

Phospholipid Fatty Acid Composition and Arachidonic Acid Metabolism in Selected Tissues of Adult *Tenebrio molitor* (Coleoptera: Tenebrionidae)

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ABSTRACT The fatty acid composition of selected tissues from adults of *Tenebrio molitor* (L.) have been analyzed. The major components were C16 and C18 saturated and unsaturated components ubiquitous to most animal species. In addition to these components, several odd-chain fatty acids (15:0, 17:0, 17:1, and 19:0), one hydroxy fatty acid 2-OH-20:0, and two prostaglandin precursor fatty acids, 20:3n-6 and 20:4n-6 were found. Injected radioactive 20:4n-6 was incorporated into excretory, alimentary, and reproductive tissues. Within each tissue, most radioactivity was associated with phospholipids, with lesser incorporation into mono-, di-, and triacylglycerides and cholesteryl esters. In addition, substantial proportions of injected radioactive 20:4n-6 were converted into three prostaglandins, PGD₂, PGE₂, and PGF_{2α}, as well as into other, more polar oxygenated derivatives.

KEY WORDS Insecta, prostaglandins, fatty acids, *Tenebrio molitor*

RECENT WORK on the composition and significance of fatty acids in insect tissues has focused on C20 polyunsaturated fatty acids (C20 PUFAs). Although pre- and early gas chromatographic studies rarely reported on the occurrence of C20 PUFAs, Stanley-Samuelson & Dadd (1983) suggested that most, if not all, insect tissues could be shown to include at least trace levels of C20 PUFAs if appropriate methods and instrumentation were used. The absolute abundances of C20 PUFAs vary widely among various insect groups and tissues. In aquatic insects, for example, arachidonic (20:4n-6) and eicosapentaenoic (20:5n-3) acids commonly make up 10-20% of tissue fatty acids (Hanson et al. 1985). Terrestrial insects typically have far lower proportions of C20 PUFAs (Stanley-Samuelson & Dadd 1983, Stanley-Samuelson et al. 1988), often <1%. Specific phospholipid (PL) fractions from certain individual tissues of terrestrial insects may, however, have quite high proportions of longer-chain components. Examples include the retinal PL fatty acids from the butterfly *Deilephila elpenor* L., where 20:5n-3 makes up about 40% of the phosphatidylethanolamine fatty acids (Zinkler 1975). Also, 20:4n-6 makes up about 25% of the spermatophore phosphatidylcholine fatty acids from the cricket *Teleogryllus commodus* Walker (Stanley-Samuelson & Loher 1983).

Within most insect species that have been examined, however, C20 PUFAs have not been recorded at all or have been reported to occur in only very low to trace levels. Examples of occurrence in the range of 0.1-1% of PL fatty acids include *Periplaneta americana* (L.) and the grasshopper *Taeniopoda equis* (Burmeister) (Stanley-Samuelson & Dadd 1983). In the house fly, *Musca domestica* L., C20 PUFAs occur in extremely low proportions, with 20:4n-6 occurring at only 0.04% of PL fatty acids from newly emerged male flies. This is the lowest proportion recorded so far in any insect (Wakayama et al. 1985). At these levels, C20 PUFAs may appear to be of little, if any, physiological significance. These C20 PUFAs are precursors, however, to prostaglandins (PGs), and other eicosanoids, and they have in recent years been shown to be involved in a wide diversity of physiological and pharmacological functions in most invertebrate phyla (Stanley-Samuelson 1987, Stanley-Samuelson & Loher 1989). This suggests that the occurrence of these C20 PUFAs in insects, even at low levels, may be of considerable biological importance (Dadd 1985).

