests the beginning of a plateau in the PL level of this constituent. Such a plateau would be expected if a fatty acid was selectively deposited in a certain lipid until a suitable balance was reached.

The 18:3 found in the PL but not the TG of insects fed only 18:2 is another indication of the strong tendency for PUFA to be used in the PL. This linolenic acid originated as a dietary contaminant and averaged only 0.3% of the total fatty acids.

Trilinolenin resulted in more PL 18:3 than did the methyl ester at both 0.15 and 0.43% of the diet. There was no difference in the TG between these sources of 18:3. Again, this indicates that trilinolenin is utilized more efficiently than methyl linolenate. Support for the greater efficiency of trilinolenin also comes from a previous observation that the triglyceride permitted a greater number of perfect moths than the same level of methyl ester.

When 18:3 is the only dietary PUFA it constitutes approximately the same relative percent of the PL fatty acids as does 18:2 under

similar circumstances. Also, as with 18:2 in the diet, 18:3 made up a greater relative portion of the TG fatty acids as the dietary level increased. When dietary linolenate increased approximately three-fold, TG linolenate increased an average of 3.31 times. Concurrently, PL 18:3 showed only a 1.34-fold increase, indicating the development of a plateau in this lipid fraction.

There is a much greater quantity of TG than PL in T. ni lipids and since each TG molecule contains more sites of attachment, there may actually be a greater percent PUFA by weight in the TG. However, the quantity of PUFA in the PL results in a much greater relative level of these acids in this class. The level of dietary 18:3 which resulted in only a few severely deformed moths was between 0.25 and 0.43% (Table 12). Thus it appears that until these dietary levels are reached, there is a selective deposition of this EFA in the phospholipids. With further dietary increases, a plateau appears to be reached in the PL and 18:3 becomes more available to the depot lipids.

Table 18 lists the results of similar experiments with H. zea. In spite of the low amount of contaminant PUFA in the unsupplemented diet, they accounted for nearly six percent of the PL fatty acids. This may explain the more favorable growth of H. zea than the loopers which was noted earlier with the unsupplemented wheat-germ residue diets. Apparently the earworm is highly efficient in removing PUFA

Table 18. Pupal fatty acids of the phospholipid and triglyceride classes in H. zea reared on PUFA supplemented wheat-germ residue diets. [1/]

Supplement	Amount (mg/100g)	Lipid Class	Relative Percent of Fatty Acids					
			16:0	16:1	18:0	18:1	18:2	18:3
None	---	PL	11.1	18.0	5.2	59.9	4.4	1.4
"	"	TG	25.6	16.2	0.9	56.5	0.5	0.3
Methyl linoleate	250	PL	13.2	7.0	5.1	20.5	52.5	1.7
"	"	TG	38.6	12.6	0.4	39.2	9.2	0
Trilinolein	250	PL	15.8	5.8	6.0	17.7	53.0	1.7
"	"	TG	38.2	12.3	0.6	39.6	9.2	Tr
Methyl linolenate	25	PL	14.0	19.9	5.8	46.7	4.0	9.6
"	"	TG	34.4	22.9	0.5	41.7	0.2	0.3
"	50	PL	13.3	15.5	5.8	41.5	3.2	20.7
"	"	TG	33.7	22.4	0.6	42.6	Tr [2/]	0.6
Trilinolenin	250	PL	15.3	5.1	5.7	34.5	3.1	36.3
"	"	TG	36.8	14.8	0.4	46.0	0.4	1.6

[1/] All pupae from 30°C, 95% RH and within 1 day of eclosion.

[2/] Tr = <0.2%.

from its diet. Palmitoleate and oleate were rather uniformly distributed between the PL and TG of insects receiving unsupplemented diets. As the diet improved, these acids were displaced in the PL by PUFA.

A comparison of methyl linoleate and trilinolein at 250 mg/100 g of diet showed that both forms resulted in identical amounts of PL and TG linoleic acid. The PL and TG of H. zea contained approximately the same amount of 18:2 when it constituted 0.25% in the diet as in T. ni when the diet contained 0.46%.

The amount of PL and TG 18:3 doubled as the dietary level increased from 0.025 to 0.050%, but not beyond this level. The fact that H. zea and T. ni contained about the same level of PL 18:3 at dietary levels of 0.15 and 0.25%, respectively, confirms the greater ability of the former species to use PUFA. Heliothis zea was previously shown to require much less dietary 18:3 for normal wings than T. ni (Tables 12 and 13).

At the 0.25% level of 18:2 or 18:3 a large difference in the lipid class distribution occurred. These acids contributed heavily to the PL class but relatively little to the TG. A similar affinity for 18:3 was also seen in the PL of T. ni (Table 17).

In the discussion of the total fatty acid measurements it was noted that an increase in tissue PUFA resulted in a corresponding decrease in monoenoic acids. An inspection of Table 17 and 18

shows that this relationship was also maintained within the PL and TG. There were no appreciable changes in the saturated fatty acids with an increase of dietary PUFA. Since it has been seen that the PUFA are more important components of the PL (Tables 17 and 18), it follows that this class should have the largest decrease in monoenoic fatty acids. This was the result during the five-fold increase in dietary 18:3 as seen in Figure 2, which shows the unsaturated fatty acids of T. ni PL and TG.

Thus, the demonstration of monoene replacement by polyene, seen in the results of the total fatty acid analyses, was mostly an indication of PL metabolism. The TG unsaturates consisted of nearly all monoenoic acids. Figure 3 shows the same relationships in the unsaturates of H. zea pupae over a larger range of dietary 18:3. These figures re-emphasize the major result of the lipid class studies--that the PL are the lipids most responsive to the available PUFA in the insect diet if the relative make-up of the classes are compared.

Short-Term Feeding Studies with Linolenate and Other PUFA

Critical Stage for Dietary Linolenate. Table 19 shows the results of tests to determine if the EFA requirement of T. ni could be satisfied by feeding methyl linolenate to fifth instar larvae previously reared on diets free of linolenate. Consumption of diet containing

