METABOLISM OF FATTY ACIDS IN *IPS PARACONFUSUS* LANIER (COLEOPTERA:SCOLYTIDAE): *IN VIVO* SYNTHESIS OF FATTY ACIDS FROM ACETATE-1-^{14}C IN FRESHLY EMERGED FEMALES

by

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Title of Thesis: Metabolism of fatty acids in *Ips paraconfusus* Lanier (Coleoptera: Scolytidae): *In Vivo* synthesis of fatty acids from Acetate-1-^{14}C in freshly emerged females

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ABSTRACT

The fatty acid composition of freshly emerged male and female Ips paraconfusus Lanier (Coleoptera:Scolytidae) was similar, however the levels of C 14:0 and C 16:0 were significantly higher in the males (P = .10). In the female the composition was found to be C 14:0, 0.4%; C 16:0, 22.4%; C 16:1, 5.8%; C 18:0, 3.1%; C 18:1, 55.3%; C 18:2, 9.6%; C 18:3, 2.2%. The composition of the male was C 14:0, 0.7%; C 16:0, 24.1%; C 16:1, 6.2%; C 18:0, 3.0%; C 18:1, 54.9%; C 18:2, 8.9%; C 18:3, 2.1%. The quantitative fatty acid composition was significantly changed in reproducing adults excised from Ponderosa Pine logs after six days. After injections of acetate-1-^{14}C into freshly emerged females, for exposures of 5, 15 and 30 minutes, radioactivity was detected in all the saturate and monounsaturate fatty acids. Calculated specific activities (dpm/μg of fatty acid), for the various exposure times indicate the magnitude of turnover of individual fatty acids, C 14:0 (13.9, 44.4, 88.0); C 16:0 (1.3, 3.2, 5.3); C 16:1 (1.5, 14.7, 15.9); C 18:0 (5.1, 8.8, 29.2); C 18:1 (0.3, 1.0, 1.5). Formation of 9-octadecenoic acid was by the desaturation of octadecanoic acid (C 18:0).

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To all students and faculty of the Department of Biological Sciences who aided me during this period, I am also very thankful.

INTRODUCTION

Changes in the structure of internal organs of maturing adult insects have been observed in many species. In Trypodendron lineatum, which were boring galleries, the flight muscles degenerated, the fat body decreased in size, and the gonads developed (Chapman 1956). Borden and Slater (1969) showed that similar internal changes, as found in other Scolytids (Reid 1958; McCambridge and Mata 1969), also occurred in Ips paraconfusus[1] Lanier. Flight muscle degeneration in I. paraconfusus (Bhakthan, Borden and Nair 1970) can be induced by synthetic juvenile hormone (Borden and Slater 1968). This suggested that the fat body changes and subsequent fatty acid metabolism may also be controlled by juvenile hormone.

An ultrastructural study of I. paraconfusus (N. M. G. Bhakthan, personal communication) showed that reproducing adults underwent dramatic changes in the cellular structure of the fat body. The synthesis of protein materials presumably for yolk deposition was greatly increased and the metabolism of lipid was also altered during ovary development.

Work on the lipid metabolism of Scolytid beetles has

[1] Sierra Nevada population, previously known as Ips confusus (Le Conte) but recently revealed to be an undescribed species (Lanier, in press).

to investigate the synthesis of fatty acids in freshly emerged I. paraconfusus female adults prior to the commencement of sexual maturation and to explore the changes in fatty acids in reproducing insects.

LITERATURE REVIEW

For many years the predominant theory for the synthesis of fatty acids was that the degradative and synthetic pathways were parts of one reversible process. Wakil (1963) reviewed the historical work on fatty acid synthesis. Two observations supported a modified β oxidation scheme; a) crotonyl-CoA could be reduced by NADPH in the presence of an enzyme found in the soluble fraction of rat liver; and b) stearate synthesis from acetyl-CoA and palmityl-CoA was catalyzed by mitochondrial enzymes in the presence of NADPH and NADH. This system, located in the mitochondria, was primarily concerned with the elongation of medium chain length fatty acids and has been named the "mitochondrial" or "elongation" system. However Lynen and Ochoa (1953) found a β oxidation scheme which was fully reversible. An in vitro system was developed using the highly purified enzymes of a β oxidation system. Intact mitochondria of pigeon, rat or beef liver could synthesize long chain fatty acids from acetyl-CoA provided the conditions were anaerobic.

Commonly fatty acids are synthesized from acetyl-CoA in the presence of ATP, Mn^{+2}, CO_2 and NADPH (Wakil 1963). This system, from avian liver, is associated with particles smaller than microsomes. The chief intermediate is malonyl-CoA and for this reason the path has been termed the "non-

mitochondrial" or malonyl-CoA pathway.

The first intermediate of this system is malonate, and its synthesis is dependent on Mn^{+2}, Adenosine Triphosphate (ATP) and HCO_3^-.

$$CO_2 + CH_3\overset{O}{\overset{\|}{C}}SCoA + ATP \rightarrow HOOCCH_2\overset{O}{\overset{\|}{C}}SCoA + ADP + P_i$$

Biotin is also essential as a cofactor for activation of the acetyl-CoA carboxylase,

$$CO_2 + ATP + \text{biotin-enzyme} \longrightarrow CO_2 \sim \text{biotin-enzyme} + ADP + P_i$$

$$CO_2 \sim \text{biotin-enzyme} + \text{acceptor} \longrightarrow \text{HOOC-acceptor} + \text{biotin-enzyme}$$

In the case of palmitate synthesis, the requirement for acetyl-CoA in the conversion of malonyl-CoA to palmitic acid is absolute. By stoichiometric relationships it was shown that one C_2-unit of palmitate was derived from acetyl-CoA and the remaining 14-carbons were derived from malonyl-CoA.

With a soluble extract of pigeon liver, free of cytochromes and cytochrome oxidase, it was possible to demonstrate the occurrence of fatty acid synthesis. Radioactive tracers showed synthesis to be a "successive head-to-tail condensation of two carbon units". Bicarbonate was an absolute requirement for synthesis in this system but was only catalytic in nature. This requirement in many diverse organisms supported the theory that the "non-mitochondrial" system was common to

all life for the synthesis of fatty acids.

Recently, confusion has arisen concerning the location of the enzyme systems. Experiments seem to indicate rather clearly that the fatty acid synthesizing system present in mitochondria can be readily extracted into the supernatant (Christ and Hülsmann 1962). It is conceivable that the soluble enzyme present in the pigeon-liver supernatant fraction is at least partially mitochondrial in origin. Possibly, the microsomal fatty acid synthesis reported by various workers, is due to the presence in these fractions of small particles of mitochondrial origin.

The physical location of fatty acid synthesis differs from organism to organism. Heart sarcosomes catalyze the incorporation of acetate by malonyl-CoA intermediates into a mixture of saturated and unsaturated long chained fatty acids (Hülsmann 1962). Tissue slices of mammary glands from lactating rats and sheep can synthesize fatty acids from acetate-1-^{14}C when stimulated by glucose, pyruvate and compounds of the citric acid cycle, providing ATP is present and the incubation is aerobic (Popjak and Tietz 1954).

Synthesis de novo

The malonyl-CoA pathway of fatty acid synthesis involves a complex of enzymes in many living organisms. In yeast and pigeon liver this pathway involves six to seven different

tightly bound proteins (Olson 1966).

In Clostridium kluyverii, Escherichia coli and several plant systems, these proteins are only loosely bound to the cell membranes (Wakil, loc. cit.). The protein of most interest is the acyl carrier protein (ACP), which is heat stable and binds acyl intermediates during the formation of long chain fatty acids. Since arsenite inhibits fatty acid synthesis in the pigeon liver system, a mechanism for fatty acid synthesis involving vicinal sulphydryl groups on a single enzyme has been proposed (Olson 1966).

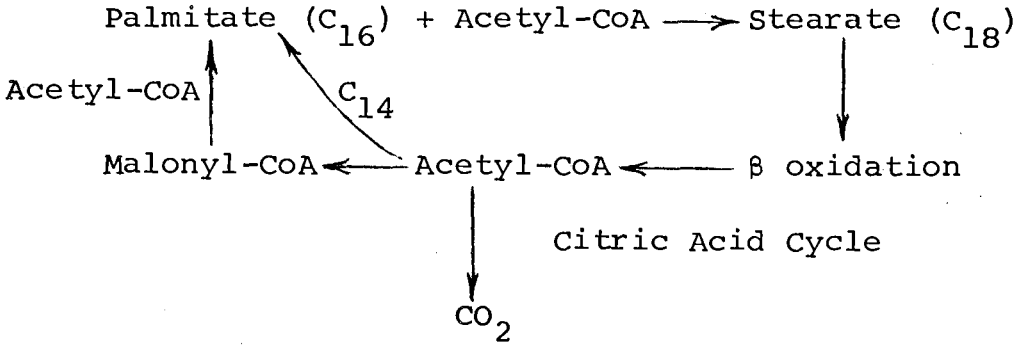
Fatty Acid Elongation

In addition to the de novo formation of fatty acids by cytoplasmic enzyme complexes, a mechanism exists for the elongation of long chain acyl-CoA derivatives in particulate fractions. An avidin-insensitive (non-biotin requiring) system exists in mitochondria by which acetyl-CoA was added to long chain acyl-CoA derivatives. In microsomes both saturated and unsaturated acyl-CoA derivatives were elongated by the malonyl-CoA pathway.

In some bacteria, the elongation produces some extremely long chain fatty acids that are formed by condensation of acyl-CoA derivatives of various chain lengths. Some of these chains may be up to 82 carbons long. Using labelled acetate-1-^{14}C CoA it was shown that, in the mitochondria, elongation

occurs by successive addition of acetyl-CoA to pre-existing short-chain fatty acids.

The shift from palmitate to stearate occurs by a C_2 addition, but the reverse reaction does not occur by simple β oxidation, as once oxidation is started on any molecule it goes to completion, as shown:



Fatty Acid Desaturation

Although there appear to be uniform pathways in plants and animals for synthesis de novo and elongation, the same cannot be said for the desaturation processes. Using ^{3}H-acetate and ^{14}C-acetate some progress has been made toward the goal of locating individual steps in the desaturation of long chain fatty acids. In vivo studies in adult rats, on the rate of incorporation of labelled acetate into fatty acids have shown that oleic acid becomes labelled much earlier than stearic acid (Raju and Reiser 1969). This indicates a route for biosynthesis of oleate other than desaturation of stearate, possibly a β, γ desaturation of laurate followed

by elongation.

An isolated double bond may be formed in a long chain fatty acid by either an aerobic or anaerobic pathway (Meyer and Meyer 1969). In most organisms the aerobic path requires molecular oxygen and a reduced pyridine nucleotide, although a ferrous ion and a flavin nucleotide are needed in some bacteria. The monooxygenase catalyzing this reaction acts on acyl-CoA or acyl-ACP derivatives and is associated with microsomes in animal tissue or a particulate fraction in microorganisms. The anaerobic pathway consists of a β, γ-dehydration of a medium chain β-hydroxyacyl-CoA derivative, followed by elongation.