Prostaglandins occur in hemimetabolous and holometabolous insects, including species from the orders Orthoptera, Isoptera, Hemiptera, Coleoptera, Lepidoptera, Hymenoptera, and Diptera (Stanley-Samuelson & Loher 1986). In addition, the defensive secretions of many arthropods contain potent prostaglandin synthetase inhibitors that have been postulated to have direct ecological effects of the releasers' parasites and predators (Howard et al. 1986; Jurenka et al. 1986, 1989; Howard & Mueller 1987). However, to date, the only physiological role of PGs that has been clearly estab-

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lished in insects is the release of egg-laying behavior in the crickets *Acheta domesticus* (L.) (Destephano et al. 1974) and *T. commodus* (Loher et al. 1981), and possibly the silkworm, *Bombyx mori* (L.) (Yamaja Setty & Ramaiah 1980). In contrast, in many other invertebrate phyla, PGs and other eicosanoids play important roles in a multiplicity of functions, including host penetration behavior by cercariae of schistosomes, egg production in snails, regulation of ion flux in marine and freshwater bivalves, oocyte maturation in starfish, and hatching in barnacles (Stanley-Samuels 1987). It follows from these considerations that PGs and other eicosanoids also might be expected to play prominent roles in a similar diversity of insect physiological systems.

We tested this hypothesis using the yellow mealworm, *Tenebrio molitor* L. Because PGs are formed from C20 PUFAs, it is crucial to establish a base of information on the occurrence and metabolism of PG precursor fatty acids in this insect. In this paper, we report on the PL fatty acid compositions of selected excretory, digestive, and reproductive tissues on the uptake and lipid fraction distribution of radio-labeled 20:4n-6 and on organismal-level formation of several primary PGs from injected 20:4n-6.

Materials and Methods

Insects. Adults (2–3 wk old) of *T. molitor* were taken from a culture maintained at 30°C, 70% RH, and 8:16 (L:D) photoperiod at the USDA-ARS, U.S. Grain Marketing Research Laboratory. Sexes of pupae were determined, and the pupae were reared in individual containers to provide adults of known age and sex.

Gas Chromatography (GC) of Tissue Fatty Acids. Lipid extraction, separation of lipid fractions, formation of fatty acid methyl esters (FAMES), and GC analysis closely followed methods described by Stanley-Samuels & Dadd (1983, 1984). Individual tissues and body components were isolated from adults, then were placed in tissue grinders containing chloroform/methanol (2:1, vol/vol). Fifty μ l of 2% butylated hydroxytoluene (BHT) in chloroform were added to each tube to reduce autoxidation of PUFAs. Total lipids were extracted three times by the method of Bligh & Dyer (1959). The total lipid extracts were dried under a stream of N_2 , then applied to thin-layer chromatographic (TLC) plates (0.25-mm silica gel plates; Aldrich, Milwaukee, Wis.) and visualized by spraying with rhodamine 6G (Aldrich). Individual lipid fractions were isolated by developing the plates in petroleum ether/ethyl ether/acetic acid (80:20:1). The lipid fractions were scraped into tubes and transmethylated by refluxing in acidified methanol for 90 min. FAMES were extracted three times in petroleum ether, then chromatographed isothermally at 190°C on a Hewlett-Packard HP-5890 gas chromatograph (Hewlett-Packard, Palo Alto, Calif.) equipped with

a SP-2330 capillary column (0.25 mm by 30 M, 0.2 μ m film thickness; Supelco, Bellefonte, Pa.), a flame-ionization detector, and a HP-3396A recording integrator. A split ratio of 45:1 was used, and separations were carried out with He_2 at 0.6 ml/min. Major FAME components were tentatively identified by comparisons of retention times with authentic standards from Sigma Chemical Company, St. Louis.

