

# Phospholipid Fatty Acid Composition and Distribution Patterns of Prostaglandins in Malpighian Tubules of the Yellow Mealworm (Coleoptera: Tenebrionidae)

RALPH W. HOWARD, NANCY A. WITTERS,<sup>1</sup> AND DAVID W. STANLEY-SAMUELSON<sup>1</sup>

U.S. Grain Marketing Research Laboratory, USDA-ARS, 1515 College Avenue, Manhattan, Kansas 66502

Ann. Entomol. Soc. Am. 85(4): 489-498 (1992)

**ABSTRACT** Fatty acid compositions were determined for four phospholipid fractions (phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine-phosphatidylinositol and cardiolipin) extracted from Malpighian tubules of laboratory and feral populations of the yellow mealworm, *Tenebrio molitor*. These compositions were compared with each other and to the fatty acid composition of standard diet used for the laboratory population. The insect lipids, but not the dietary ones, showed multiple positional isomers for the monoenoic acids, and the presence of polyunsaturated fatty acids. Among the four phospholipid classes there were distinct relative abundance differences, and for all classes except phosphatidylserine-phosphatidylinositol and cardiolipin, the feral population contained greater proportions of unsaturated fatty acids (especially polyunsaturated fatty acids) than did the laboratory population. These results indicate selective fatty acid incorporation into individual Malpighian tubule phospholipid classes. Studies with radioactive arachidonic acid using the laboratory population similarly indicate selective absorption of arachidonic acid into Malpighian tubule phospholipid classes. Under our conditions, 1.2% of total radioactivity was incorporated into the Malpighian tubule phospholipids. Within phospholipids, most of the radioactivity was associated with phosphatidylcholine (76.9%), and less with phosphatidylethanolamine (9.5%) and phosphatidylserine-phosphatidylinositol (11.9%). Very little radioactivity was recovered in cardiolipin (1.8%). Finally, immunohistochemical studies with intact Malpighian tubules of *T. molitor* larvae demonstrated distinctive distribution patterns for the prostaglandins PGE<sub>2</sub> and PGF<sub>2α</sub>, which are two major cyclooxygenase products from phospholipid polyunsaturated fatty acids suspected to be involved in *T. molitor* renal functions.

**KEY WORDS** Insecta, eicosanoids, water regulation, ion transport

THE PHYSIOLOGICAL SIGNIFICANCE of eicosanoids is well established in the vertebrate literature (Moore 1985). Their distribution and physiological significance in invertebrates are less appreciated (Stanley-Samuelson 1987, 1991). Among insects, prostaglandins have now been found in at least four orders (Stanley-Samuelson & Loher 1983, Stanley-Samuelson 1987). They play important roles in egg laying behavior (DeStephano et al. 1974, Loher et al. 1981, Yamaja Setty & Ramaiah 1980), in immunity (Stanley-Samuelson et al. 1991), in thermoregulation (Stanley-Samuelson et al. 1990), and in renal function (Petzel & Stanley-Samuelson 1992). The presence of eicosanoids other than prostaglandins in insects has only been observed so far as a series of lipoxigenase products in the firebrat, *Thermobia domestica* (Packard) (Thysanura) (Ragab et al. 1987, 1991).

Eicosanoids in vertebrate systems are primarily thought of as "local hormones" because of their rapid degradation in the circulatory system (Moore 1985). The circulatory system of insects is open, however, and eicosanoids have somewhat longer half-lives than in vertebrate systems (Stanley-Samuelson & Loher 1983, Stanley-Samuelson 1987). Even so, their half-lives in insects are on the order of a few minutes and many of their roles may be conducted solely at a local tissue or organ level as suggested for mosquito Malpighian tubules (Petzel & Stanley-Samuelson 1992). Because eicosanoids may not be globally circulated, when physiological functions for eicosanoids in a given tissue or organ are postulated, it is necessary to show that the precursor C20 polyunsaturated fatty acids are locally available. It is also necessary to identify the phospholipid source of the C20 polyunsaturated fatty acids and to demonstrate that the given tissue or organ is capable of biosynthesizing the postulated eicosanoids.

As noted above, eicosanoids have been implicated in Malpighian tubule function in the mosquito *Aedes aegypti* L. (Petzel & Stanley-

This article reports the result of research only. Mention of a proprietary product does not constitute an endorsement or recommendation for its use by USDA.

<sup>1</sup> Department of Entomology, 202 Plant Industry Building, University of Nebraska-East Campus, Lincoln, Nebr. 68583.

x D. Cou-  
nus digo-  
shed plant  
. Environ.

for natu-  
lant bugs,  
. Graham  
al control  
America.

amily Mir-  
Henry &  
eteroptera,  
ital United

Adelpho-  
Miridae  
duction in  
Tech. Bul.