Although the formation of monoenoic acids in plants was oxygen dependent, the direct conversion of stearate to oleate has not been shown (Olson 1966). In the ice plant, Carpolrotus chilense, myristate was directly desaturated in the presence of oxygen, and light. In starved and carbohydrate refed rats the incorporation of an additional double bond in oleate in the β, γ-position requires a further monooxygenase reaction. The oleate was activated to oleyl-CoA which was then converted to linoleyl-CoA.

The conversion of saturated long-chain fatty acids in yeast to the monounsaturated analogs requires molecular oxygen, reduced NADPH and two enzyme fractions, (one soluble

and one bound) (Bloomfield and Bloch 1960). The aerobic process uses a saturated acid as the precursor of the olefinic acid. The mechanism is thought to be a two step process: a) an hydroxylation by molecular O_2, b) dehydration of the hydroxyl derivative to the olefin.

$$\text{Palmitate} \xrightarrow[Mg^{+2}]{\text{CoA, ATP}} \text{palmityl-CoA} \xrightarrow[\text{NADPH}]{O_2} \text{[oxypalmityl-CoA]}$$

$$\text{[oxypalmityl-CoA]} \longrightarrow \text{palmitoleyl-CoA} \xrightarrow[\text{CoA}]{\text{Thiolase}} \text{palmitoleic acid}$$

Thus, unsaturated fatty acids are characteristically formed directly from long chain precursors and not from smaller units by an independent de novo process.

In Chlorella vulgaris another system for desaturation has been elucidated (Gurr, Robinson and James 1969). Labelled oleate was rapidly desaturated to linoleate and then incorporated into phospholipids. The unesterified acid must be activated before the oxidative process can occur. Sterculic acid inhibited the formation of unsaturated fatty acid from stearate by inhibiting transfer of acyl-CoA to the acyl carrier protein but not from acetate, suggesting that fatty acyl-CoA esters are not the true substrate for the desaturase. The ACP-ester is the true substrate in association with phosphatidyl choline. There was a time lag before free labelled linoleate appeared.

The activity was associated with phosphatidyl choline suggesting a lipid bound complex.

Work done on Drosophila melanogaster (Keith 1967) using acetate-1-^{14}C and acetate-2-^{3}H has shown that there are two possible pathways for synthesis of monounsaturate fatty acids. One pathway elongated a saturate fatty acid by addition of acetate and then desaturated, while the other elongated a desaturated precursor by the two carbons.

The spirochete, Treponema zuelzerae, uses still another mechanism for synthesizing its unsaturated fatty acids (Meyer et al. 1969). An anaerobic pathway introduces the double bond into the fatty acids during the process of chain elongation. Using 1-^{14}C-acetate all unsaturated fatty acids become radioactive throughout the entire chain indicating de novo synthesis. In this case the esterified fatty acid once released from the ACP complex is not "picked-up" again for desaturation.

Regulation of Fatty Acid Metabolism

It has been suggested by Levy (1963) that the location of glucose in intermediate metabolism places it in a critical position with respect to the metabolic control of the fatty acids.

A unique regulatory system for fatty acid metabolism has been proposed for the cell free extracts of lactating rabbit mammary gland (Tame and Dils 1969). The fatty acids

had a wide range of chain length, and the chromatographic pattern was influenced by the cofactors, especially the malonyl-CoA concentration. Therefore control of the fatty acid chain length could have occurred through the activity of the rate limiting enzyme acetyl-CoA carboxylase. Increasing the activity of acetyl-CoA carboxylase increased both the rate of acetate incorporation and the proportion of palmitate synthesized. A marked decrease in chain length of the fatty acids synthesized can be explained in part by a decreased concentration of malonyl-CoA available to the fatty acid synthetase.

Nothing is known about either the location of the two synthetic systems, elongation or de novo synthesis, or the relationship of the two systems to each other and the metabolism of the mitochondria. After fractionation the mitochondrial de novo synthesizing reactions are predominantly associated with the outer membranes, and elongation reactions by all of the fractions (Howard 1968). The de novo synthetic system can be measured only when no exogenous ATP is present. After adding ATP, elongation becomes the major synthetic pathway and the amount of substrate incorporation increases.

The availability of ATP within the mitochondria may serve as one control mechanism of the mode of fatty acid synthesis and the amounts of acids synthesized. In the outer membrane

of the mitochondria the fatty acid synthesis was also controlled by ATP (Howard 1968). Fatty acyl-CoA synthetase, located in the outer membranes, activated fatty acids to their CoA-derivatives utilizing ATP. The activated acids could then be substrates along with acetyl-CoA for the elongation reactions. Without ATP present the fatty acid activating system would not be functional and the de novo synthetic system would predominate.

Sufficient evidence is available to speculate about some of the mechanisms that may regulate the biosynthesis of unsaturated fatty acids in the microsomes and consequently regulate the fatty acid composition of the cell (Brenner and Peluffo 1969). Fatty acids all compete for the desaturase enzyme. This competition would constitute a preliminary mechanism regulating the relative proportions of these different fatty acids which reach the microsomes. Consequently the competition determines the relative proportions of the corresponding more highly unsaturated fatty acids which are formed. A second regulatory mechanism in the synthesis of the linolenic acid and the other intermediates of this or other series, is the feedback effect.

A third postulated regulatory mechanism in the microsomal synthesis of unsaturated fatty acids is the competition between the process of desaturation and transacylation of fatty acids.

Another factor involved most likely is the enzymatic induction controlled in some way by insulin, as well as diet.

In the adult male desert locust the activities associated with lipogenesis change during the maturation process (Walker and Bailey 1970). The changes in enzyme activities explain the overall changes found in the fat body metabolism and changes in lipid and carbohydrate levels. High glycolytic rates yield excess acetate which is converted to lipids which are stored in the fat body.

The hormonal control of insect fat body has generally been studied with respect to proteins and not fatty acids. It has been shown however that extracts from the corpora allata of Leucophaea produced a decreased rate of lipid biosynthesis in vitro (Gilbert 1967). Applications of juvenile hormone or farnesyl methyl ether caused a similar result in the regulation of lipid synthesis and there was an increase in fatty acid oxidation in certain insects (Gilbert, loc. cit.). However in I. paraconfusus topical applications of synthetic juvenile hormone cause an increase in lipid synthesis in the adult female (N. M. G. Bhakthan, personal communication). This observation suggests a complex hormonal regulation of the metabolism of lipid storage products in the living insect.

In D. melanogaster the concentration of polyunsaturates

in the diet did not appear to affect the incorporation of acetate-1-^{14}C into saturate and monounsaturate fatty acids (Keith 1967). The addition of polyunsaturates did however result in the appearance of short chain dienoic acids which were assumed to be oxidation products.

MATERIALS AND METHODS

Ips paraconfusus adults were obtained as they emerged from laboratory maintained rearing logs of Pinus ponderosa and were stored in a refrigerator at 6 C until used. All insects were used within one week of emergence for each experiment.

Experiments on reproducing insects were accomplished by establishing culture logs and excising the adults. Males were introduced individually one day prior to introduction of the females. The insect pairs were allowed to bore for several days, 6 days for the males and 4½ - 5 days for the females. The excised insects were starved twelve hours prior to sacrifice to eliminate gut contents.

To study the synthesis of fatty acids from acetate-1-^{14}C, I. paraconfusus females were placed in sequentially numbered and stoppered 10x75 mm culture tubes. These tubes were placed in an ice water bath at 4 C to lessen the physical movement of the insects. Injections of 1 μCi/μl of water, were carried out using a 31 gauge needle attached to a 0.25 ml Gilmont micrometer syringe. The cooled insects were turned dorsal surface up and the needle was passed through the posterior end into the haemocoel in proximity to the fat body. The 1 μl volume of sodium acetate-1-^{14}C was expelled into the insect and the needle remained in place approximately 30

seconds; the insects were then placed back in the stoppered tubes and into the ice-water bath. The injection time for each insect was noted. The insects were sacrificed after varying exposure times by submersing the stoppered tube in an acetone-dry ice bath at -78 C.

To determine the time required for satisfactory incorporation of the radioactive acetate into lipid, exposure times from 5 minutes to 11.5 hours were used. As the "gross lipid incorporation" curve (Fig. 7) was linear up to 30 minutes, the incorporation of acetate-1-^{14}C into the fatty acids of females was investigated at exposure times of 5, 15 and 30 minutes. For each replicate, 80 insects were injected and pooled for analysis.

The lipid extract from the 15 minute exposure was dissolved in hexane and distilled at a reduced pressure. The resultant distillate was radioassayed and found to have no activity, showing that no free acetate-1-^{14}C remained in the gross lipid extract after separation.

After exposure, the insects were homogenized in a tissue homogenizer and the lipids extracted (Bligh and Dyer 1959), saponified (after Journal A. O. A. C. 9th ed. 1965), and methylated with diazomethane (Shlenk and Gellerman 1960). The radioactive fatty acids were resolved according to the degree of unsaturation on a 1.5 g column of silicic acid

impregnated with silver nitrate (De Vries 1963).

Analysis of fatty acids was carried out on a Carlo-Erba gas liquid chromatograph with hot wire detector and fraction collector. The glass columns (2 meters long, 4 mm I.D.) were packed with 15% diethylene glycol succinate on Chromosorb W (A W) 60/80 mesh, and were maintained at 190 - 195 C. The carrier gas was helium.

The methylated fatty acids were qualitatively and quantitatively identified with a standard fatty acid methyl ester mixture which was injected before each sample. The standard mixture contained myristic (C 14:0),[2] palmitic (C 16:0), palmitoleic (C 16:1), stearic (C 18:0), oleic (C 18:1), linoleic (C 18:2) and linolenic (C 18:3) acids. No attempt was made to identify geometric isomers of the unsaturated fatty acids other than by the chain length and degree of unsaturation.

Each fatty acid to be radioassayed was collected from the sample effluent in a side arm collection tube immersed in an acetone-dry ice bath. The side arm collection tubes were rinsed into scintillation vials containing a PPO-toluene cocktail and these were counted on a Beckman LS-250 liquid scintillation spectrometer. To determine the location of the radioactivity and double bonds, the monounsaturated

[2] The first number refers to carbon number, the second to the number of double bonds.

fatty acids, C 16:1 and C 18:1, were collected on the GLC fractionator. They were oxidized (Tinoco and Miljanich 1965), re-esterified (Shlenk and Gellerman 1960) and separated by GLC. The collected oxidized products were radioassayed as before and qualitatively identified with reference to a standard mixture of short chain monocarboxyl and dicarboxyl fatty acid methyl esters. In order to trap the volatile short chain compounds, the collection tubes were partially filled with the toluene cocktail to be used for the radio-assay.

The fatty acids of the phloem of Ponderosa Pine culture logs were separated and quantified.

Although I. paraconfusus adults generally host a moderate number of nematodes, no experiments were done to determine if these influenced the incorporation of acetate-1-^{14}C into the fatty acids. However the frequency of heavy parasitism was generally less than two insects per 80 to be injected, and the effects of the nematodes were judged to be negligible.

RESULTS

The analysis of the long, straight chain, fatty acids of both sexes of freshly emerged I. paraconfusus showed no major sexual dimorphism (Table I) with respect to the relative concentrations in males and females (Fig. 1 and Table A-I). The levels of myristic (C 14:0) and palmitic (C 16:0) acids are significantly higher in the males ($P = .10$). In reproducing insects (Figs. 1 to 4; Tables I, A-II) palmitic acid and the percentage total lipid of the insect wet weight (Fig. 5) were significantly lower ($P = .15$) in the reproducing females than in the reproducing males.