Gas Chromatography-Mass Spectrometry of FAMES. Analyses were conducted by capillary GC electron impact mass spectrometry on a Hewlett-Packard 5790 GC equipped with a SP-2330 column of the same specifications as described above. The GC was interfaced to a Hewlett-Packard 5970 mass selective detector operated at 70 eV. A 45-s splitless injection was used, and all GC runs used temperature programming from 170 to 200°C at 1°C/min, with an initial 2-min hold period. Ultrapure He_2 was the carrier gas, with a flow rate of 1 ml/min. Retention times and total ion EI-mass spectra of even-carbon-number FAMES were compared with FAME standards obtained from Sigma Chemical Company. Odd-carbon-number FAMES and hydroxy FAMES were characterized by comparison with published EI-mass spectra (McCloskey 1970, Ryhage & Stenhagen 1963). Selected ion monitoring experiments also were conducted to verify the presence of 20:3n-6 and 20:4n-6 in tissue extracts. These experiments were conducted using PL FAMES, m/z 79, 318, and 320 were monitored. Dwell times were 150 ms for each ion mass.

Incorporation of Radioactive Arachidonic Acid into Individual Tissues and Lipid Fractions. Tritiated arachidonic acid (3H -5,6,8,9,11,12,14,15-20:4n-6, specific activity 666–888 $\times 10^7$ Bq/mmol; New England Nuclear, Cambridge, Mass.) was purified by elution with toluene from a silica gel minicolumn (Bio-Sil A, 100–200 mesh; Bio-Rad, Richmond, Calif.), then made up in 100% EtOH to a specific activity of 3.7×10^4 Bq/ μ l. 3.7×10^4 Bq of tritiated 20:4n-6 was injected into individual adults by inserting the needle (27 gauge, beveled) of a 10- μ l Hamilton 701 syringe (Hamilton, Reno, Nev.) between the second and third dorsal abdominal sclerites. The needle was pushed into the abdomen about 3 mm, then the plunger was depressed.

At 18 h after injection, Malpighian tubules, testes, hindgut-rectum complex, and foregut-midgut were isolated, and radiolabel incorporation into total lipids and individual lipid classes was measured. Total lipids were extracted as described above. An aliquot (5 of 100 μ l) of each total lipid extract was transferred to liquid scintillation vials. The remaining total lipid extracts were separated into PL, monoacylglycerols (MG), diacylglycerols (DG), free fatty acids (FFAs), triacylglycerols (TG) and cholesterol esters (CE) by TLC as described above. Individual TLC fractions were identified by comparison with authentic standards, then scraped into liquid scintillation vials. Radioactivity was assayed

by liquid scintillation counting on a Pharmacia-LKB 1209 LS counter (Pharmacia-LKB, Gaithersburg, Md.) equipped with chemiluminescence discrimination at 70% counting efficiency for tritium.

Biosynthesis of PGs. 1.85×10^4 Bq of tritiated 20:4n-6 was injected into the abdomens of adult beetles, then after 1, 5, or 30 min, the 20:4n-6 and oxygenated metabolites were extracted three times from individual whole animals by homogenization in 3 ml of ethyl acetate acidified with 1 ml of 0.1 N HCl. The pooled organic phases from each extraction were evaporated under N_2 . Extracts were separated into PGD₂, PGE₂, PGF_{2 α} , and more polar metabolites by developing TLC plates in the saturated organic layer formed by mixing ethyl acetate/isooctane/water/acetic acid (55:25:50:10, vol/vol) (Hurst et al. 1987). Standard PG mixtures were added to the origin of each sample as carriers, and individual PG standards (Biomol Research Laboratories, Plymouth Meeting, Pa.) were spotted in separate lanes for identification. Individual fractions were visualized by exposing the TLC plates to I₂ vapors and were scraped into counting vials. Radioactivity was assayed as described above.

Statistical Methods and Voucher Specimens. Summary statistics and one-way analyses of variance (ANOVA) were conducted using the PC version of Minitab (Minitab, Reading, Mass.). Significance tests were conducted at the $\alpha = 0.05$ level. Voucher specimens have been deposited in the research collection of the Department of Entomology, Kansas State University, Manhattan.