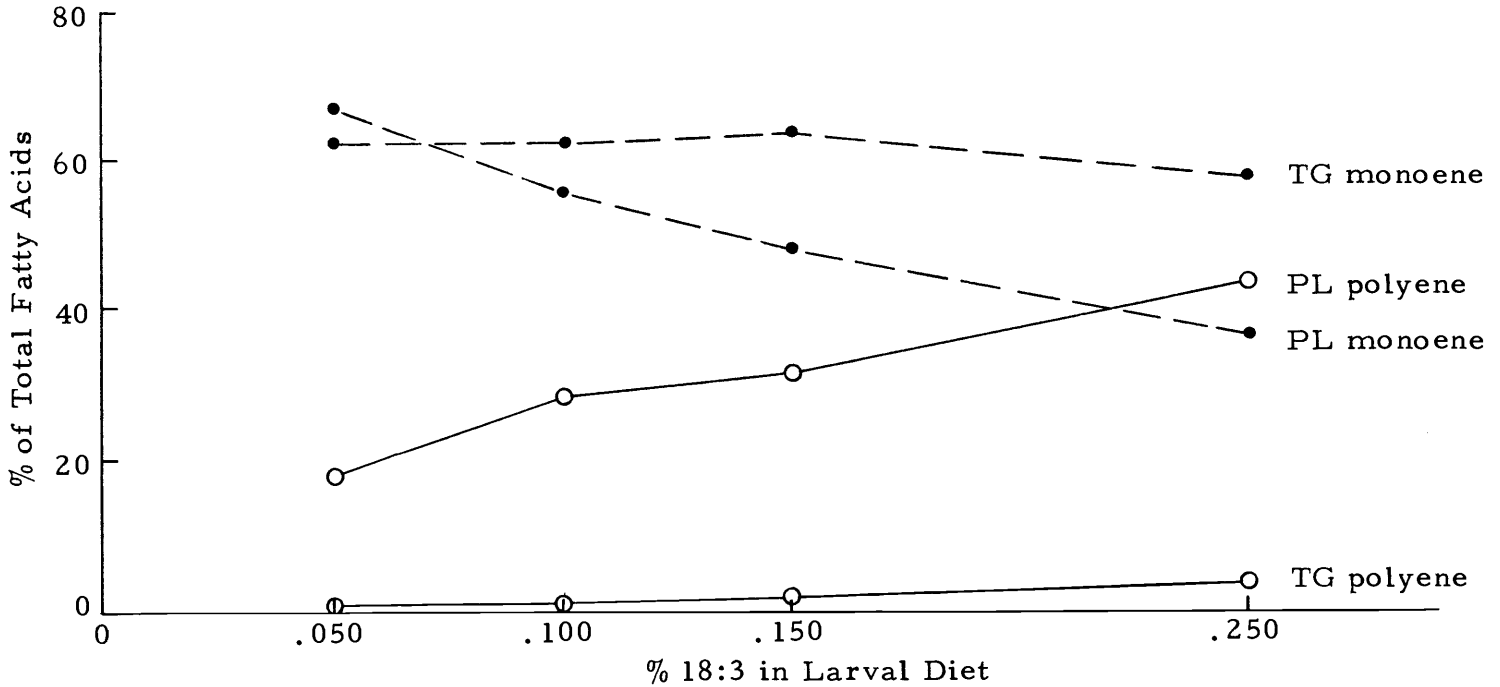


Figure 2. Change in monoene and polyene fatty acid content of phospholipids and triglycerides in T. ni pupae from larvae reared on wheat-germ residue diets containing increasing levels of linolenic acid. Pupae were reared at 30°C and 95% RH and analyzed within 1 day of eclosion.

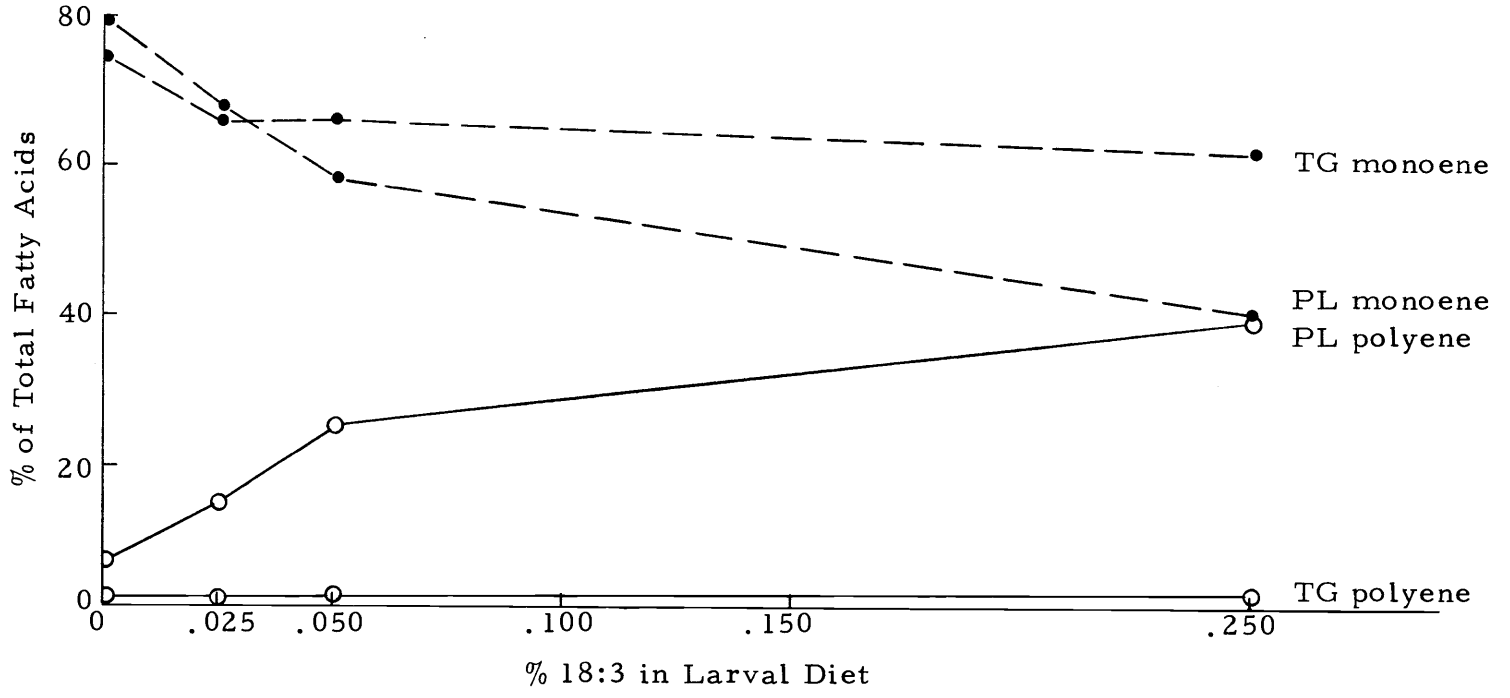


Figure 3. Change in monoene and polyene fatty acid content of phospholipids and triglycerides in H. zea pupae from larvae reared on wheat-germ residue diets containing increasing levels of linolenic acid. Pupae were reared at 30°C and 95% RH and analyzed within 1 day of eclosion.

Table 19. Prepupal feeding time on 18:3 deficient and adequate diets and extent of wing abnormality in T. ni adults. [1/]

% 18:3 in Diet		Time on [2/] Prepupal Diet, Hrs.	Number of Specimens	% Normal [3/] Wings
Previous	Prepupal			
0	0.25	0	6	0
0	0.25	36	4	25
0	0.25	48	12	84
0	0.50	12	4	25
0	0.50	24	4	25
0	0.50	42	30	90
0.15	0	0	4	100
0.15	0	12	4	100
0.15	0	36	8	25
0.15	0	54	14	0

[1/] T. ni reared on 18:3 deficient or adequate diet prior to transfer, during 5th instar, to indicated prepupal diet.

[2/] Time on diet prior to beginning of prepupal period.

[3/] Sum of A + B wings.

the EFA prior to the larval-pupal molt was tantamount to rearing the entire larval stage on an adequate diet. As expected a low level of dietary 18:3 necessitated a longer feeding period to alleviate the deformity symptom. Approximately 42 hours of feeding was necessary on a diet of 0.50% linolenate for nearly all the adults to have normal wings.

Similar results were obtained when A. californica larvae, taken from a deficient diet, were fed 0.50% methyl linolenate for 36 to 60 hours prior to pupation. Of 13 larvae, all produced perfectly formed moths.

On the basis of previous experience an initial rapid incorporation of linolenate into the PL fraction would be predicted. Indeed, Table 20 shows that 18:3 made up about 25% of the PL fatty acids after only 24 hours of feeding on a diet of 0.50% linolenate. During this period, only 1.9% of the TG fatty acids consisted of 18:3. After 48 hours of feeding the emerging adults were normal and pupal PL and TG contained approximately 37 and 4% linolenate, respectively.

The red-banded leaf roller is also able to obtain sufficient dietary EFA during the last larval instar (Rock, Patton, and Glass, 1965). These observations are in accord with EFA studies in the rat, as tail growth resumed and tail lesions disappeared when fat was added to the previously fat-free diet (Burr and Burr, 1929).

Table 20. Linolenate content of T. ni phospholipids, triglycerides, and total fatty acids after fifth instar feeding on 18:3 supplemented wheat-germ residue diets. 1/

Lipid	Hrs. Fed Prior to Pupation 2/			
	24	48	64	70
Phospholipids	24.6	36.9	43.1	41.1
Triglycerides	1.9	4.2	6.2	5.4
Total fatty acids	4.1	8.4	9.4	11.9

1/ All larvae fed 18:2 at 0.15% until placed on experimental diet, pupae analyzed 1 day prior to eclosion.