Statistics,  
Off., Wash-

species of  
e Palearc-  
: 280-288.  
ris lineola-  
ridae). En-

icroctonus  
ilus (Nees)  
parasites of  
ulionidae).

orine para-  
menoptera:  
pp. 69-75.  
, Economic  
Lygus and  
ARS-64.

se of caged  
omparative  
fa. J. Econ.

alfalfa plant  
tance in al-

l reproduc-  
parthenoge-  
chn.) Ann.

hods. Iowa

R. F. Luck-  
us forms of  
chogramma-  
-481.

logg. 1990.  
(Hemiptera:  
lsfoot trefoll  
Entomol. 83:

91; accepted

Samuelson 1992). R.W.H. & D.W.S. have also investigated the possible role of eicosanoids in renal functions of an insect with rather different ion and water requirements, the yellow mealworm, *Tenebrio molitor* L. Chromatographic procedures have shown that this insect can biosynthesize prostaglandins and other eicosanoids at the whole body level (Howard & Stanley-Samuelson 1990). Chromatographic and mass spectral methods have also shown that the requisite C20 polyunsaturated fatty acids are present in Malpighian tubules, although at low levels on a whole-organ basis (Howard & Stanley-Samuelson 1990). These C20 polyunsaturated fatty acids could very well be present in somewhat greater quantities in individual phospholipid classes, however. To test this hypothesis, we isolated the four major phospholipid classes from the Malpighian tubules of *T. molitor* and determined their fatty acid compositions. We also compared these compositions between a 3-yr-old laboratory population and a freshly collected feral population to assess the possibility that previously reported low levels of C20 polyunsaturated fatty acids in Malpighian tubules of these insects were an artifact of laboratory culture conditions. We examined the incorporation of radioactive arachidonic acid into each of the four phospholipid classes by Malpighian tubule preparations. Finally, the presence and gross distribution pattern of PGE<sub>2</sub> and PGF<sub>2α</sub> in larval *T. molitor* Malpighian tubules was shown using immunohistochemical procedures.

#### Materials and Methods

**Insects.** *T. molitor* adults or late-instars were either from a laboratory culture obtained ≈3 yr ago from Carolina Biological supply (Burlington, N.C.) and maintained at 30°C, 70% RH, and 8:16 (L:D) photoperiod (designated the laboratory population) or were feral adults collected in July 1991 from a farm in Pottawatomie County, Kansas and used within 1 d of collection (designated the feral population). The laboratory population was sampled as adults (2–3 wk old) or late instars and the feral population adults were of unknown age. Sexes were not segregated before analysis. The laboratory population was maintained on a diet of 35% oatmeal, 35% unbleached white flour, 15% ground milo, 10% poultry layer mash (15% protein content), and 5% torula yeast (all on a weight basis). The feral population was not fed before analysis.

**Extraction and Analysis of Lipids.** Malpighian tubules were dissected from beetles in Ringers solution and transferred immediately to tissue grinders that contained chloroform-methanol (2:1, by vol) and 50 μl of 2% butylated hydroxytoluene to reduce autoxidation of polyunsaturated fatty acids. Total lipids were extracted three times by the method of Bligh & Dyer

(1959). Total lipid extracts were concentrated under a stream of N<sub>2</sub> to dryness, redissolved in chloroform, and applied to thin-layer chromatography plates (TLC) (0.25-mm silica gel plates; Aldrich, Milwaukee, Wis.). Plates were developed in chloroform-methanol-water (65:25:4, by vol). Selected fractions, phosphatidylethanolamine, phosphatidylcholine, phosphatidylserine, phosphatidylinositol, and cardiolipin corresponding in R<sub>f</sub> values to authentic standards (Sigma) were scraped into 15-ml reaction tubes with teflon-lined caps. Because phospholipids do not separate into pure fractions with one-dimensional TLC, the system we used will possibly contain traces of phosphatidyl-N-methyl-ethanolamine or phosphatidyl-N,N-dimethylethanolamine in the phosphatidylethanolamine fraction, and the phosphatidylcholine fraction may contain traces of phosphatidylglycerol. Phosphatidylethanolamine and phosphatidylcholine are well known as major cellular phospholipids (Hawthorne & Ansell 1982) and we regard the two fractions described in this paper as phosphatidylethanolamine- and phosphatidylcholine-rich fractions. Fatty acid methyl esters were formed by transesterification of the isolated phospholipids in refluxing methanol-sulfuric acid for 90 min (Stanley-Samuelson & Dadd 1983). Analysis of the laboratory population diet involved extracting three 1-g aliquots in chloroform-methanol (2:1) and transesterification as described above.