Results

The major fatty acid components of PLs isolated from heads, thoraces, Malpighian tubules, midgut-foregut and hindgut-rectum complexes, and testes are 16:0, 18:0, 18:1, and 18:2 (Table 1), as is seen in the generality of insect studies (Stanley-Samuelson et al. 1988). Smaller proportions of 20:0, 20:1, 20:2, 22:0, and 24:0 also were found.

Aside from these common components, other fatty acids not usually reported were detected. These include several odd-carbon-numbered acids: 15:0, 17:0, 17:1, and 19:0. These odd-chain components made up a very small proportion of total fatty acids and were identified by their retention times and by mass spectrometry (McCloskey 1970, Ryhage & Stenhagen 1963). The other unusual fatty acid was 2-hydroxyicosanoic acid (Fig. 1). This compound shows a distinct molecular ion at m/z 342 and a prominent α -cleavage ion fragment at m/z 283 (M-59) diagnostic for 2-hydroxy fatty acids (McCloskey 1970). In addition to the fatty acids reported above, two PG precursor PUFAs were present in all tissues: homo-gamma-linolenic acid (20:3n-6), precursor to the 1-series of PGs, and arachidonic acid, precursor to the 2-series of PGs. The quantity of arachidonic acid in these tissues was so low, however, as to be usually undetectable by FID. Detectable quantities of 20:4n-6 were found

Table 1. Proportions of fatty acids as percentage of total fatty acids in the phospholipid fractions prepared from total lipid extracts of selected body segments and tissues from adults of *T. molitor*

Acid	Tissue				
	Head	Thorax	Hindgut	Malpighian tubules	Testes
14:0	0.5	0.3	0.4	0.3	0.3
15:0	<0.1	<0.1	<0.1	<0.1	<0.1
16:0	11.6	9.6	9.5	6.7	7.9
16:1	0.8	0.6	0.4	0.8	0.1
17:0	0.3	0.2	0.3	0.4	0.9
17:1	0.2	<0.1	<0.1	<0.1	0.2
18:0	11.5	8.4	11.8	15.5	16.1
18:1	22.3	22.6	31.4	22.5	25.4
18:2	47.9	55.3	40.0	50.9	36.0
18:3n-6	1.4	0.1	<0.1	<0.1	0.4
19:0	0.5	0.4	0.5	<0.1	<0.1
20:0	0.1	0.2	1.9	1.5	3.1
2-OH-20:0	<0.1	<0.1	<0.1	<0.1	0.6
20:1	0.1	0.1	<0.1	<0.1	0.5
20:2	<0.1	<0.1	0.1	<0.1	0.3
20:3n-6	1.0	0.7	0.2	<0.1	0.3
20:4n-6	<0.1	<0.1	<0.1	<0.1	<0.1
22:0	<0.1	<0.1	1.8	0.4	0.8
24:0	<0.1	<0.1	<0.1	<0.1	<0.1

by total ion mass spectrometry. Signal/noise ratios were low enough, however, that verification of this tentative total ion GC-MS identification of 20:4n-6 was required. We conducted SIM experiments using ion fragments specific to arachidonic acid and showed that the small presumptive 20:4n-6 peak coeluted with authentic arachidonic acid. This method thus provided unequivocal evidence for the presence of 20:4n-6 in all *T. molitor* tissues examined, albeit in sub-ng levels. A similar SIM experiment also was conducted to confirm the identity of the homo-gamma-linolenic acid.

With the exception of 18:1 and 18:2, the PLs of the body segments and tissues we examined had fairly similar fatty acid profiles, with about 7-10% of 16:0, about 9-16% of 18:0, about 0.5% of 20:3n-6, trace quantities of arachidonic acid, and consistently low proportions of the remaining components. The head, thorax, and Malpighian tubules were highest in PL 18:2 (about 50%), and lower proportions (about 38%) of 18:2 were found in the hindgut and testes. Tissues with higher proportions of PL 18:2 had lower relative proportions of 18:1. These sorts of differences have been observed in other studies of tissue fatty acid compositions (Stanley-Samuelson & Dadd 1983, Stanley-Samuelson et al. 1988) and are taken to indicate tissue-regulated incorporation of PUFAs for yet-undefined physiological purposes.