2/ Diet contained 0.50% 18:3.

The persistence of 18:3 in the last instar was also studied. Table 19 shows that 18:3 could be depleted below the threshold for normal wings when such larvae were deprived of this EFA. The larvae were reared through the fourth instar on a diet containing 0.15% methyl linolenate. This is enough 18:3 to enable approximately three-fourths of the adults to have normal wings if feeding continued until pupation. After only 12 hours on the deficient diet the wing deformity increased sharply.

Pupae from larvae reared on the deficient diet so as to cause the adults to fail in escaping the pupal case contained only 8.2% 18:3 in the PL and 1.4% in the total fatty acids. Normally, as seen in Table 20, the levels would be approximately 37 and 8%, respectively, from a diet of 0.50% linolenate for 48 hours.

Sufficient incorporation of the essential component at the unknown site or in the critical tissue occurred just prior to pupation. It might be expected that a nutrient so vital to the development of the organism would be conserved more efficiently. Apparently 18:3 is available to the general pool of metabolic systems and is not spared when in limited quantities.

Results with Esters of Other Lipids. Table 21 lists some additional esters which were examined for EFA activity in T. ni using the last instar feeding method. The cholesterol ester of linolenic acid at 0.25% did not result in normal moths through 80 hours of feeding. This molecule is probably hydrolyzed in the gut and the sterol and fatty acid absorbed separately, but it was tested on the possibility that it was directly absorbed or that additional dietary sterol was necessary for the transport of 18:3. Even though the linolenate makes up less than half of the molecular weight of this ester, better results were expected. For example, in previous assays with methyl linolenate at 0.10% (Table 13), some normal wings occurred. Apparently, 18:3 was used less effectively in this form. Only C and D wing classes resulted from larvae fed throughout the 80 hour period. There is no indication that a longer feeding period would have changed the outcome of the experiment.

The percent of 18:3 in the pupal PL, TG and total fatty acid portions was 8.8, 0.4 and 1.8, respectively, for insects fed the steryl

Table 21. Wing condition in *T. ni* after feeding fifth instar larvae on some methyl linolenate substitutes. [1/]

Substitute Lipid					Wing Condition, Percent			
Chemical Name	Abbreviated Designation	Amount (mg/100g)	No. Larvae	Hrs. Fed	A	B	C	D
Cholesteryl 9, 12, 15-octadecatrienoate	Cholesteryl 18:3 (ω 3)	250	21	48-80	0	0	67	33
Methyl 6, 9, 12-octadecatrienoate	$CH_3$18:3 (ω 6)	250	14	12-84	0	0	0	100
Methyl 5, 8, 11, 14-eicosatetraenoate	$CH_3$20:4 (ω 6)	250	16	12-84	0	0	0	100
Methyl 5, 8, 11, 14, 17-eicosapentaenoate	$CH_3$20:5 (ω 3)	250	12	36-84	0	0	0	100
Methyl 4, 7, 10, 13, 16, 19-docosahexaenoate	$CH_3$22:6 (ω 3)	250	28	12-84	0	0	0	100

[1/] All larvae fed either methyl linoleate at 0.15% or cottonseed oil at 0.75% until placed on experimental diet.

ester. These levels are considerably below the thresholds previously established for normal wings. Evidently, little uptake of 18:3 is possible when presented in this form.

To test the possibility that another 18 carbon acid with three double bonds would replace linolenate for T. ni, methyl 6, 9, 12-octadecatrienoate (18:3 ω6 or γ linolenate) was assayed at 0.25% in the diet. This fatty acid differs from linolenate (18:3 ω 3) only in the position of the double bonds. The results in Table 21 show that the location of the double bonds is critical since no moths emerged from larvae fed this fat. Pupae from this experiment were analyzed for fatty acids to determine if γ linolenate was incorporated into the insect lipids. The results showed a distribution much like that of 18:3 (ω 3) taking into account the dietary concentration and the feeding period. The γ linolenate accounted for approximately 22, 4 and 6% of the PL, TG and total fatty acids, respectively, in pupae. Thus, this fatty acid isomer of linolenate entered the same lipids as the normal 18:3 but did not allow the slightest positive response.

Methyl 5, 8, 11, 14-eicosatetraenoate (arachidonate) is an EFA for the rat but when this PUFA was fed to T. ni, no EFA activity was evident (Table 21). Likewise, methyl 5, 8, 11, 14, 17-eicosapentaenoate and methyl 4, 7, 10, 13, 16, 19-docosahexanoate, metabolites of linolenic acid in vertebrates did not relieve the deficiency when assayed at a level of 0.25% in the larval diet. These two PUFA were

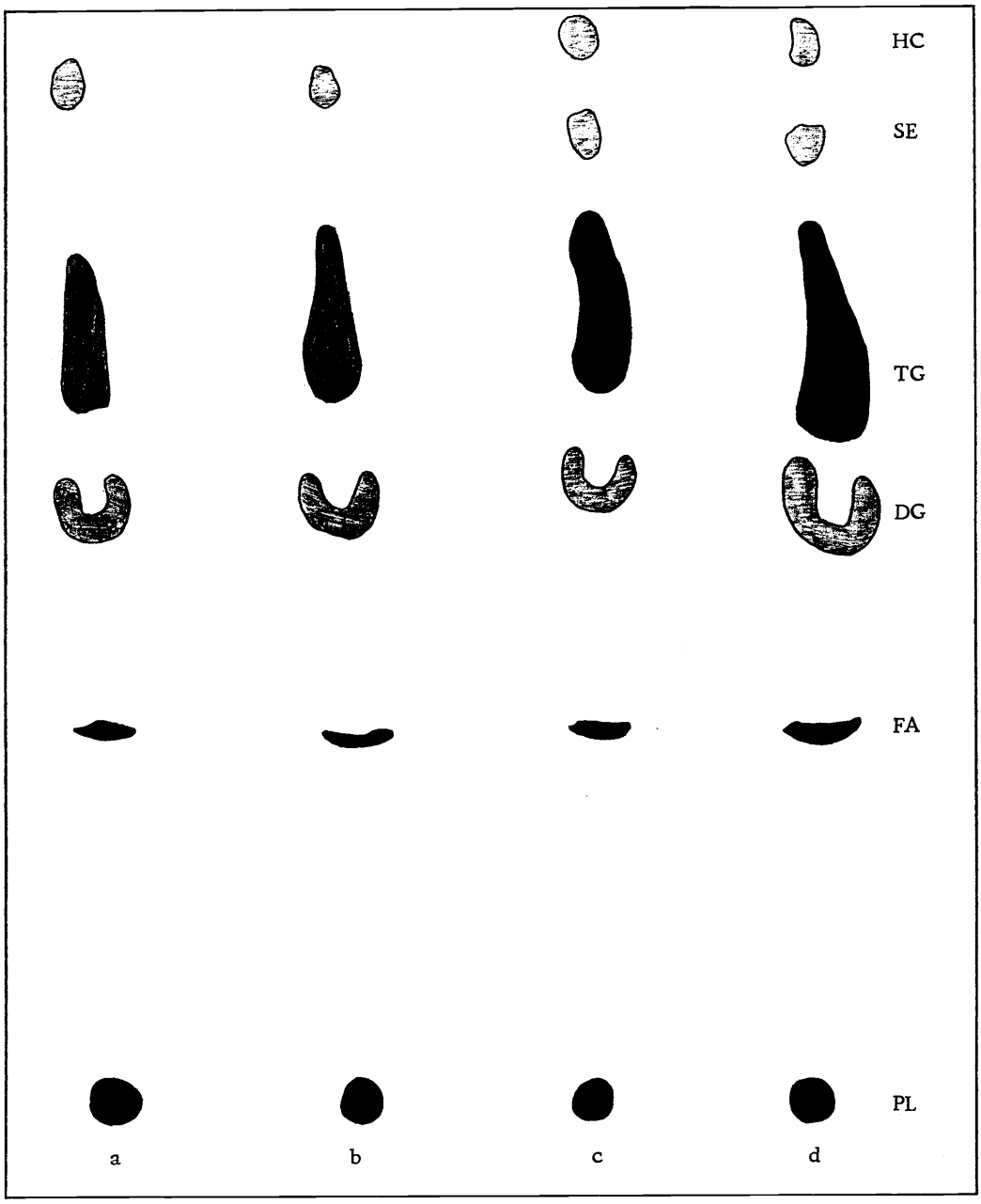
tested by Chippendale, Beck and Strong (1964) with the same result but these workers were uncertain of the validity of the experiment due to the possibility of oxidative degradation of the PUFA during the larval feeding period. Since a maximum of 84 hours of feeding took place during the present study, this factor is greatly minimized.