Comparison of the fatty acid compositions of the control and reproducing males and females showed significant changes in each sex (Table I). The monounsaturated fatty acids of the reproducing males decreased as did the percentage lipid while the saturate (C 16:0) and polyunsaturates, linoleic (C 18:2) and linolenic acid (C 18:3) levels, increased in relative percentage with respect to the freshly emerged males. In reproducing females the levels of C 14:0, C 18:0, C 18:2 and C 18:3 increasing significantly, while C 16:0, C 16:1, C 18:1 and the percentage of lipid material decreased.

In Ponderosa Pine phloem the percentage of unsaturated fatty acids was found to be almost 90% (Figs. 1,6; Table A-III). Two peaks were unidentified (Fig. 6) and might

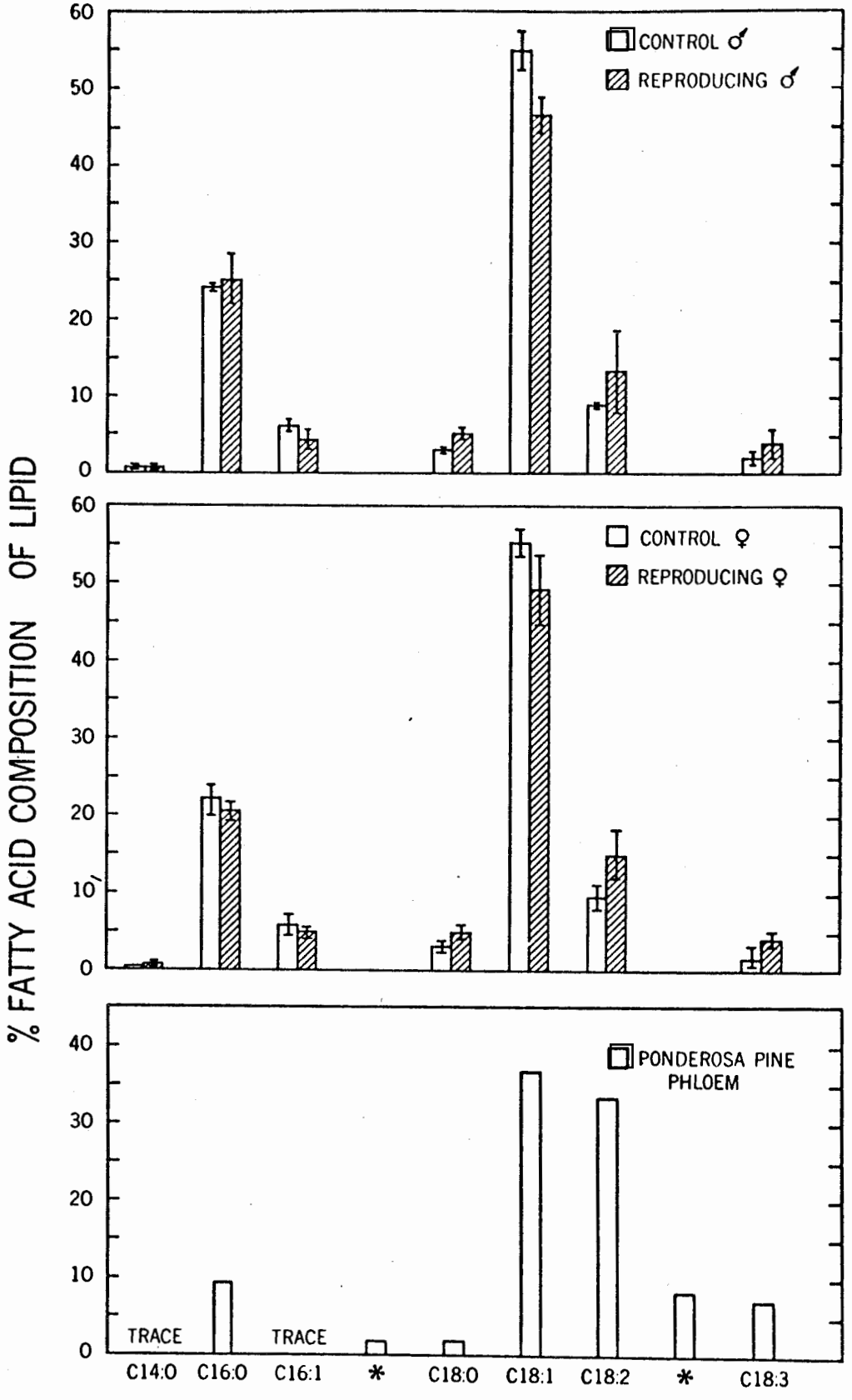
TABLE I

t - Analysis of the quantitative composition of the long chained fatty acids of freshly emerged and mating male and female Ips paraconfusus.

	Control ♂ (C_1) vs Control ♀ (C_2)	Control ♂ (C_1) vs Reproducing ♂ (R_1)	Control ♀ (C_2) vs Reproducing ♀ (R_2)	Reproducing ♂ (R_1) vs Reproducing ♀ (R_2)
14:0	P = .10	*	P = .05	*
	$C_1 > C_2$		$C_2 < R_2$	
16:0	P = .10	*	P = .15	P = .15
	$C_1 > C_2$		$C_2 > R_2$	$R_1 > R_2$
16:1	*	P = .05	P = .15	*
		$C_1 > R_1$	$C_2 > R_2$	
18:0	*	P = .005	P = .01	*
		$C_1 < R_1$	$C_2 < R_2$	
18:1	*	P = .005	P = .025	*
		$C_1 > R_1$	$C_2 > R_2$	
18.2	*	P = .10	P = .01	*
		$C_1 < R_1$	$C_2 < R_2$	
18.3	*	P = .10	P = .05	*
		$C_1 < M_1$	$C_2 < M_2$	
% lipid	*	P = .10	P = .005	P = .15
		$C_1 > M_1$	$C_2 > M_2$	$M_1 > M_2$

* P > .15

Figure 1. Fatty Acid Composition of Freshly Emerged and Reproducing Adult Ips paraconfusus, and the Ponderosa Pine Culture Log (with the standard deviation indicated).



* Unidentified

Figure 2. Chromatograph of the Fatty Acids of Freshly Emerged Female *Ips paraconfusus*.

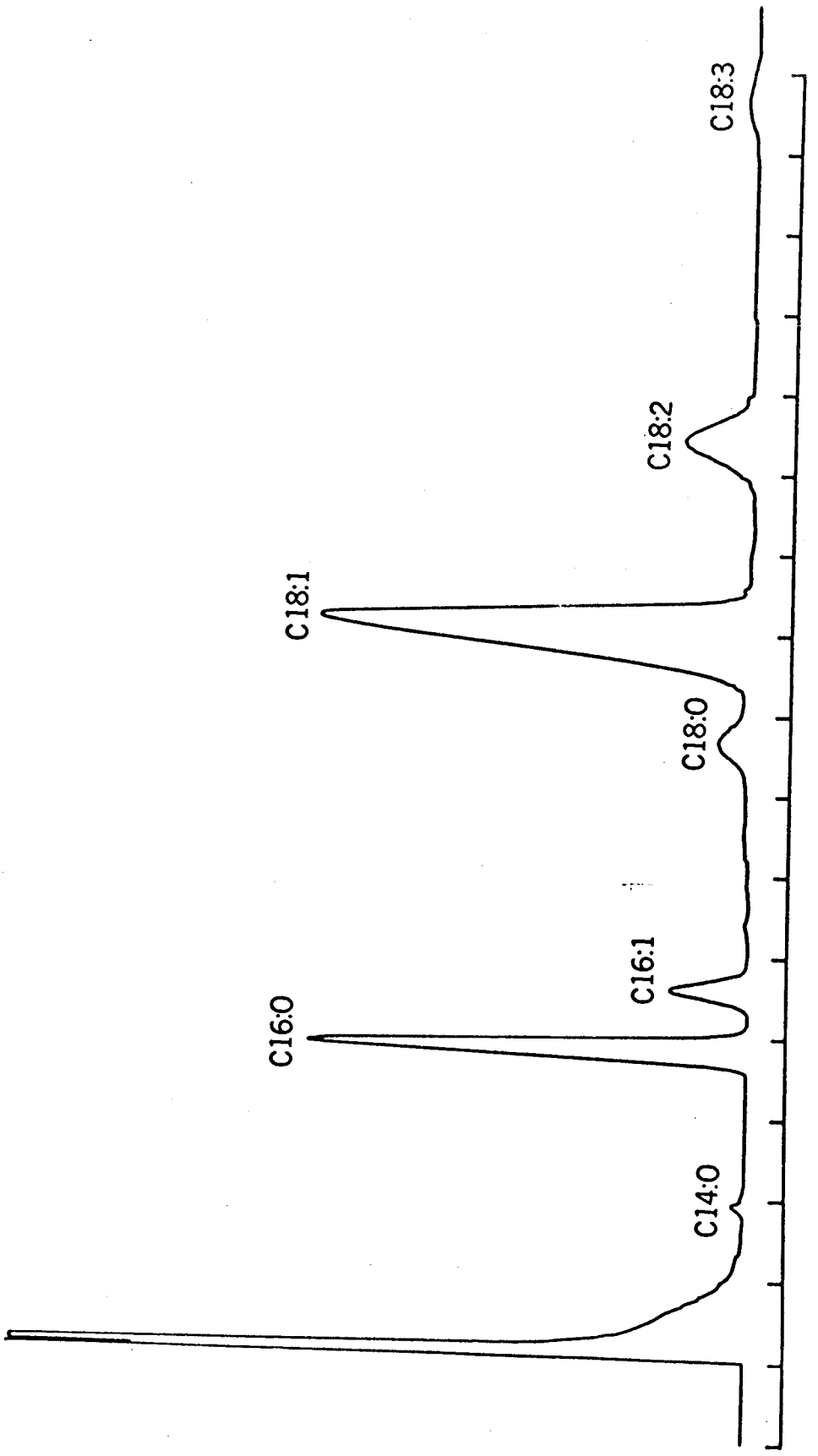

C14:0
C16:0
C16:1
C18:0
C18:1
C18:2
C18:3

Figure 3. Chromatograph of the Fatty Acids of Reproducing Male Ips paraconfusus, Excised from Culture Logs After 6 Days.