Tritiated 20:4n-6 was taken up and incorporated into alimentary, excretory, and testicular tissues by *T. molitor* (Table 2). In males, 35% of recovered radioactivity was found in testes, about 30% in the hindgut-rectum complex, 15% in Malpighian tubules, and 18% in foregut-midgut. Reproductive tissues were not considered in females, where about

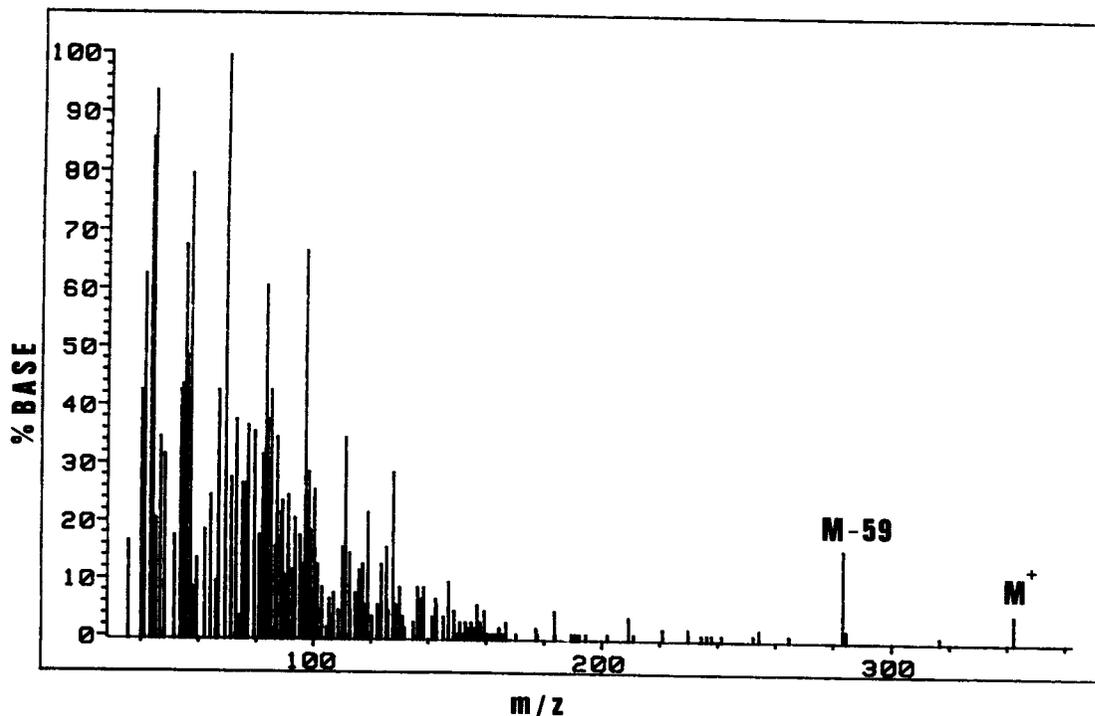


Fig. 1. Electron impact-mass spectrum of 2-hydroxyeicansanoic acid methyl ester isolated from *T. molitor* adults.

one-third of recovered radioactivity was found in each of three tissues: Malpighian tubules, foregut-midgut, and hindgut-rectum. Most of the radioactivity recovered from either male or female tissues was associated with PL (Table 3). In testes, Malpighian tubules, and hindgut-rectum, >70% of recovered radioactivity was associated with PL; <5% with MG, DG, FFA; <10% with TG; and 11-17% with CE. Radioactivity recovered from foregut-midgut was distributed slightly differently, with about 60% in PL and 25% in CE.