Studies with 1-^{14}C-Linolenic Acid

Several experiments with ^{14}C-linolenic acid in the larval diet served a dual purpose: providing another test of previous experiments; and permitting the tracing of the ingested 18:3 into the various lipids.

Plate VIII illustrates an autoradiogram showing a typical TLC separation of the total pupal lipids into the various lipid classes. Deposition of ^{14}C was readily visible in the PL, FA, DG and TG classes, while only traces were visible in the steryl ester and hydrocarbon regions. No label was detected in the monoglyceride or cholesterol regions. It is known that these insects are unable to biosynthesize cholesterol.

Trichoplusia ni larvae were fed the wheat-germ residue diet containing 0.50% unlabeled linolenate containing sufficient labeled 18:3 for appropriate counting statistics. Three lengths of feeding were compared. The pupae were extracted as usual for lipid study. Table 22 summarizes the results of liquid scintillation counting of the lipid classes after separation by TLC. It is readily apparent



Drawing of an autoradiogram of TLC separation of lipids from T. ni pupae after 5th instar larvae were fed 1-^{14}C-linolenic acid. Percentage of dietary 18:3 and hours of larval feeding: a) 0.05, 70; b) 0.05, 45; c) 0.50, 80; d) 0.50, 68. Load: approx. 16,000 dpm. Solid support: silica gel G; developing solvent: diethyl ether-benzene-ethanol-acetic acid 40:50:2:0.2. followed by diethyl ether-hexane 6:94. Abbreviations: HC, hydrocarbons; SE, steryl ester; TG, triglyceride; DG, diglyceride; FA, free fatty acid; PL, phospholipid.

that the majority of the ^{14}C was found in the glyceride and phospholipid fractions. It was necessary to count DG and TG together as these TLC spots were not well separated. Later, more complete, separations showed that only four to five percent of the activity was actually in the diglyceride fraction. Very little of the carbon label was found in the SE or FA classes and length of feeding had no influence on the amount present. When larvae fed 40-50 hours prior to pupation there was slightly more ^{14}C in the PL than in the glycerides. After larvae had fed 60-70 hours prior to this stage, the glycerides contained considerably more label than the PL. The trend continued on longer feeding.

Table 22. Distribution of ^{14}C in lipid classes after TLC resolution of extracts of T. ni pupae. 1/

Larval Feeding (Hrs)	% of ^{14}C in Lipid Classes 2/				% of ^{14}C on TLC Plate
	SE	FA	DG + TG	PL	
40 - 50	0.7	1.8	45.8	51.5	99.8
60 - 70	0.4	1.8	58.9	38.5	99.6
80 - 90	0.5	1.8	60.6	36.7	99.5

1/ Larval diet contained 0.50% linolenate and tracer amounts of ^{14}C linolenate.

2/ Average of 3-6 assays.

Table 23 shows the percent of the label in the PL and glycerides under conditions known to result in normal and deformed wings. At

0.05% dietary linolenate, a level resulting in moths with deformed wings, approximately two-thirds of the lipid ^{14}C was found in the PL whether larvae fed 40 or 70 hours before pupating. This preferential deposition in the PL also occurred at 0.25%, a dietary level expected to allow about half of the moths to have normal wings when larvae feed for 40 hours. However, at 0.50%, a level at which virtually all moths were normal when larvae fed 40 hours, there was nearly as much label in the glycerides as in the PL, and with 70 hours of pre-pupation feeding, there was more.

Table 23. Percent of lipid ^{14}C in phospholipid and glyceride fractions of T. ni pupae reared on 18:3 supplemented diet. 1/

% Dietary 18:3	40 Hrs. Feeding		70 Hrs. Feeding	
	PL	DG+TG	PL	DG+TG
0.05	65.7	30.6	69.1	25.9
0.25	68.8	29.1	----	----
0.50	52.0	46.1	38.5	58.9

1/ Diets contained tracer levels of ^{14}C linolenate. Average of 3-6 assays.

Figure 4 depicts the ^{14}C content of pupal PL as larval feeding time was varied. The nearly constant level of ^{14}C in the PL from the 0.05% diet is again seen over a long period of feeding. As the dietary level of 18:3 or the feeding period is increased, there is less label in this lipid fraction. Thus, when dietary 18:3 was below