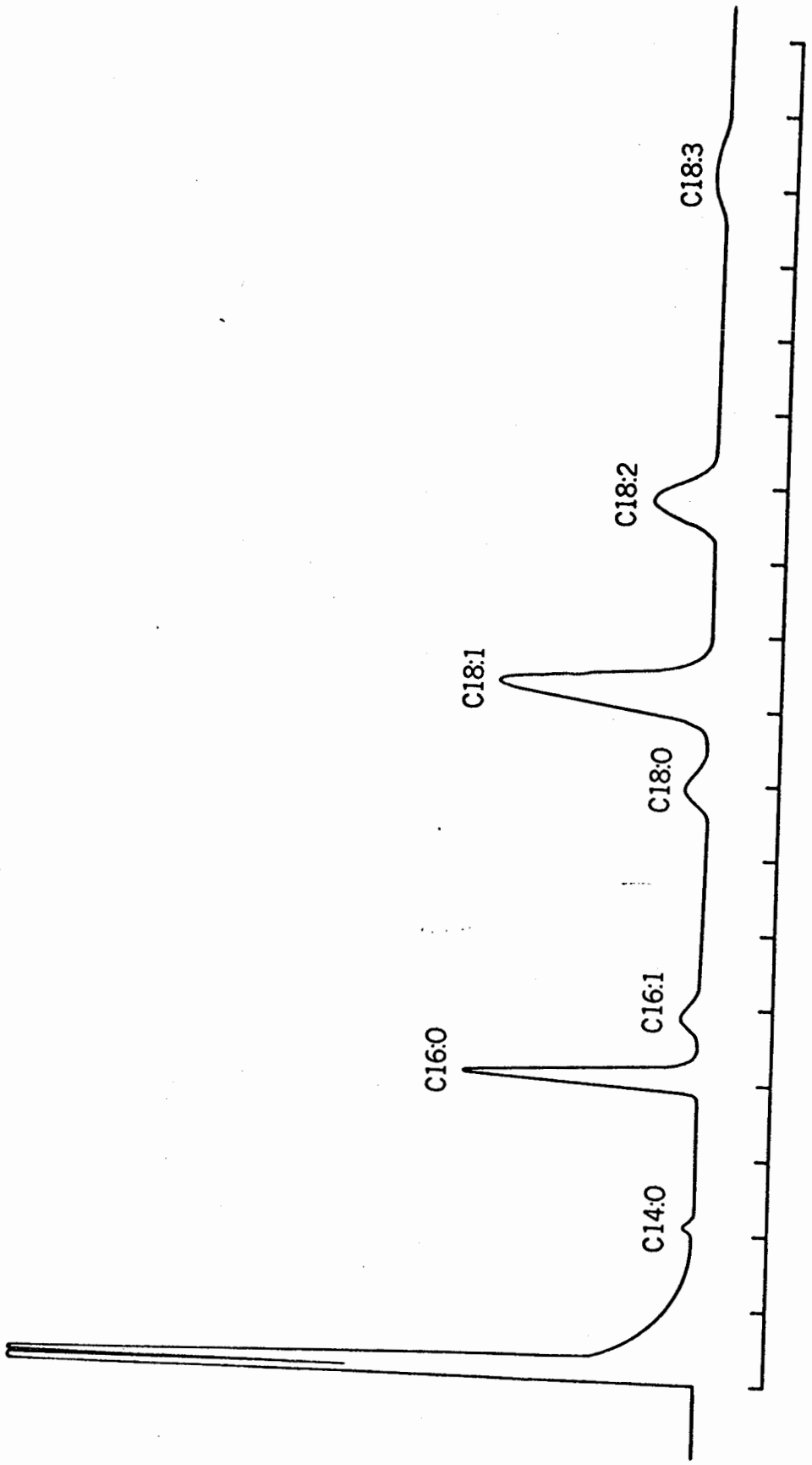

C14:0
C16:0
C16:1
C18:0
C18:1
C18:2
C18:3

Figure 4. Chromatograph of the Fatty Acids of Reproducing Female Ips paraconfusus, Excised from Culture Logs After 4½ - 5 Days.

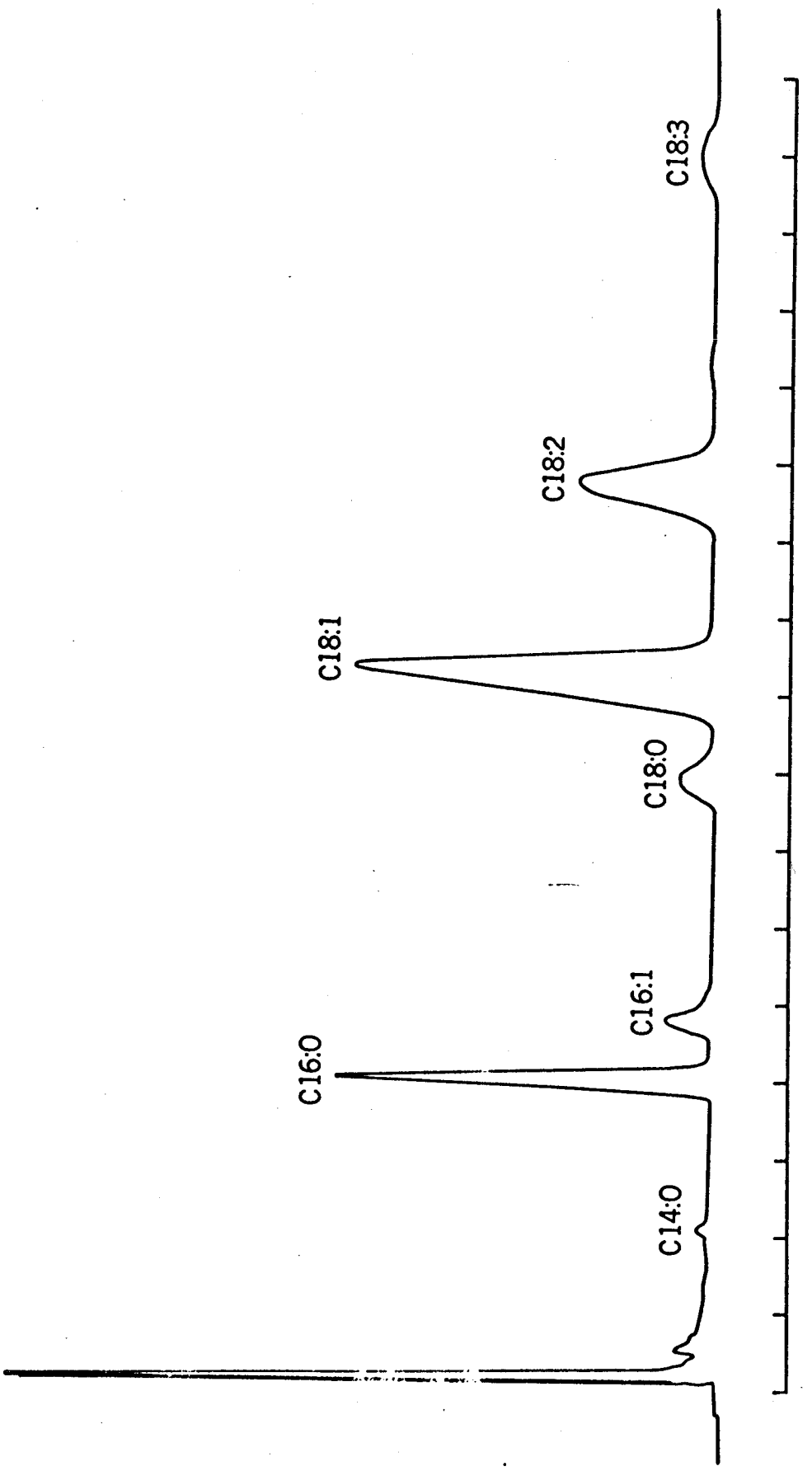
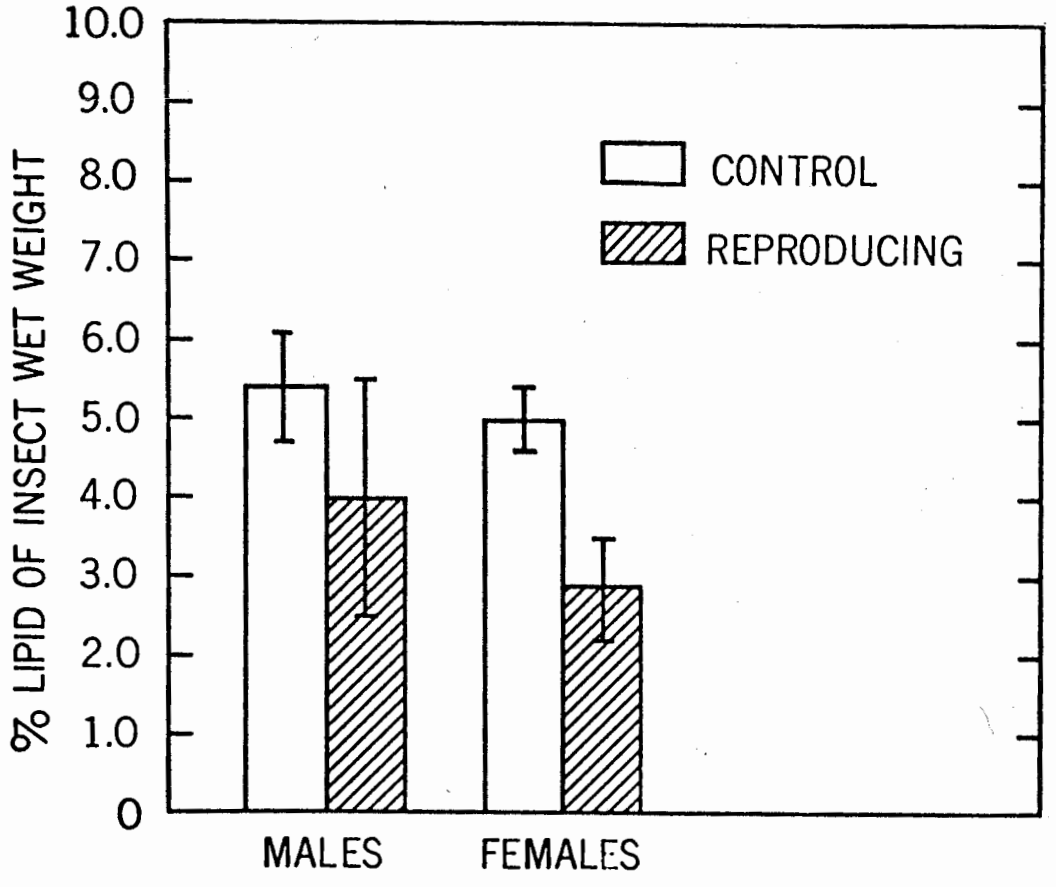

C14:0
C16:0
C16:1
C18:0
C18:1
C18:2
C18:3

Figure 5. Lipid Content of Freshly Emerged and Reproducing Adult Ips paraconfusus. (with standard deviation indicated)


10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0
% LIPID OF INSECT WET WEIGHT
CONTROL
REPRODUCING
MALES
FEMALES

possibly be branched chain fatty acids.

Injections of 1 μCi acetate-1-^{14}C/insect proved sufficient to detect radioactivity in the fatty acids of adult female I. paraconfusus. The gross lipid incorporation curve (Fig. 7) appears to be a combination of two major phases, a linear phase to about 45 minutes, and a subsequent portion from 3½ hours to the end of the experimental time possibly representing another sequence of reactions hidden by the rapid initial incorporation of acetate-1-^{14}C. The acetate-1-^{14}C was incorporated in all the saturate (Fig. 8) and monounsaturated (Fig. 9) fatty acids even after only 5 minutes (no activity was detected in the unsaponifiable fractions of the 5, 15 and 30 minute incorporations). Trace amounts of radioactivity were detected in the polyunsaturated C 18:2 and C 18:3 after a 30 minute exposure (Table A-IV).