Adults of *T. molitor* biosynthesized three primary PGs (PGD_2 , PGE_2 , and $PGF_{2\alpha}$) from injected radioactive 20:4n-6. No sex-related differences in PG biosynthesis were obtained, so the data set forth in Table 4 show pooled values for PG biosynthesis by males and females. At 1, 5, and 30 min, most radioactivity associated with the primary PGs was recovered as PGD_2 , with PGE_2 and $PGF_{2\alpha}$ each at about one-half the level of PGD_2 . In addition to the primary PGs, radioactivity was recovered as more polar metabolites. These products are taken

to represent lipoxygenase products not yet characterized, as well as more polar products of PG oxidation. There was a fivefold increase in these products as incubation periods were extended from 1 to 30 min. The proportion of recovered radioactivity was similar at 1 and 5 min (about 60% recovery) and decreased to about one-half of that by 30 min.

Discussion

Palmitic, stearic, oleic, and linoleic acids make up >80% of PL fatty acids from whole *T. molitor* adults, in line with reports from earlier analyses of *T. molitor* (Albrecht 1961, Fast 1966, Thompson & Barlow 1970). In addition, we recorded substantial proportions of 20:3n-6 (2-3% of PL fatty acids) and trace levels (<0.1%) of 20:4n-6, both of which are PG precursor fatty acids. The 20:3n-6 but not 20:4n-6 was routinely observed by GLC using FID. This may seem at variance with earlier work on *T. molitor* (Stanley-Samuelson & Dadd 1983),

Table 2. Incorporation of 3H -arachidonic acid into selected tissues of *T. molitor* adults

Sex	n	Mean (\pm SE) % distribution			
		Malpighian tubules	Hindgut/rectum	Foregut/midgut	Testes
Male	3	15.12 (4.49)	31.28 (6.09)	18.59 (7.30)	35.0 (15.9)
Female	3	33.44 (4.24)	31.78 (4.35)	34.78 (5.25)	—

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Table 3. Incorporation of ³H-arachidonic acid into four lipid classes by tissues of adult *T. molitor*

Tissue	n	Mean (±SE) % composition			
		Phospholipid	Diglycerides	Triglycerides	Cholesteryl esters
Malpighian tubules	6	75.97 (2.86)	4.82 (0.90)	8.18 (1.79)	11.07 (1.69)
Testes	3	70.87 (3.00)	2.77 (0.47)	8.83 (1.74)	17.47 (1.59)
Hindgut-rectum	6	73.92 (2.28)	2.97 (0.50)	7.75 (2.39)	15.33 (2.34)
Foregut-midgut	6	57.90 (6.98)	6.98 (1.06)	9.88 (2.09)	25.30 (5.79)

where several tenths of a percentage of 20:4n-6 were recorded during GC analysis. The differences are quantitative, however, because in the earlier work, analyses were performed on fatty acids prepared from the PL fraction from several whole adult beetles. In the current work, we analyzed small pools of individual tissues, which did not provide enough material for detection by FID (<1 ng). Hence, analysis using more sensitive instrumentation, with detection in the picogram range, was required.

Our analyses showed several odd-chain fatty acids, including 15:0, 17:0, 17:1, and 19:0, as well as 2-hydroxy-20:0, all of which are rarely recorded in analyses of insect fatty acids. These components are far more common in microbes (Harwood & Russell 1984), where they are associated with membrane and cell wall lipids. These acids occur in only very low levels in higher organisms, and their biological significance is not well appreciated. It is not clear if they are of microbial origin or if they are indeed formed as endogenous products of insect fatty acid metabolism. The odd-chain fatty acids we found may be metabolically related to the hydroxy fatty acids, however, because oxidative decarboxylation of α -hydroxy fatty acids have been shown in microbes to lead to odd-chain analogues (Sweeley 1985).