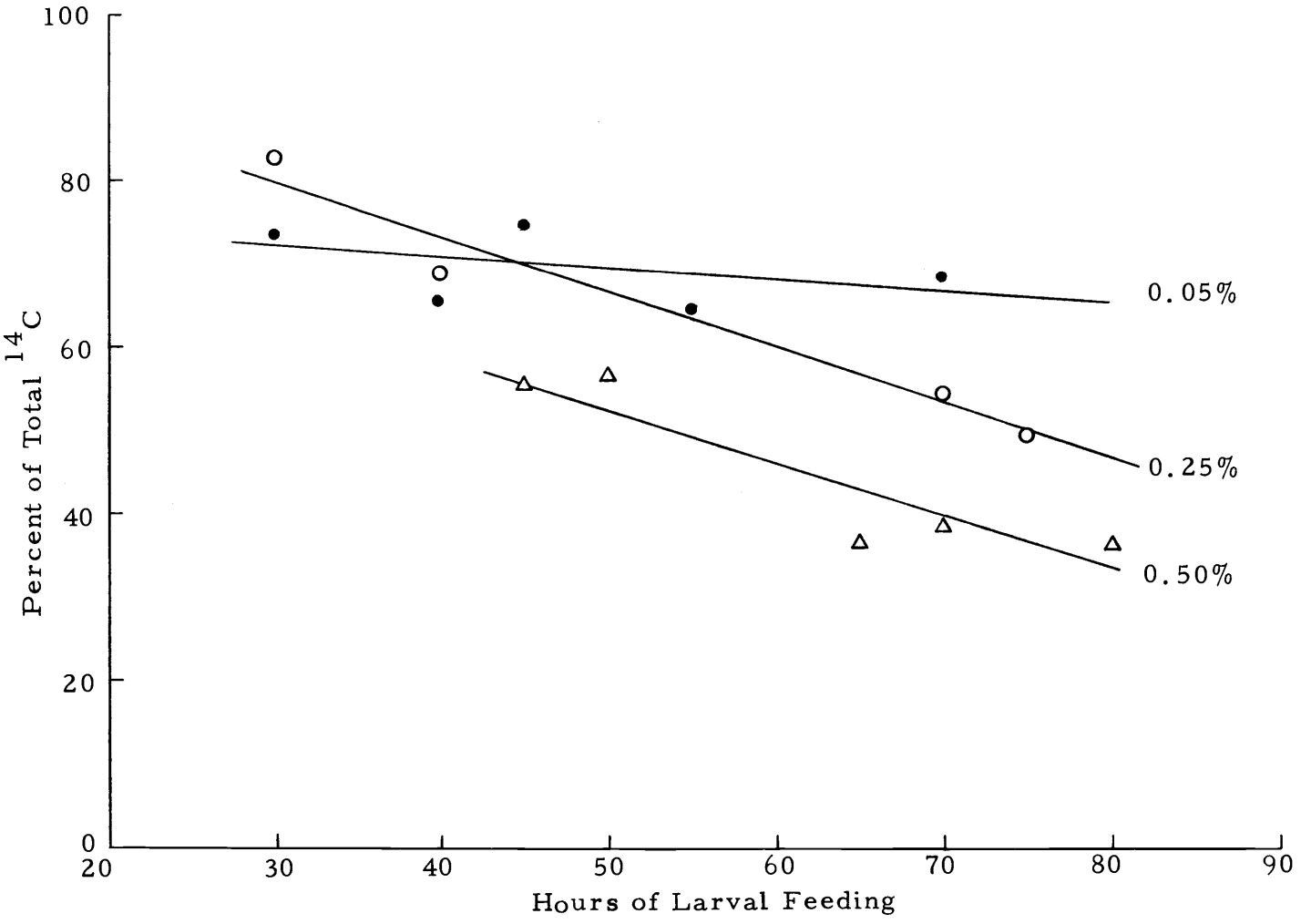


Figure 4. Change in ^{14}C distribution of phospholipids of T. ni pupae when fifth instar larvae were reared on diets containing 0.05, 0.25, and 0.50% linolenate.

optimum or the feeding period was short, label was preferentially deposited in the PL. The amount deposited is striking when it is remembered that the PL constitutes only a minor portion of the total lipids in these insects.

The foregoing experiments identified only the location of ^{14}C within lipid classes and not its chemical form. To obtain this information samples of total fatty acids, glycerides and phospholipids were transesterified to methyl esters and the esters were separated by TLC. Subsequent counting of the areas on the TLC plates showed that an average of 97% of the radioactivity associated with the lipids was in the form of fatty acid esters. Practically none of the labeled carbon was found in the glycerol moiety.

The labeled esters of the fatty acids were separated by TLC or by GLC and individual esters were collected and quantitated (see Methods). Table 24 shows the results of these separations. The methods gave good agreement and indicate that most of the radioactivity consumed by the larvae remained as 18:3 since 86-96% occurred in this acid. The saturated acids 16:0 and 18:0 and oleic acid contained nearly all of the remaining activity.

When the individual fatty acids of the PL and TG were analyzed for ^{14}C, it was found that the label associated with the PL was almost entirely 18:3 (Table 25). Insects from 0.05% dietary linolenate contained an average of 94.3% of the dpm in this acid and those from

Table 24. Radioactive fatty acids in T. ni pupae reared on 18:3 supplemented diet, as determined by two methods. 1/

18:3 in Diet, %	Hours Fed	Method of Separation	Percent ^{14}C as			
			16:0 + 18:0	18:1	18:2	18:3
0.05	40	TLC	1.5	1.6	0.6	95.1
"	"	GLC	1.5	1.0	0	96.5
0.25	"	TLC	3.0	3.3	0.5	92.3
"	"	GLC	0	2.1	3.1	95.8
0.05	75	TLC	4.0	6.2	0.6	88.4
"	"	GLC	3.7	8.0	0	88.3
0.25	"	TLC	3.1	3.4	0.6	91.2
"	"	GLC	3.5	4.8	0.8	86.3

1/ Analyses are of the total pupal fatty acids.

Table 25. Distribution of ^{14}C fatty acids of phospholipids and triglycerides of T. ni pupae reared on 18:3 supplemented diet. 1/

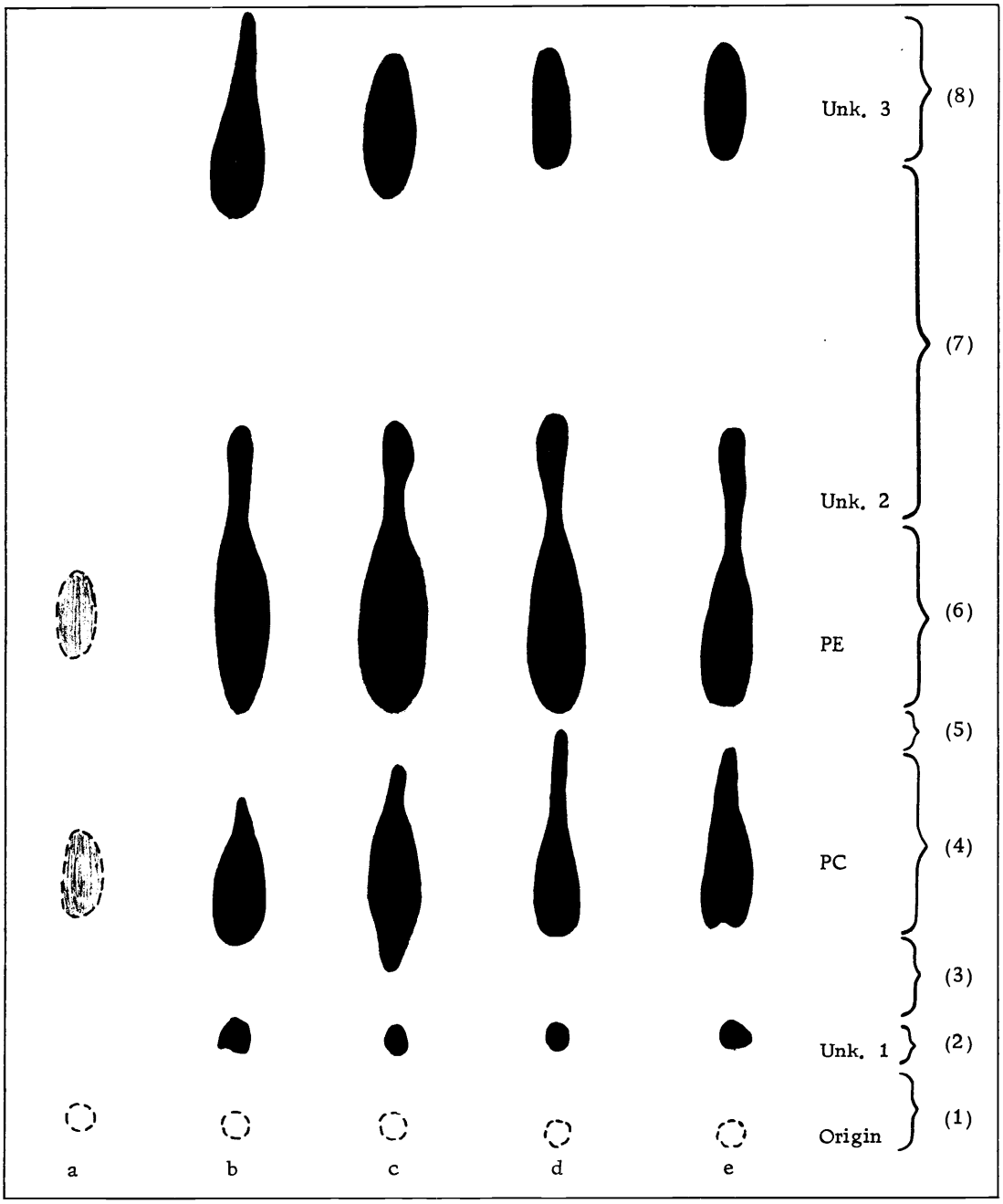
18:3 in Diet, %; Hrs. Fed	Lipid Class	Percent ^{14}C as							
		<16:0	16:0	16:1	18:0	18:1	18:2	18:3	>18:3
0.05, 45	PL	0.5	0	0.6	0.8	0.6	0.4	94.3	2.8
"	TG	7.7	14.2	7.1	1.4	17.1	0	48.5	4.0
0.50, 70	PL	0	0	0	0	0	0	98.5	1.5
"	TG	2.6	3.6	1.3	0	4.1	0	83.3	4.6

1/ Average of two assays.