Oxidation of the monounsaturated fatty acids C 16:1 and C 18:1, extracted from insects exposed to acetate-1-^{14}C for 30 minutes showed different levels of radioactivity (Table A-V) in the resultant products. There were no quantitative determinations made on the collected products because of the difficulty of trapping the shorter chain fatty acid methyl esters. The oxidized C 16:1 (Fig. 10) was chromatographed and the products were identified as C 7:0 (which contained 10 dpm), and Nonanedioc acid (which contained 20 dpm). The

Figure 6. Chromatograph of the Fatty Acids of the Ponderosa Pine Phloem.

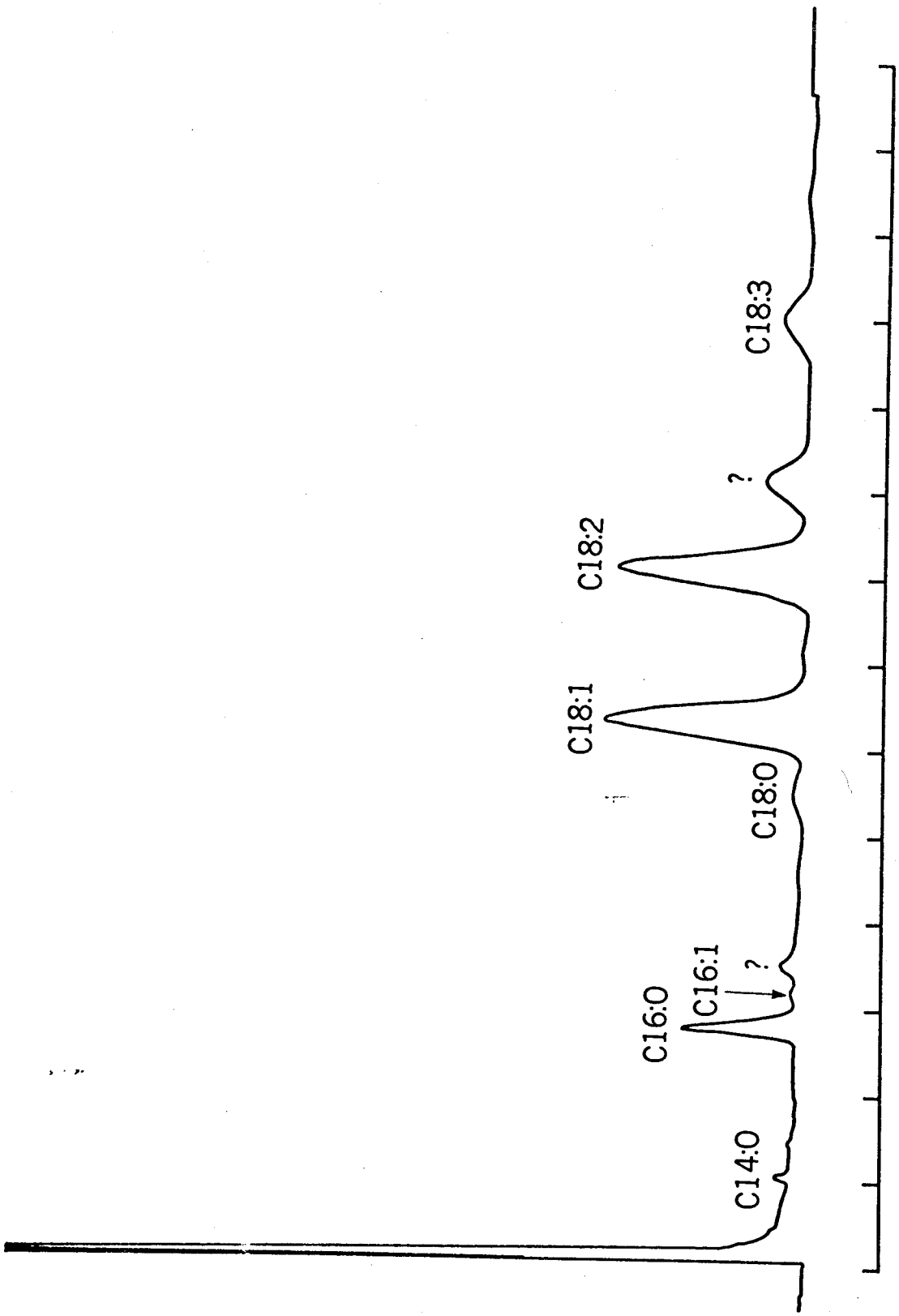

C14:0
C16:0
C16:1
?
C18:0
C18:1
C18:2
?
C18:3

Figure 7. Incorporation of Acetate-1-^{14}C into the Lipids of Freshly Emerged Female Ips paraconfusus.

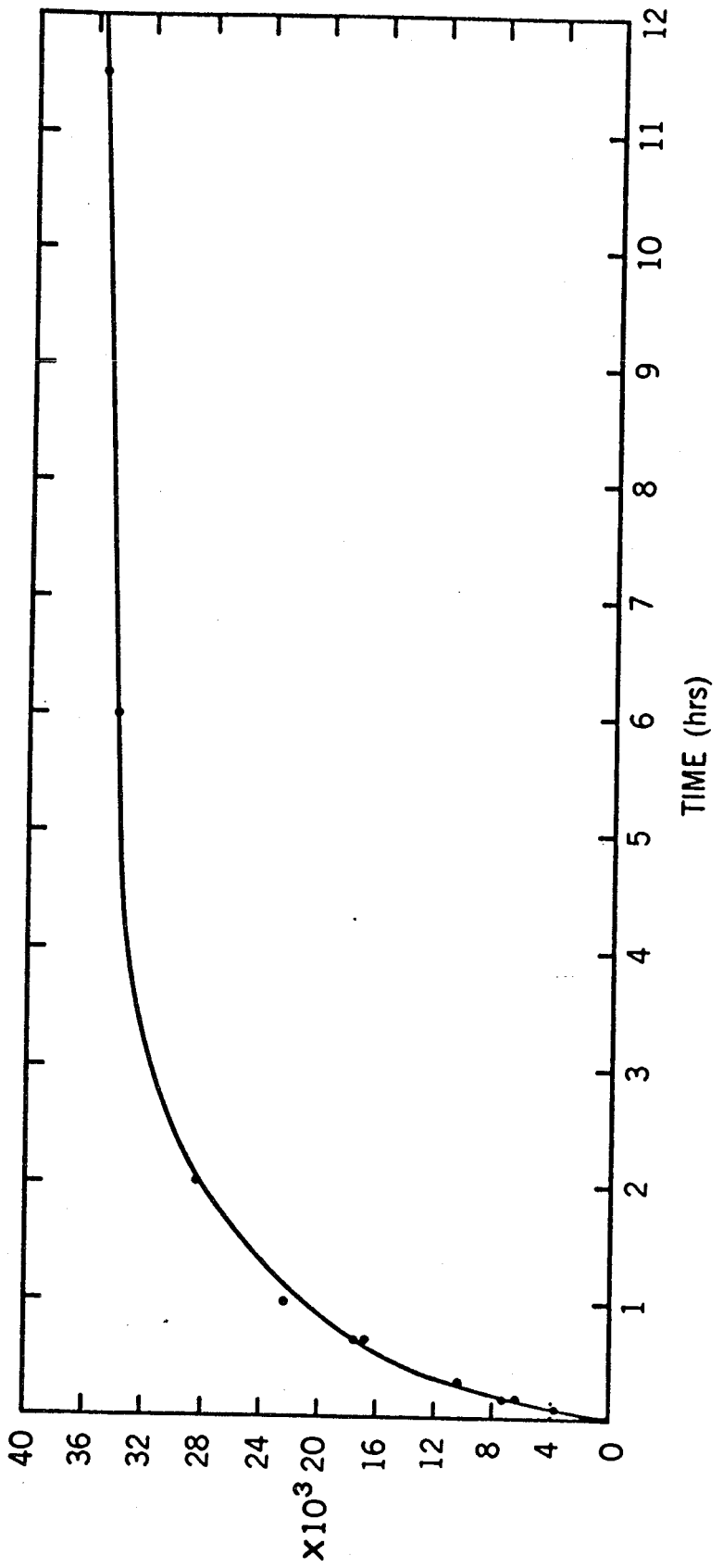

SPECIFIC ACTIVITY (dpm/mg of lipid)
x10³
0
4
8
12
16
20
24
28
32
36
40
1
2
3
4
5
6
7
8
9
10
11
12
TIME (hrs)

Figure 8. Incorporation of Acetate-1-^{14}C into the Saturated Fatty Acids of Freshly Emerged Female Ips paraconfusus (x represents the mean with standard deviation indicated).

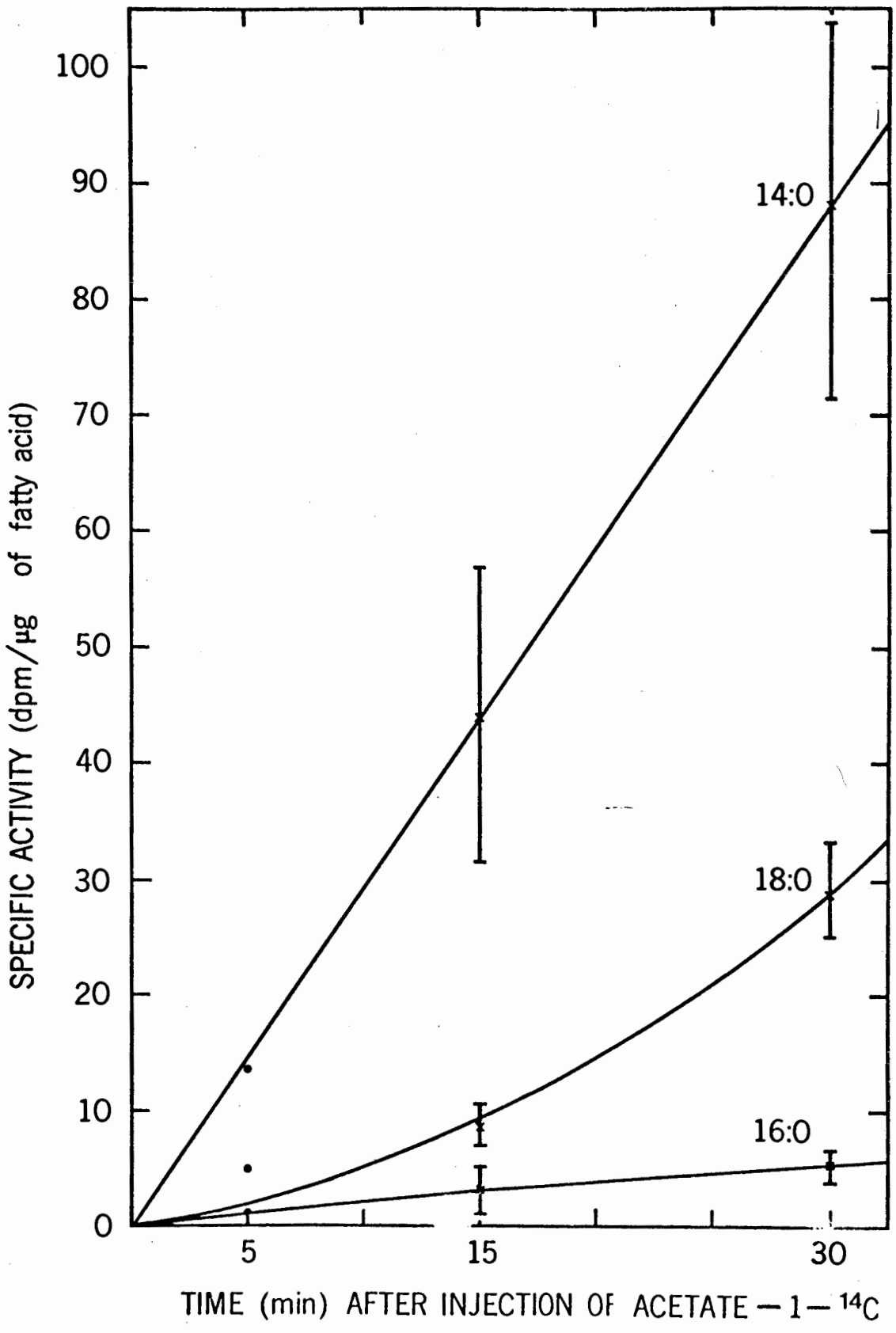

100
90
80
70
60
50
40
30
20
10
0
SPECIFIC ACTIVITY (dpm/µg of fatty acid)
14:0
18:0
16:0
5
15
30
TIME (min) AFTER INJECTION OF ACETATE — 1 — 14C

Figure 9. Incorporation of Acetate-1-^{14}C into the Unsaturated Fatty Acids of Freshly Emerged Female Ips paraconfusus (x represents the mean with standard deviation indicated).