Another complicating factor in assessing lipid compositional profiles in insects is that tissue fatty acid compositions are sometimes heavily influenced by environmental parameters, such as diet (Stanley-Samuelson et al. 1988). For example, in the greater wax moth, *Galleria mellonella* (L.), proportions of 20:5n-3 in PL from whole adults increased linearly with larval dietary concentrations of 18:3n-3, a metabolic relative (Stanley-Sam-

uelson & Dadd 1984). Similarly, adults of the mosquito *Culex pipiens* L. had greater proportions of arachidonic acid when more arachidonic acid was included in the larval diets (Stanley-Samuelson & Dadd 1981, Dadd et al. 1987). It follows that, within a species, apparent levels of any given acid may be quite variable, depending on the dietary history of the particular exemplars under study.

Radioactivity associated with injected, radio-labeled arachidonic acid was taken up by all the tissues of adult males and females we examined. In females, Malpighian tubules, hindgut-rectum, and foregut-midgut accumulated about equal proportions of the recovered radioactivity. This suggests that all these tissues have the enzymes needed for C20 PUFA uptake and incorporation. These data are not directly comparable with results from the males, however, because we included testes with the latter. About 35% of the recovered radioactivity in males was found in the testes. This value is similar to testicular uptake of injected 20:4n-6 by males of the cricket *T. commodus* (Stanley-Samuelson et al. 1986). These data thus indicate considerable capability for preferential uptake of 20:4n-6 by testes. Substantial levels of radioactivity in the males also were recovered from Malpighian tubules, hindgut-rectum complex, and foregut-midgut, again indicating considerable potential for uptake and incorporation of injected 20:4n-6.

Within the tissues we examined, radioactivity from labeled 20:4n-6 was associated with several lipid fractions, including PL, MG, DG, FFA, TG, and CE. In all tissues, most radioactivity was found in PL. This is consistent with the preferential incorporation of radioactive 20:4n-6 into PL previously seen in such other insect systems as the whole-animal PL of *M. domestica* (Wakayama et al. 1985)

Table 4. Incorporation of ³H-arachidonic acid into prostaglandins by adult *T. molitor*

Incubation time, min	Sample size ^a	% Counts recovered	% (±SE) distribution of recovered counts		
			PGD ₂	PGE ₂	PGF ₂
1	7 (3 ♂♂, 4 ♀♀)	59.3a	0.32 (0.08)a	0.16 (0.04)a	0.13 (0.02)a
5	3 (2 ♂♂, 1 ♀)	64.2a	0.58 (0.03)a	0.34 (0.06)b	0.26 (0.04)b
30	5 (5 ♀♀)	29.0b	0.40 (0.05)a	0.21 (0.03)a	0.24 (0.06)b

Means within a column followed by the same letter are not significantly different from each other at the $\alpha = 0.05$ level. ANOVA and mean separations were conducted on arcsin square root of proportions transformations. Reported means and SE's are on the untransformed data.

^a No significant ($P < 0.05$) sex-related differences (paired *t* test) were found for the 1-min analysis, so the data from both sexes at each time interval were pooled in all subsequent analyses.

and the testicular PL of *T. commodus* (Stanley-Samuels et al. 1986). Nutritional studies have similarly shown preferential incorporation of dietary PUFAs into PL. Examples are incorporation of dietary C18 PUFAs in adult PL by *G. mellonella* (Stanley-Samuels & Dadd 1984) and *C. pipiens* (Stanley-Samuels & Dadd 1981, Dadd et al. 1987). Finally, studies of insect fatty acid compositions have consistently shown that PLs contain higher proportion of PUFAs than do other lipid fractions (Stanley-Samuels et al. 1988). All these points are consistent with the notion that PUFAs express their physiological significance in association with PLs.