0.50% 18:3 diets contained 98.5% as linolenate. The TG fraction from the 0.50% diet was also high in 18:3 (83.3%) but that from the 0.05% diet contained only 48.5%. The remainder of the ^{14}C atoms were distributed among the acids synthesized by T. ni. Palmitate and oleate contained more of the label than the other acids. It is possible that under the conditions of lipid stress (0.05% 18:3 in the diet) this lipid, even though an essential fatty acid, is metabolized to a greater than normal extent.

One experiment provided information on the distribution of the labeled linolenic acid in the phospholipid fraction. Phospholipid samples were separated by TLC and the chromatograms compared with those of two standard insect phospholipids, phosphatidylcholine (PC) and phosphatidylethanolamine (PE). Plate IX is a tracing of an autoradiogram of these separations. Each area of the plate which showed the presence of lipid when sprayed with Rhodamine 6 G also contained ^{14}C. In addition to PC and PE, there were at least three other radioactive regions. Unknowns one and three were always distinct spots, whereas unknown two did not separate cleanly from PE. Table 26 gives the relative distribution of ^{14}C-18:3 in these areas. Phosphatidylcholine contained the largest amount of linolenic acid regardless of the dietary regimen or period of larval feeding. There was no apparent difference between 0.05% and 0.50% dietary 18:3 after the 40 hour feeding period. Phosphatidylethanolamine also

Plate IX



Drawing of an autoradiogram of TLC separation of pupal phospholipids of T. ni after larvae were fed 1-^{14}C-linolenic acid. Percentage of dietary 18:3 and hours of larval feeding: b) 0.05, 45; c) 0.05, 45; d) 0.50, 75; e) 0.50, 45. Standards: a) phosophatidylcholine (PC); phosphatidylethanolamine (PE). Regions assayed for ^{14}C (Table 26) designated by numbers. Solid support: silica gel G; developing solvent: chloroform-methanol-water 65:40:5.

Table 26. Distribution of ^{14}C in components of phospholipids of T. ni pupae as separated by TLC.

18:3 in Diet, %; Hrs. Fed	Percent ^{14}C in TLC Region[1/]							
	(1) Origin	(2) Unk. 1	(3)	(4) PC	(5)	(6) PE	(7) Unk. 2	(8) Unk. 3
0.05, 40	0.3	1.6	1.4	38.6	1.5	28.8	8.9	18.9
0.05, 70	0.6	3.3	1.2	38.4	1.9	26.4	9.5	18.7
0.50, 50	0.7	3.1	1.9	41.2	1.3	31.7	8.5	11.6
0.50, 90	0.9	3.5	1.8	35.9	1.9	27.6	11.7	16.7

[1/] Refer to Plate IX for areas sampled, PC = phosphatidylcholine; PE = phosphatidylethanolamine

contained considerable amounts of 18:3 which appeared unrelated to dietary level or length of feeding. These two components accounted for 63.5 to 72.9% of the radioactivity in the phospholipid. Of the unidentified lipids, unknown three had the most radioactivity. Thus, it appears that all major components of the pupal phospholipids, already known to contain a large amount of linolenic acid, will incorporate this acid from a deficient or adequate dietary level if the larvae are allowed to feed 40 hours before pupating.

SUMMARY

The fatty acid nutrition of these species has proven to be a challenging problem for study. Many of the questions asked initially have been answered and basic information necessary for the solution of other problems has been gathered. The major accomplishments of the work can be summarized in the following statements:

1. Linolenic acid is essential for normal wing development in all three species. A. californica having the greatest and H. zea the least requirement. None of the isomeric forms, steryl esters, or derivative acids tested can replace this essential fatty acid. The nutrient must be available during the last day of larval feeding but it can be omitted from the diet prior to this point. The wing deformity condition may be the only result of 18:3 deficiency.

2. Linolenic acid must be in the larval diet at a minimal level, at least 0.05% for H. zea and up to 0.25% for A. californica. As the dietary level increases, the tissue lipid level in the insects also increases, indicating that conversion to other lipids is inefficient. The triglyceride is probably the best dietary form of the fatty acid and other dietary lipids appear to have a sparing effect on the amount of 18:3 required.

3. When in limited supply, linolenic acid is used first in the synthesis of phospholipids, spilling over into the triglycerides and

other lipids as the stress is relieved. There is no evidence of conversion to other fat soluble materials.

4. There is a relationship between linolenic acid nutrition and conditions of rearing, either high or low humidity or high temperature antagonizing the fatty acid deficiency. This antagonism is not due to increased utilization of 18:3 under the stress condition. Either humidity or temperature induced wing deformities can be overcome by improving the level of 18:3 nutrition.

The actual means by which 18:3 acts to provide its essentiality remains unknown. What is so unique about the 18:3 (ω3) structure which enables only this molecule to satisfy wing development physiology? The splitting open and escape from the pupal case by the adult is primarily a physical process. If lipid is required at this point in the life cycle it may be serving as a lubricant for eclosion. It is readily observed that the wings (or at least the wing scales) are stuck within the pupal wing pads in 18:3 deficient insects. However, it is difficult to rationalize the observed structural specificity with such a need.

The present research points to the phospholipid class as the key to further understanding of the EFA requirement. Because phospholipids are major components of membranes, speculations of possible membrane involvement are easily formed especially since the insect wing is a double membrane. Perhaps 18:3 is needed in the

structural make-up of this tissue. Another possibility is an involvement of linolenic acid in the little understood ecdysial membrane which separates the new and old cuticular tissues prior to ecdysis. Linolenate is not essential for the larval molts, however.

If linolenic acid could be provided during the pupal stage in a form suitable for normal metabolism, the critical site of action might be further defined. A more specific identification of the linolenate-containing phospholipids and the utilization of inhibitors in their metabolism could serve to aid in the confirmation or denial of their role as key constituents.

BIBLIOGRAPHY

Bade, M. L. 1964. Biosynthesis of fatty acids in the roach Eurycotis floridana. Journal of Insect Physiology 10:333-341.

Barlow, J.S. 1964. Fatty acids in some insect and spider fats. Canadian Journal of Biochemistry 42:1365-1374.

Beck, S.D., J.H. Lilly and J.F. Stauffer. 1949. Nutrition of the cornborer Pyrausta nubilalis (Hbn). I. Development of a satisfactory purified diet for larval growth. Annals of the Entomological Society of America 42:249.

Bligh, E.G. and W.J. Dyer. 1959. A rapid method of total lipide extraction and purification. Canadian Journal of Biochemistry and Physiology 37:911-917.

Brookes, V.J. and G. Fraenkel. 1958. The nutrition of the larva of the housefly Musca domestica L. Physiological Zoology 31:208-233.

Burr, G.G. and M.M. Burr. 1929. A new deficiency disease produced by the rigid exclusion of fat from the diet. Journal of Biological Chemistry 82:345-350.

_____ 1930. On the nature and role of the fatty acids essential in nutrition. Journal of Biological Chemistry 86:587-621.

Cherry, L.M. 1959. Fat metabolism and temperature acclimatization in the fly Phormia terraenovae R.-D. Entomologia Experimentalis et Applicata 2:68-76.

Chippendale, G.M. and S.D. Beck. 1965. A method for rearing the cabbage looper, Trichoplusia ni, on a meridic diet. Journal of Economic Entomology 58:377-378.

Chippendale, G.M., S.D. Beck and F.M. Strong. 1964. Methyl linolenate as an essential nutrient for the cabbage looper, Trichoplusia ni (Hübner). Nature 204:710-711.

Chippendale, G. M., S. D. Beck and F. M. Strong. 1965. Nutrition of the cabbage looper, Trichoplusia ni (Hubn.) I. Some requirements for larval growth and wing development. Journal of Insect Physiology 11:211-223.

Clayton, R. B. 1964. The utilization of sterols by insects. Journal of Lipid Research 5:3-19.