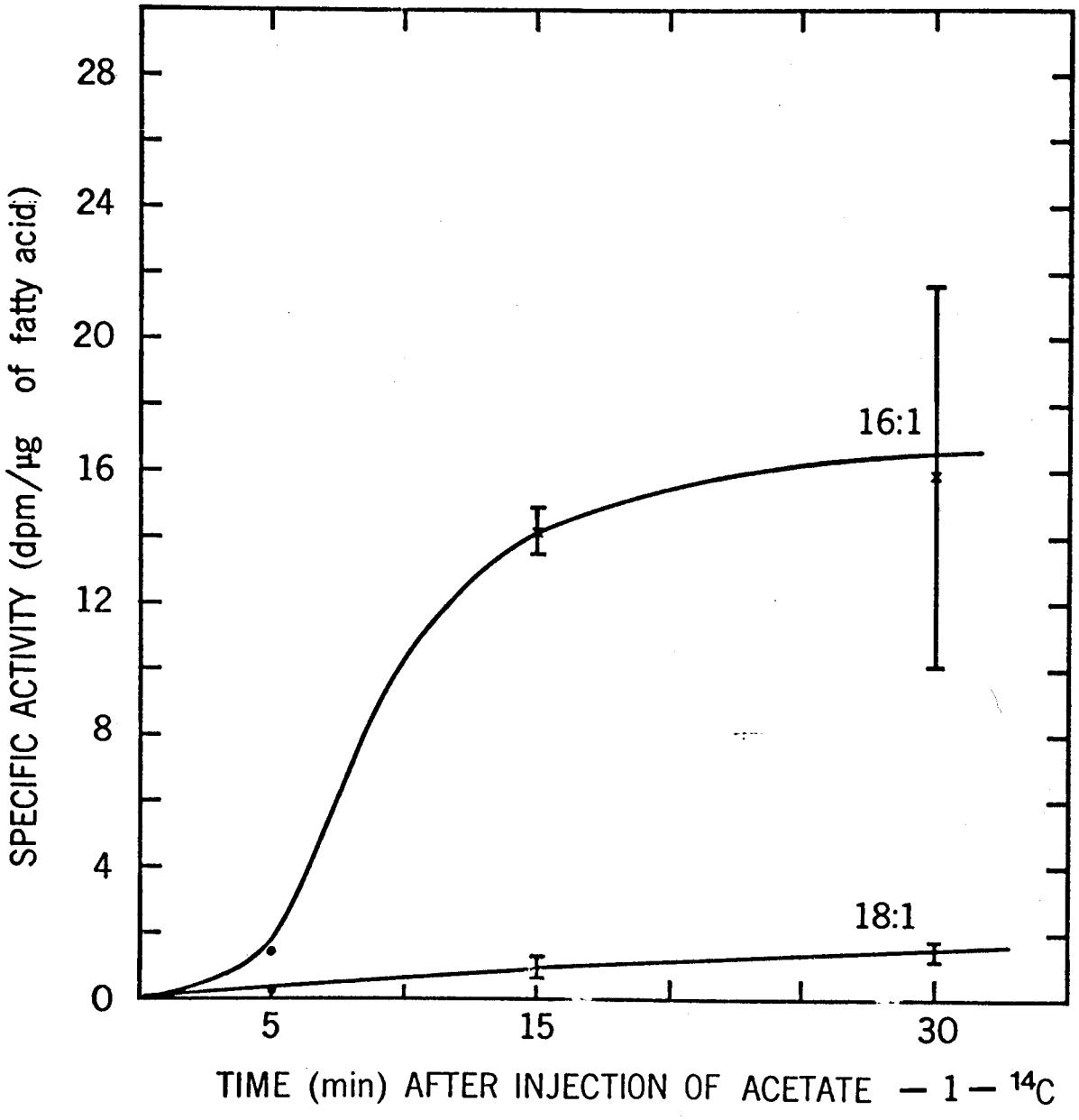
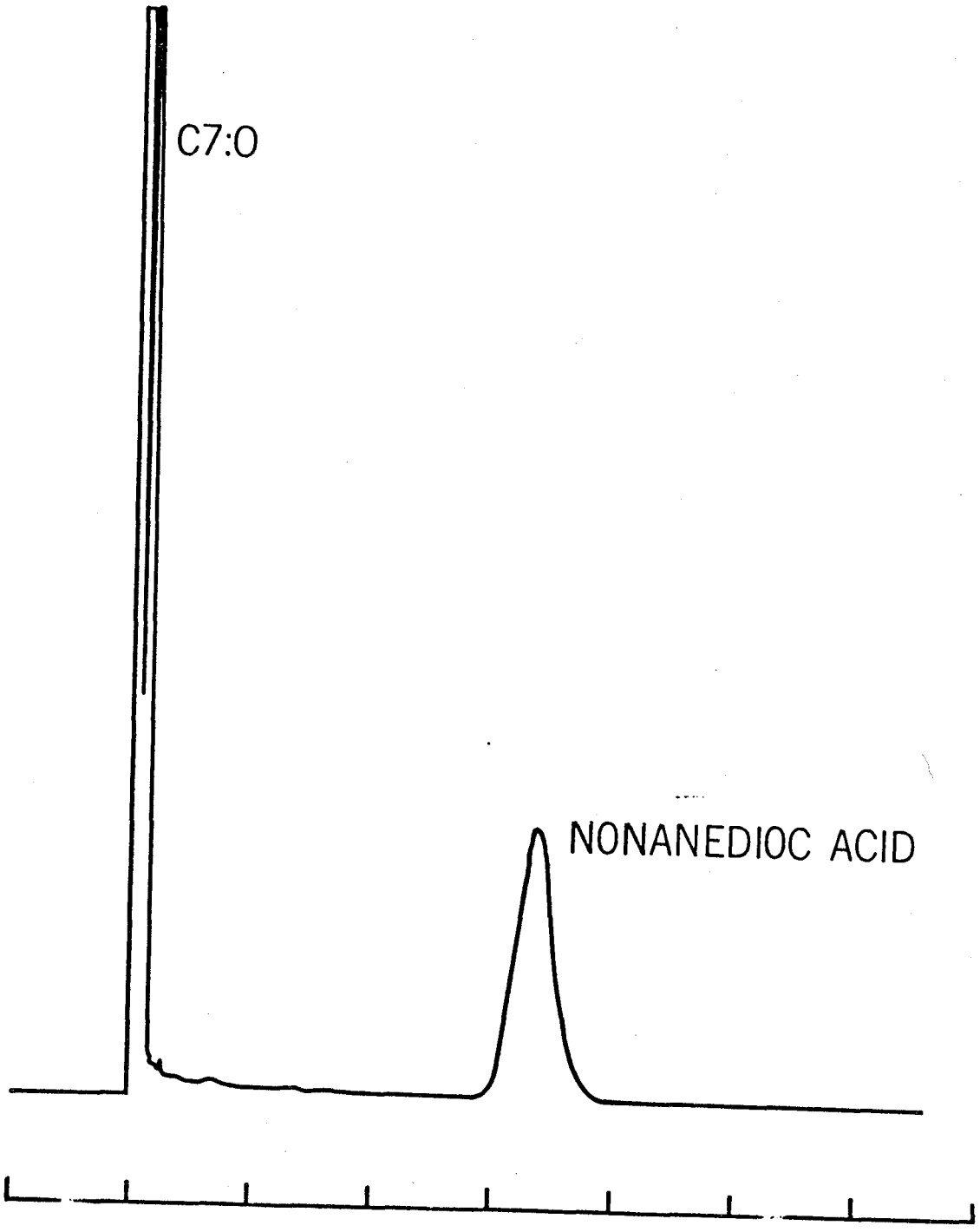

28
24
20
16
12
8
4
0
SPECIFIC ACTIVITY (dpm/µg of fatty acid)
16:1
18:1
5
15
30
TIME (min) AFTER INJECTION OF ACETATE — 1 — ^{14}C