PUFAs are generally thought to serve in two broad areas of physiology (Hansen 1989). On the one hand, PUFAs make up crucial structural components of cellular and subcellular membrane PLs. As components of biomembranes, PUFAs are involved in those physiological functions that are specific to membranes, such as ion, nutrient, and water transport, creating microenvironments for enzyme and other membrane-associated proteins and creating a physical demarcation between cells. On the other hand, certain PUFAs are known to be released from membrane PLs as free fatty acids by the action of phospholipases. When any of three specific PUFAs (20:3n-6, 20:4n-6, or 20:5n-3) is released as a free acid, it is likely to become available for the biosynthesis of PGs and related eicosanoids.

Prostaglandins have been detected in many insect species by several analytical methods (Stanley-Samuels & Loher 1986). Two PGs, PGE₂ and PGF_{2α}, have been detected previously in *T. molitor* by radioimmunoassay (Murtaugh & Denlinger 1982). Here, we add more support for the role of 20:4n-6 as a PG precursor PUFA in *T. molitor* by showing that injected, radio-labeled arachidonic acid is converted into PGD₂, PGE₂, and PGF_{2α}, as well as into other more polar arachidonic acid metabolites. Among studies of PGs in insects, PGE₂ and PGF_{2α} are the most commonly detected arachidonic acid metabolites. This is probably because the RIA methods available at the time of early exploration into arachidonic acid metabolism within insects favored detection of these two compounds. In addition to these two metabolites, there are a few reports of other PGs and related eicosanoids in insect tissues. The lipoxigenase product LTB₄ was detected by RIA in extracts from larval and adult *C. pipiens* (D. W. Stanley-Samuels & R. H. Dadd, unpublished observations). Material that co-chromatographed with PGA₂ on TLC and HPLC was detected in the ovaries of *T. commodus* (Stanley-Samuels et al. 1983). Also, studies on biosynthesis of eicosanoids from radioactive 20:4n-6 have shown PGD₂ biosynthesis in the mosquito *Aedes aegypti* (L.) (Stanley-Samuels & Loher 1989) and lipoxigenase activity in the firebrat, *Thermobia domestica* (Packard) (Ragab et al. 1987). Our finding of PGD₂ biosynthesis in *T. molitor* is consistent with the growing number of arachidonic

acid metabolites associated with invertebrate systems.

The broad background for the biological significance of eicosanoids comes from intense efforts driven by the clinical and veterinary importance of these compounds. A crucial concept that has emerged from the vast amount of work on mammalian systems is that different eicosanoids play separately identifiable roles in different tissue systems. For example, within the same organism, many lipoxigenase metabolites of arachidonic acid are associated with host defense functions, and other arachidonic acid metabolites are associated with regulation of basic physiological processes, such as contraction of smooth muscle. In comparison with mammalian studies, where several systems within a single organism are studied, work on invertebrates has focused on only one system within any given species. In *T. molitor* and in *A. aegypti*, where analytical systems were optimized to show a range of arachidonic acid metabolites, we have evidence for biosynthesis of a large number of eicosanoids by whole animals. This range of arachidonic acid metabolites suggests, first, that eicosanoids probably are involved in a number of independent physiological systems and, second, that the regulatory roles of eicosanoids may be expressed in terms of quantitative ratios of one eicosanoid to another. Thus, in the egg-laying behavior in *T. commodus*, circulating levels of PGE₂ and PGF₂ are inverted following mating, leading to the idea that it is not biosynthesis of PGE₂ alone that releases egg-laying behavior but rather the combined synthesis of PGE₂ coupled with decreased levels of PGF_{2α} (Stanley-Samuels & Loher 1986).

We feel that substantial progress in appreciation of the significance of eicosanoids in invertebrates will arise only from studies of several physiological systems within the same organism. *T. molitor* is an organism of choice for such studies because it is relatively easy to maintain in culture, it has a deep history of detailed physiological work already in place, and our preliminary studies clearly indicate that this insect carries out a complex range of arachidonic acid metabolism.

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