Coniglio, J. G., J. T. Davis and S. Aylward. 1964. Metabolism of ethyl arachidonate-1-C^{14} in rats fed complete or fat-free diets. Journal of Nutrition 84:265-271.

Coots, R. H. 1965. Metabolism of arachidonic acid-1-^{14}C in the rat. Journal of Lipid Research 6:494-497.

Dadd, R. H. 1960. The nutritional requirements of locusts. I. Development of synthetic diets and lipid requirements. Journal of Insect Physiology 4:319-347.

_____ 1961. The nutritional requirements of locusts. V. Observations on essential fatty acids, chlorophyll, nutritional salt mixtures and proteins or amino acid components of synthetic diets. Journal of Insect Physiology 6:126-145.

_____ 1964. A study of carbohydrate and lipid nutrition in the wax moth, Galleria mellonella (L.), using partially synthetic diets. Journal of Insect Physiology 10:161-178.

Ditman, L. P. 1938. Metabolism in the corn ear worm. I. Studies on fat and water. Bulletin of the University of Maryland Agricultural Experiment Station 414:183-206.

Earle, N. W., B. Slatten and M. L. Burks, Jr. 1967. Essential fatty acids in the diet of the boll weevil, Anthonomus grandis Boheman (Coleoptera: Curculionidae). Journal of Insect Physiology 13:187-200.

Edwards, H. M., Jr. and J. E. Marion. 1963. Influence of dietary menhaden oil on growth rate and tissue fatty acids of the chick. Journal of Nutrition 81:123-130.

Fast, P. G. 1964. Insect lipids: A review. Memoirs of the Entomological Society of Canada 37:1-50.

Fawzi, M., M. F. H. Osman, and G. H. Schmidt. 1961. Analyse der körperfette von imaginalen Wanderheuschrecken der Art Locusta migratoria migratoriodes L. Biochemische Zeitschrift 344:441-450.

Folch, J., M. Lees and G. H. Sloane-Stanley. 1957. A simple method for the isolation and purification of total lipides from animal tissues. Journal of Biological Chemistry 226:497-509.

Fraenkel, G. and M. Blewett. 1946. Linoleic acid, vitamin E and other fat-soluble substances in the nutrition of certain insects, Ephestia kuehniella, E. elutella, E. cautella and Plodia interpunctella (Lep.). Journal of Experimental Biology 22:172-190.

_____ 1947. Linoleic acid and arachidonic acid in the metabolism of the insects, Ephestia kuehniella and Tenebrio molitor. Biochemistry Journal 41:475-478.

Fraenkel, G. and H. S. Hopf. 1940. The physiological action of abnormally high temperatures on poikilothermic animals. I. Temperature adaptations and the degree of saturation of the phosphatides. Biochemistry Journal 34:1085-1092.

Freeman, C. P. and D. West. 1966. Complete separation of lipid classes on a single thin-layer plate. Journal of Lipid Research 7:324-327.

Fulco, A. J. and J. F. Mead. 1959. Metabolism of essential fatty acids. VIII. Origin of 5, 8, 11-eicosatrienoic acid in the fat-deficient rat. Journal of Biological Chemistry 234:1411-1416.

Gilbert, L. I. 1967. Lipid metabolism and function in insects. Advances in Insect Physiology 4:69-211.

Gordon, H. T. 1959. Minimal nutritional requirements of the German roach, Blattella germanica L. Annals of the New York Academy of Science 77:290-352.

Grau, P. A. and L. C. Terriere. 1967. A temperature-dependent factor for normal wing development in the cabbage looper, Trichoplusia ni (Lepidoptera: Noctuidae). Annals of the Entomological Society of America 60:549-552.

Hammarstrand, K. 1968. Collecting GLC effluent with cigarette filter tips. Varian Aerograph Research Notes. February:3.

Harwood, R. F. and N. Takata. 1965. Effect of photoperiod and temperature on fatty acid composition of the mosquito, Culex tarsalis. Journal of Insect Physiology 11:711-716.

Holman, R. T. 1964. Nutritional and metabolic interrelationships between fatty acids. Federation Proceedings 23:1062-1067.

_____ 1968. Essential fatty acid deficiency. Progress in the Chemistry of Fats and Other Lipids 9:275-348.

Holman, R. T. and H. Mohrhauer. 1963. A hypothesis involving competitive inhibitions in the metabolism of polyunsaturated fatty acids. Acta chemica Scandinavica 17:584-590.

Holman, R. T. and J. J. Rahm. Analysis and characterization of polyunsaturated fatty acids. In: Progress in the chemistry of fats and other lipids, ed. by R. T. Holman. Vol. 9. part 1. New York, Pergamon Press 1966. p. 15-90.

Horie, Y., S. Nakasone and T. Ito. 1968. The conversion of ^{14}C-carbohydrates into CO_2 and lipid by the silkworm, Bombyx mori. Journal of Insect Physiology 14:971-981.

Horning, E. C. et al. 1964. Quantitative analysis of fatty acids by gas-liquid chromatography. Journal of Lipid Research 5:20-27.

House, H. L. 1965. Insect nutrition. In: Physiology of Insecta. Vol. 2, ed. by M. Rockstein, New York, Academic Press. p. 769-814.

House, H. L. and J. S. Barlow. 1961. Effects of oleic and other fatty acids on the growth rate of Agria affinis (Fall.) (Diptera: Sarcophagidae). Journal of Nutrition 72:409-414.

Keith, A. D. 1967. Fatty acid metabolism in Drosophila melanogaster: Interaction between dietary fatty acids and de novo synthesis. Comparative Biochemistry and Physiology 21:587-600.

Klenk, E. and K. Oette. 1960. The C_{20} and C_{22} polyenoic acids of rat liver phosphatides after the addition of linoleic or linolenic acid to a fat-free diet. Zeitschrift fuer Physiologische Chemie 318:86-99

Klenk, E. and H. Pflüger. 1963. The biogenesis of $\Delta^{5, 8, 11}$-eicosatrienoic acid in rats on a fat free diet. Zeitschrift fuer Physiologische Chemie 335:53-62.

Knipprath, W. G. and J. F. Mead. 1968. Effect of the environmental temperature on the fatty acid composition and on the in vivo incorporation of 1-^{14}C-acetate in goldfish (Carassius auratus L.). Lipids 3:121-128.

Kodicek, E. and Z. H. Levinson. 1960. Metabolism of β-sitosterol and other lipids in the presence of acetate-2-C^{14} by blowfly larvae. Nature 188:1023-1025.

Lamb, N. J. and R. E. Monroe. 1968. Studies of complex lipids synthesized from acetate-1-C^{14} by the cereal leaf beetle, Oulema melanopus. Annals of the Entomological Society of America 61:1167-1169.

Lambremont, E. N. 1965. Biosynthesis of fatty acids in aseptically reared insects. Comparative Biochemistry and Physiology 14:419-424.

Lambremont, E. N. and A. F. Bennett. 1966. Lipid biosynthesis in the boll weevil. Formation of the acetate precursor for lipid synthesis from glucose and related carbohydrates. Canadian Journal of Biochemistry 44:1597-1607.

Lambremont, E. N. and M. S. Blum. 1963. Fatty acids of the boll weevil. Annals of the Entomological Society of America 56: 612-616.

Levinson, Z. H. and E. D. Bergmann. 1957. Steroid utilization and fatty acid synthesis by the larva of the housefly Musca vicina. Biochemistry Journal 65:254-266.

Lewis, R. W. 1962. Temperature and pressure effects on the fatty acids of some marine ectotherms. Comparative Biochemistry and Physiology 6:75-89.

Louloudes, S.J. et al. 1961. Lipogenesis from C^{14}-acetate by the American cockroach. Annals of the Entomological Society of America 54:99-103.

Malins, D.C. 1966. Recent developments in the thin-layer chromatography of lipids. Progress in the Chemistry of Fats and Other Lipids 8:303-358.