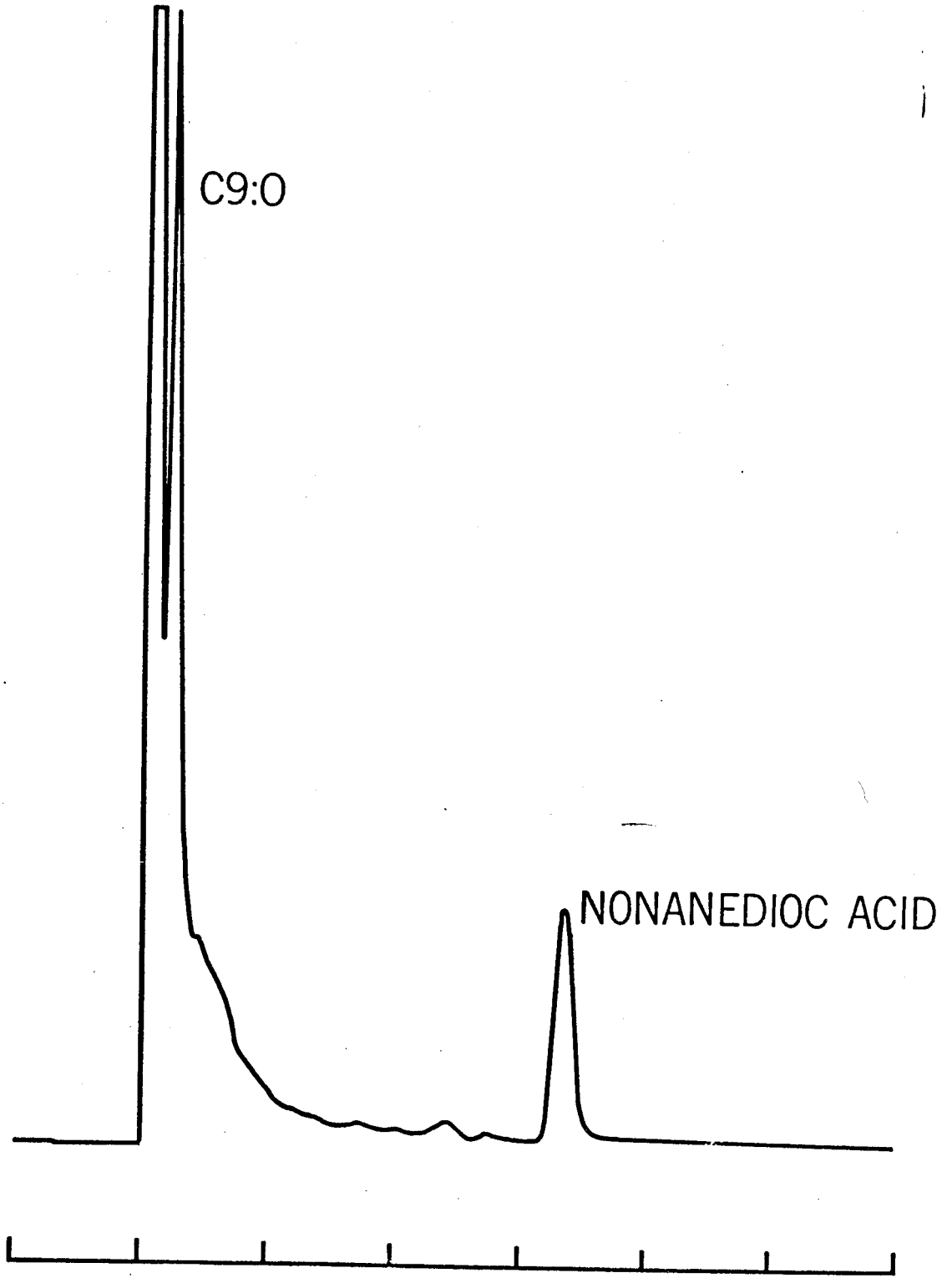
Figure 10. Chromatograph of the Oxidation Products of Palmitoleic Acid from Female Ips paraconfusus.


C7:O
NONANEDIOC ACID

observation of only the two oxidation products indicated that all the detectable palmitoleic acid of the control females was the Δ 9, 10 isomer. The oxidation of C 18:1 also yielded only two detectable products (Fig. 11) which were identified as C 9:0 (which contained 10 dpm) and Nonanedioc acid (which contained 40 dpm). This again indicates only the Δ 9, 10 isomer of oleic acid was present in the control females. The absence of any Δ 11, 12 C 18:1 indicates that no elongation of Δ 9, 10 C 16:1 occurred and that the monounsaturated fatty acids were the desaturation products of the corresponding saturated fatty acids.

The apparent radioactivity content of the various fatty acids, as measured by changes in specific activity with time, are net values. Both synthesis and conversion of the fatty acids are considered in the resultant values of radioactive content. The changes in the specific activities of the various fatty acids are therefore a measure of the turnover rate for each acid.

Figure 11. Chromatograph of the Oxidation Products of Oleic Acid from Female Ips paraconfusus.


C9:O
NONANEDIOC ACID

DISCUSSION

Although the fatty acids of the few Coleoptera analyzed (Barlow 1964) showed a similar high concentration of C 18:1 as that found in I. paraconfusus, no other similarities were obvious. In another Scolytid beetle, Dendroctonus pseudotsugae (S. N. Thompson, personal communication) the pattern of fatty acid composition was very similar to I. paraconfusus except that the concentration of C 16:1 was about three times higher at 17%.

The apparent two phase incorporation of radioactive acetate (Fig. 7) is very different than that found in the larva of Galleria mellonella in which a distinct lag was followed by an exponential increase in the incorporated acetate-1-^{14}C (Thompson 1970). In I. paraconfusus the injection of acetate-1-^{14}C was made directly into the haemocoel and fat body, which is the major site of lipid synthesis. There may, therefore, be a two pool effect that results in a change in the rate of incorporation. The rapid initial phase may be due to the high concentration of label at the site of synthesis followed by diffusion throughout the haemocoel. The diffusion would dilute the available acetate, resulting in a decreased rate of label incorporation, although the synthesis rate should not change. Another alternative is

that the available supply of labelled acetate is used in alternate metabolic pathways resulting in a pulse like incorporation.

Little radioactivity was incorporated into the non-fatty acid fraction of I. paraconfusus lipids indicating that the biphasic incorporation of acetate (Fig. 7) was into fatty acids. The incorporation of acetate-1-^{14}C in Drosophila melanogaster was distinctly different in the saturate and unsaturate fatty acids (Keith, Gauslaa and Anderson 1967). The saturate fatty acids incorporated acetate-1-^{14}C to a greater extent in the first hour and unsaturate fatty acids incorporated acetate-1-^{14}C to a much greater extent from one to nine hours after exposure. This represents another explanation of the biphasic incorporation.

Only after 30 minutes were traces of radioactivity detected in C 18:2 and C 18:3 in I. paraconfusus. Since insects are not known to synthesize polyunsaturates (Bade 1964), but accumulate them, there are two explanations for the radioactivity present. In collecting labelled fatty acids Nelson and Sukkestad (1968) found activity associated with C 18:2 and C 18:3 was contamination from C 18:1. Other in vitro work by Barron (1966) showed that labelled acetate in a central pool could undergo an exchange with the terminal acetates (carbons 1 and 2) of fatty acids and fatty acids

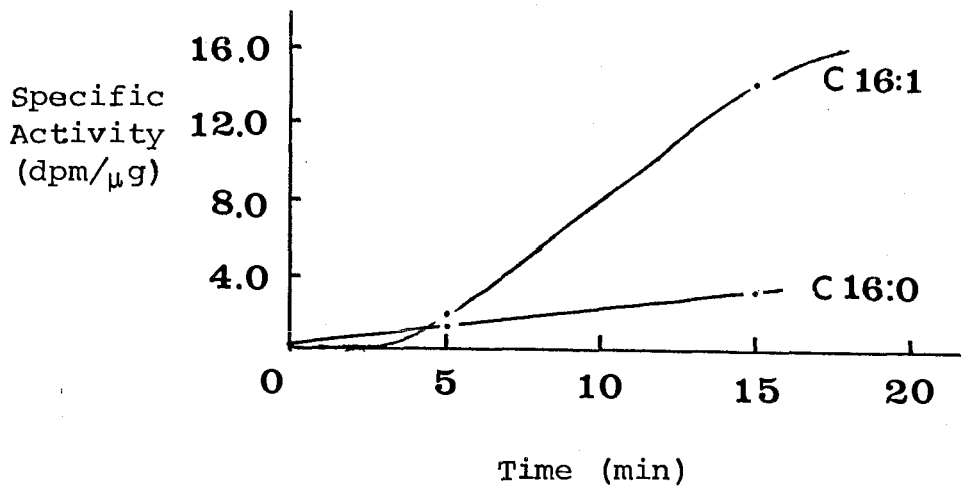
could also exchange terminal acetates with one another. If this occurs *in vivo* in *I. paraconfusus* it represents a small effect as seen in the trace values of activity found in the polyunsaturated fatty acids.

The incorporation studies were done at 4 C to resolve the synthesis reactions by slowing them down. At 4 C the reactions proceeded at a rate which made separation impossible, suggesting that the rates of the fatty acid synthesis are very rapid.

In *G. mellonella* (Thompson 1970) there was no evidence for *de novo* synthesis until 4 hours after injection of acetate-1-^{14}C. In *I. paraconfusus* the synthesis of myristic and palmitic acids, the *de novo* synthesis end products, was found to be occurring rapidly, as radioactivity was detected in them after only 5 minutes, with no lag period (Fig. 8). Therefore, there is little delay in the formation of malonyl CoA from the injected acetate. As the *de novo* synthesis is cytoplasmic (Wakil 1963), it appears that the injection of acetate-1-^{14}C in *I. paraconfusus* was made directly into the synthesis area. The increase in specific activity of stearic acid (C 18:0) is not linear but the rate of incorporation of the acetate-1-^{14}C increases with time (Fig. 8). The lag in the incorporation of acetate-1-^{14}C into C 18:0 (Fig. 8) could be expected since elongation reactions of *de novo* products occur in the mitochondria, and in order to transport the

acyl CoA product across the organelle membrane it must be converted to the carnitine derivative. The inability to separate fatty acid synthesis reactions means that the comparison of the specific activities of fatty acids does not indicate the precursor-product relationship characteristic of *in vitro* work. The specific activity is a function of the fractional turnover rate of the individual fatty acids. However, the specific activities of C 16:0 and C 18:0 show the incorporation of radioactive acetate into C 18:0 from C 16:0 (Fig. 8). Because of the inability to eliminate *de novo* synthesis, traces of activity in C 18:0 may not be due just to elongation reactions.

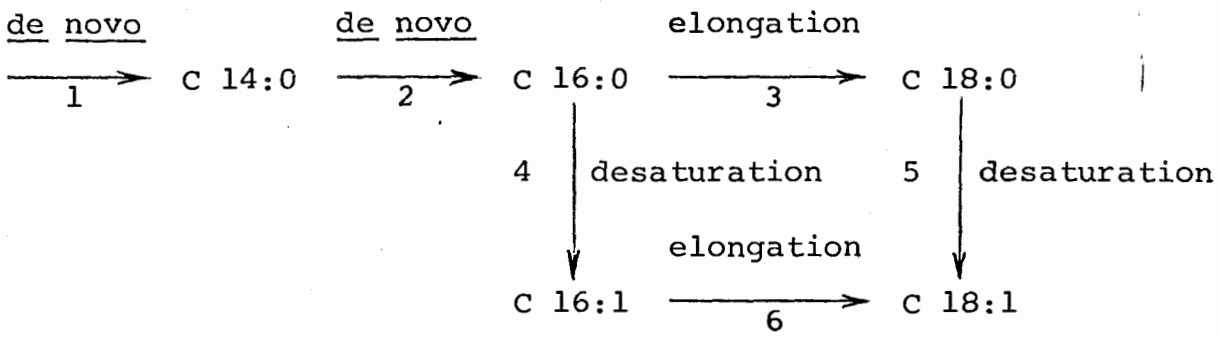
The desaturation reactions producing C 16:1 and C 18:1 were also observed to be occurring in the period from 5 to 30 minutes (Fig. 9). The specific activities of the monounsaturates reveal a product-precursor relationship as the desaturated acid incorporates the activity after it appears in the saturated precursor. In the C 16:1 there is a lag before radioactivity is isolated in the unsaturated acid but no such lag in the incorporation into C 16:0 occurs, as shown:



The slower turnover rate of C 16:1 therefore results in a higher specific activity because of the small pool size; however, after an extended exposure the specific activity of C 16:0 would probably again be higher than C 16:1. The same type of crossover was not observed in the conversion of C 18:0 to C 18:1 apparently because of the large pool size which C 18:1 represents.