Marco, G.J. et al. 1961. Dietary effects of fats upon fatty acid composition of the mitochondria. Archives of Biochemistry and Biophysics 94:115-120.

Marr, A.G. and J.L. Ingraham. 1962. Effect of temperature on the composition of fatty acids in Escherichia coli. Journal of Bacteriology 84:1260-1267.

Mead, J.F. 1968. The metabolism of the polyunsaturated fatty acids. Progress in the Chemistry of Fats and Other Lipids 9:161-192.

Miura, K. et al. 1965. Biosynthesis of unsaturated and saturated fatty acids in aseptically reared larvae of Calliphora erythrocephala (Meig.). Archives Internationales de Physiologie et de Biochemie 73:65-72.

Mohrhauer, H. and R.T. Holman. 1963a. The effect of dose level of essential fatty acids upon fatty acid composition of the rat liver. Journal of Lipid Research 4:151-159.

_____ 1963b. The effect of linolenic acid upon the metabolism of linoleic acid. Journal of Nutrition 81:67-74.

Morris, L.J. 1966. Separations of lipids by silver ion chromatography. Journal of Lipid Research 7:717-732.

Munson, S.C. 1953. Some effects of storage at different temperatures on the lipids of the American roach and on the resistance of this insect to heat. Journal of Economic Entomology 46: 657-666.

Nayar, J.K. 1964. The nutritional requirements of grasshoppers. I. Rearing of the grasshopper, Melonoplus bivittatus (Say) on a completely defined synthetic diet and some effects of different concentrations of B-vitamin mixture, linoleic acid, and β-carotene. Canadian Journal of Zoology 42:11-22.

Nelson, J.H., R.L. Glass and W.F. Geddes. 1963. The triglycerides and fatty acids of wheat. Cereal Chemistry 40:343-350.

Nelson, D.R. and D.R. Sukkestad. 1968. Fatty acid composition of the diet and larvae and biosynthesis of fatty acids from ^{14}C-acetate in the cabbage looper, Trichoplusia ni. Journal of Insect Physiology 14:293-300.

Pant, N.C. and J.C. Pant. 1961. Nutritional studies of Trogoderma granarium. V. Studies on lipid requirements. Indian Journal of Entomology 23:10-14.

Pearson, L.K. and H.S. Raper. 1927. The influence of temperature on the nature of the fat formed by living organisms. Biochemistry Journal 21:875-879.

Pepper, J.H. and E. Hastings. 1943. Biochemical studies on the sugar beet webworm Loxostege sticticalis L., with special reference to the fatty acids and their relation to diapause and sterility. Bulletin of the Montana Agricultural Experiment Station 413:1-36.

Privett, O.S. et al. 1965. Lipid analysis by quantitative thin-layer chromatography. The Journal of the American Oil Chemists' Society 42:381-392.

Rahm, J.J. and R.T. Holman. 1964a. The relation of single dietary polyunsaturated fatty acids to fatty acid composition of the rat liver. Journal of Lipid Research 5:169-176.

_____ 1964b. Studies of the metabolism of polyunsaturated acids by short-term experiments. Journal of Nutrition 84:149-154.

Rainey, R.C. 1938. On the changes in chemical composition associated with larval development in sheep blowfly. Annals of Applied Biology 25:822-835.

Ritchot, C. and J.E. MacFarlane. 1962. The effects of wheat germ oil and linoleic acid on growth and reproduction of the house cricket. Canadian Journal of Zoology 40:371-374.

Rock, G.C. 1967. Aseptic rearing of the codling moth of synthetic diets: ascorbic acid and fatty acid requirements. Journal of Economic Entomology 60:1002-1005.

Rock, G. C., R. L. Patton and E. H. Glass. 1965. Studies of the fatty acid requirements of Argyrotaenia velutinana (Walker). Journal of Insect Physiology 11:91-101.

Schafer, J., P. Grau, and L. C. Terriere. 1968. Temperature-induced wing malformations in alfalfa loopers and variegated cutworms. Journal of Economic Entomology 61:575-576.

Schmidt, G. H. 1963. Spektralphotometrischer Nachweis von Pentaensäuren in Ameisenfetten und irhe quantitativen Veränderungen warhend der Metamorphose. Naturwissenshaften 50:375-376.

Schmidt, G. H. 1964. Polyensäuren in Insektenlipiden und irhe Bedeutung fur die Metamorphose. Fette, Seifen Anstrichmittel 66:573-579.

Schmidt, G. H. and M. F. H. Osman. 1962. Analyse des Raupenöls vom Mondvogel Phalera bucephala L. (Lepidoptera: Notodontidae). Journal of Insect Physiology 8:233-240.

Scoggin, J. K. and O. E. Tauber. 1950. Survey of literature on insect lipids. Iowa State College Journal of Science 25:99-124.

Shaefer, C. H. 1968. The relationship of the fatty acid composition of Heliothis zea larvae to that of its diet. Journal of Insect Physiology 14:171-178.

Shorey, H. H. 1963a. The biology of Trichoplusia ni (Lepidoptera: Noctuidae) II. Factors affecting adult fecundity and longevity. Annals of the Entomological Society of America 56:476-480.

_____1963b. A simple artificial rearing medium for the cabbage looper. Journal of Economic Entomology 56:536-537.

Shorey, H. H. and R. L. Hale. 1965. Mass-rearing of the larvae of nine noctuid species on a simple artificial medium. Journal of Economic Entomology 58:522-524.

Silverman, P. H. and Z. H. Levinson. 1954. Lipid requirements of the larva of the housefly Musca vicina (Macq.) reared under non-aseptic conditions. Biochemistry Journal 58:291-294.

Sridhara, S. and J. V. Bhat. 1964. Incorporation of 1-^{14}C-acetate into the lipids of the silkworm Bombyx mori L. Biochemistry Journal 91:120-123.

Stoffel, W. and E. H. Ahrens, Jr. 1960. The unsaturated fatty acids in menhaden body oil: The C_{18}, C_{20}, and C_{22} series. Journal of Lipid Research 1:139-146.

Tamaki, Y. 1961. Studies on nutrition and metabolism of the smaller tea tortrix, Adoxophyes orana (Fischer von Rösler-stamm) II. An essential factor for adult emergence. Japanese Journal of Applied Entomology and Zoology 5:58-63.

Uberoi, N. K. 1960. Fatty acids in the lipid metabolism of rice moth Corcyra cephalonica Stainton. Journal of the Zoological Society of India 8:85-90.

Vanderzant, E. S. 1967. Wheat-germ diets for insects: Rearing the boll weevil and the salt-marsh caterpillar. Annals of the Entomological Society of America 60:1062-1066.

_____ 1968. Dietary requirements of the bollworm, Heliothis zea (Lepidoptera: Noctuidae), for lipids, choline, and inositol and the effect of fats and fatty acids on the composition of the body fat. Annals of the Entomological Society of America 61:120-125.

Vanderzant, E. S., D. Kerur and R. Reiser. 1957. The role of dietary fatty acids in the development of the pink bollworm. Journal of Economic Entomology 50:606-608.

Vanderzant, E. S. and C. D. Richardson. 1964. Nutrition of the adult boll weevil: lipid requirements. Journal of Insect Physiology 10:267-272.

Vanderzant, E. S., C. D. Richardson, and S. W. Fort, Jr. 1962. Rearing of the bollworm on artificial diet. Journal of Economic Entomology 55:140.

Van Handel, E. 1966. Temperature independence of the composition of triglyceride fatty acids synthesized de novo by the mosquito. Journal of Lipid Research 7:112-115.

Van Handel, E. and P. T. M. Lum. 1961. Sex as a regulator of triglyceride metabolism in the mosquito. Science 134:1979-1980.

Wigglesworth, V. B. 1942. The storage of protein, fat, glycogen and uric acid in the fat body and other tissues of mosquito larvae. Journal of Experimental Biology 19:56-77.