No evidence was obtained that suggests that saturated and unsaturated fatty acids are synthesized by separate pathways as proposed by Sedee (1961). The changes in specific activity do not indicate precursor-product relationships *in vivo* as they do *in vitro*, but indicate turnover. *In vitro* conditions can be regulated to give individual reactions.

The reactions of fatty acid synthesis may be summarized as follows:



In order to evaluate if desaturation of C 16:0 to form C 16:1 is followed by elongation (reaction 6) or if C 16:0 is elongated to form C 18:0 and then desaturated to form C 18:1 (reaction 5) the C 16:1 and C 18:1 were oxidized at the double bond.

Analysis of the oxidation products (Figs. 10, 11; Table A$_7$-V) indicates that reaction 6 of the previous sequence does not occur, but reactions 1, 2, 3, 4 and 5 do occur. The synthesis of monounsaturated fatty acids has been shown by Bade (1964) to be the result of desaturation of saturated analogs. The label distribution of the unsaturated fatty acid is therefore the same as the saturated precursor. The distribution of label in the oxidized products of C 16:1 indicates that the labelled acetate was incorporated into short chained saturated fatty acids already in the process of de novo synthesis. This labelling pattern would produce more label at the carboxyl end of C 16:0 which then was

desaturated. The label distribution of C 18:0 as observed in the oxidation products of C 18:1 is the result of elongation initially of unlabelled C 16:0 and later elongation of labelled C 16:0 produced by de novo synthesis. This incorporation process explains the heavy label at the carboxyl end of C 18:1 and therefore C 18:0.

The decrease in total lipid content of the reproducing adult (Fig. 5) may be due to several factors. In males there is no deposition of any materials so the decrease in lipid is probably due to oxidation. In females the lipid is mobilized from the fat body and deposited in the developing oöcytes. Another factor in reproducing I. paraconfusus is that the major portion of the boring is done by the females and therefore they have available a supply of phloem lipid. If the lipid material is used also as an energy source, the metabolic turnover rate of lipid would be much greater in the reproducing female than in the freshly emerged female, resulting in the lower lipid content.

Comparison of the control and reproducing males shows that in the reproducing males the level of monounsaturates is significantly decreased while the polyunsaturates and C 18:0 are increased (Fig. 1). In electron micrographs of fat body from reproducing males, there was no evidence of increased metabolic activity associated with the internal

changes and sexual maturation (N. M. G. Bhakthan, personal communication). Possibly preferential oxidation of monounsaturates could occur concurrently with preferential accumulation of C 18:2 and C 18:3 from ponderosa pine phloem. The two unidentified fatty acids in the phloem sample (Figs. 1, 6 and Table A-III) were not detected in I. paraconfusus, suggesting that uptake or accumulation of the fatty acid material from the rearing log was preferential. Such a preferential accumulation of the fatty acids from the diet is known to occur in the larvae of the cabbage looper, Trichophisia ni (Nelson et al. 1968).

In reproducing female I. paraconfusus, the fat body rapidly synthesizes material, such as protein and lipid, for deposition in the oocytes (N. M. G. Bhakthan, personal communication). The fatty acid composition of the reproducing female was very different than the control with the levels of C 16:0, C 16:1 and C 18:1 being lower (Fig. 1). As in the males, the levels of polyunsaturates appears higher due either to accumulation or lack of mobilization to the oocytes as energy stores. The decrease in total lipid content of the reproducing females (Fig. 5) combined with the observed increase in synthetic activity of the fat body leads to the conclusion that the fatty acid synthesis rate is probably much accelerated in reproducing females. Another factor, which might explain differences in fatty acid composition

between reproducing and control insects, is that the logs from which reproducing insects were excised were very fresh, whereas control insects had emerged from "old" logs in which they had matured, greatly changing the phloem characteristics in the process.

Characteristic changes in the fat body of the female and development of the ovaries can be induced by topical applications of juvenile hormone (N. M. G. Bhakthan, personal communication). Lipid mobilization in insect fat bodies can also be induced by various vertebrate hormones (Bhakthan and Gilbert 1968). Just as hormones regulate the development of the ovarian tissue (Collins 1969), they may also regulate fatty acid synthesis by regulating vital control points such as the enzymes joining glycolysis and lipogenesis (Zamkin and Hermann 1969). As reproducing I. paraconfusus has available to it a source of carbohydrate, lipid and protein in the phloem, the enzyme regulation may be a factor in the fatty acid synthesis rate of the reproducing adults. Hormonal regulation has been also observed in the mobilization of storage compounds in the female adult Pyrrohocoris apterus, during the cyclic pattern of oocyte maturation (Martin 1969). In P. apterus there is no synthesis of fatty acids during the reproductive phase as all the necessary materials have been stored.

The ponderosa pine log that was analyzed for its fatty acid composition represents a typical log in which a colony might be established in nature. In Anthonomus grandis, the fatty acids available to the larvae in the diet had a pronounced effect on the fecundity of the adults (Earle, Slatten and Burk 1967). Although the presence of certain fatty acids in the phloem is probably not a major factor in host tree selection, host fatty acid composition might (as in A. grandis) influence the fecundity or survival rate of I. paraconfusus.

SUMMARY AND CONCLUSION

1. Within 5 minutes after acetate-1-^{14}C injection, all the reactions involved in fatty acid synthesis were occurring rapidly and acetate-1-^{14}C was incorporated into all saturate and unsaturated fatty acids of the female I. paraconfusus.

2. Precursor-product relationships were not evident because of the inability to separate the various reactions, except in a pulse-like incorporation of acetate-1-^{14}C into C 16:1.

3. Synthesis of C 18:1 was by the desaturation of C 18:0 and not elongation of C 16:1 as only the Δ 9, 10 isomers of the monounsaturated fatty acids were detected in oxidation experiments.

4. The fatty acid composition of I. paraconfusus adults in the 6th day of the reproductive phase was significantly different from freshly emerged controls.

5. The plotted specific activities of the fatty acids indicate the order of magnitude of turnover, no fractional turnover rates were calculated as the synthetic reactions were not separated.

6. In vivo studies on the synthesis of fatty acids using acetate-1-^{14}C in reproducing female adults of Ips paraconfusus

would prove useful as a measure of the metabolic changes that occur during reproduction when compared with freshly emerged females.

7. The implication of juvenile hormone in the regulation of internal changes associated with sexual maturation of I. paraconfusus would yield a further field of study in the regulation of fatty acid metabolism.

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APPENDIX A

TABLE A-I

Qualitative composition and relative concentration of the long chained fatty acids of freshly emerged male and female Ips paraconfusus.

	14:0	16:0	16:1	18:0	18:1	18:2	18:3	% lipid
Males								
Rep 1	1.3	24.1	7.5	3.2	51.0	9.3	3.4	4.2
2	.6	24.1	6.1	3.1	54.9	8.6	2.5	5.5
3	.3	23.5	5.6	2.6	57.8	8.8	1.4	6.1
4	.4	24.8	5.6	3.0	56.0	9.0	1.3	5.8
Ave	.7	24.1	6.2	3.0	54.9	8.9	2.1	5.4
±σ	±.4	±.5	±.8	±.2	±2.5	±.2	±.9	±.7
Females								
Rep 1	.7	20.8	5.9	4.4	54.0	10.0	3.5	5.2
2	.6	22.6	8.7	2.9	53.9	8.9	2.3	4.4
3	.3	22.8	4.9	2.3	59.0	9.9	.7	4.8
4	.3	24.6	6.1	3.4	55.7	6.6	1.3	5.7
5	.2	21.8	4.8	2.2	53.9	12.4	4.4	4.9
6	.4	26.0	4.3	3.4	55.0	9.6	1.1	4.8
Ave	.4	22.1	5.8	3.1	55.3	9.6	2.2	5.0
±σ	±.2	±2.0	±1.4	±.7	±1.8	±1.7	±1.3	±.4

TABLE A-II

Qualitative composition and relative concentration of the long chained fatty acids of reproducing *Ips paraconfusus* excised from ponderosa pine, males after 6 days and females after 4½ - 5 days.

	14:0	16:0	16:1	18:0	18:1	18:2	18:3	% lipid
Males								
Rep 1	.7	26.0	6.0	4.7	50.0	9.3	3.1	6.4
2	1.3	30.2	5.4	6.6	47.4	7.3	1.6	2.6
3	.5	23.5	3.2	5.2	44.9	15.9	6.5	4.2
4	.6	21.7	2.8	4.8	43.9	20.8	5.1	2.9
Ave	.7	25.3	4.3	5.3	46.5	13.3	4.0	4.0
±σ	±.3	±3.2	±1.3	±.8	±2.4	±5.3	±1.9	±1.5
Females								
Rep 1	1.0	19.3	5.7	4.0	55.7	10.0	4.3	3.0
2	1.4	20.6	4.8	6.3	43.8	16.9	6.1	1.9
3	.5	22.6	3.5	4.6	46.8	18.6	3.1	3.3
4	.6	19.5	5.4	4.3	51.1	15.1	3.7	3.6
Ave	.8	20.5	4.8	4.8	49.3	15.1	4.3	2.9
±σ	±.3	±1.3	±.8	±.9	±4.5	±3.2	±1.1	±.6

TABLE A-III

Qualitative and quantitative composition of the long chained fatty acids of the ponderosa pine phloem.

Fatty Acid	Relative Retention	% Composition
14:0	.248	Trace
16:0	.497	9.3
16:1	.584	Trace
*	.634	1.9
18:0	1.00	2.0
18:1	1.15	36.8
18:2	1.48	33.4
*	1.67	8.2
18:3	2.02	7.2

* Unidentified

TABLE A-IV

Specific activity (dpm/μg of fatty acid) of the fatty acids of Ips paraconfusus females injected with acetate-1-^{14}C.

Replicate	Time	14:0	16:0	16:1	18:0	18:1	18:2	18:3
1	5 min.	13.9	1.3	1.5	5.1	.3	Tr.	0.0
1	15	58.0	5.9	13.5	10.7	1.3	0.0	0.0
2		27.3	1.2	*	6.4	*	*	*
3		48.0	2.5	14.8	9.4	.7	Tr.	0.0
Ave		44.4	3.2	14.2	8.8	1.0		
$\pm\sigma$		± 12.7	±2.0	± .7	±1.8	±.3		
1 a**	30	44.9	2.8	8.6	21.3	*	Tr.	Tr.
1 b		65.0	4.0	8.6	33.6	1.3	Tr.	Tr.
2		100.0	7.0	22.2	30.3	1.4	Tr.	Tr.
3		99.0	4.8	17.0	23.8	1.9	Tr.	*
Ave		88.0	5.3	15.9	29.2	1.5	Tr.	Tr.
$\pm\sigma$		±16.3	±1.3	±5.8	±4.1	±.3		

* lost in separation

** a and b are duplicates; a not used in statistics.

TABLE A-V

Radioactivity associated with the oxidation products of the monounsaturated fatty acids of *Ips paraconfusus* females injected with acetate-1-^{14}C for 30 minutes.

Fatty Acid	Oxidation Products	Rep I	Rep II
16:1	Heptanoic Acid	*10 dpm	10 dpm
	Nonanedioc Acid	20 dpm	20 dpm
18:1	Nonanoic Acid	10 dpm	10 dpm
	Nonanedioc Acid	40 dpm	40 dpm

* approximate values

CURRICULUM VITAE

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