**Gas Chromatography (GC) and Gas Chromatography-Mass Spectrometry (GC-MS).** The methyl esters were extracted three times from the transesterification medium with hexane, concentrated under N<sub>2</sub>, and analyzed as described by Howard & Stanley-Samuelson (1990). Methyl esters were quantitatively analyzed by chromatography on a Hewlett-Packard 5730A gas chromatograph (Hewlett-Packard, Palo Alto, Calif.) equipped with a 30 m × 0.25 mm, 0.2-μm film thickness, Supelcowax 10 capillary column (Supelco, Bellefonte, Pa.), a flame ionization detector, and a Shimadzu CR-4A Chromatopak workstation (Shimadzu, Kyoto, Japan). A 45-s splitless injection was used and all runs utilized temperature programming from 187 to 230°C at 2°C/min, with a 2-min initial hold period. Ultrapure helium was the carrier gas at a flow rate of 1 ml/min.

Identification of methyl esters was confirmed by comparison to GC retention times of authentic standards and confirmed by GC-MS analysis. Analyses were conducted on a Hewlett-Packard 5790GC equipped with a 30 m × 0.25 mm, 0.2-μm film thickness Supelcowax 10 capillary column (Supelco). The GC was interfaced to a HP 5970 electron impact mass selective detector operated at 70 eV. Chromatographic conditions included a 45-s splitless injection, a 2-min hold period, and a 1°C/min temperature program from 170 to 200°C. Ultrapure helium was the carrier gas at 1 ml/min. Retention times and total ion

mass spectra with authentic Company or mass spectra (Hagen 1963).

**Double-Bonded Esters.** Monoe by argention sired fraction three times w chloroform sol stream of N<sub>2</sub>. μl of dimethy iodine was ac 60°C for 8 h. added. The m. rated sodium dried over and Veland 1981). rated with N<sub>2</sub>. and analyzed 0.2-μm film (J & W Scient ure programm a 2-min initial hold period. scribed by Du

**Incorporatio into Phospho acid** (<sup>3</sup>H-5,6,8 tivity 8.0 TE Cambridge, N to a specific aliquot of this ual adults by beveled) of a ton, Reno, N dorsal abdor pushed into plunger depr

Malpighiar injections, ar. individual pho Phospholipid TLC as desc phospholipid were identifi authentic sta liquid scintil sayed by li Pharmacia-L LKB, Gaitl chemiluminc counting effi

**Immunohi and PGF<sub>2α</sub> and Rinsing.** from *T. mo* fixed in 4% r temperature The tubules

concentrated un-  
s, redissolved in  
layer chromatog-  
silica gel plates;  
ates were devel-  
water (65:25:4, by  
sphatidylethanol-  
osphatidylserine-  
lipin correspond-  
standards (Sigma)  
tion tubes with  
pholipids do not  
one-dimensional  
possibly contain  
-ethanolamine or  
nolamine in the  
on, and the phos-  
contain traces of  
idylethanolamine  
well known as  
(Hawthorne &  
two fractions de-  
sphatidylethanol-  
ne-rich fractions.  
ormed by transes-  
holipids in reflux-  
90 min (Stanley-  
Analysis of the  
olved extracting  
n-methanol (2:1)  
bed above.

**Gas Chromatog-  
MS).** The methyl  
s from the trans-  
ine, concentrated  
ribed by Howard  
ethyl esters were  
matography on a  
chromatograph  
Calif.) equipped  
n film thickness,  
1 (Supelco, Belle-  
-tector, and a Shi-  
-orkstation (Shi-  
-plitless injection  
temperature pro-  
2°C/min, with a  
pure helium was  
ml/min.

s was confirme-  
times of auther  
GC-MS analysis.  
Hewlett-Packard  
m × 0.25 mm.  
wax 10 capillary  
s interfaced to a  
elective detector  
aphic conditions  
on, a 2-min hold  
ure program from  
1 was the carrier  
ies and total ion

mass spectra of methyl esters were compared with authentic standards from Sigma Chemical Company or by comparison to published EI-mass spectra (McCloskey 1970, Ryhage & Stenhagen 1963).

**Double-Bond Location in Monoenoic Methyl Esters.** Monoenoic methyl esters were isolated by argentation TLC (Hurst et al. 1987). The desired fraction was scraped into tubes, extracted three times with chloroform, and the combined chloroform solutions were dried under a gentle stream of N<sub>2</sub>. The residue was dissolved in 200 μl of dimethyl disulfide, one small crystal of iodine was added, the mixture was stirred at 60°C for 8 h, cooled, and 0.5 ml hexane was added. The mixture was washed once with saturated sodium thiosulfate, once with water, and dried over anhydrous sodium sulfate (Francis & Veland 1981). The hexane solution was evaporated with N<sub>2</sub>, re-dissolved with 20 μl of hexane, and analyzed by GC-MS on a 10 m × 0.19 mm, 0.2-μm film thickness DB-5 capillary column (J & W Scientific, Folsom, Calif.), with temperature programming from 150 to 320°C at 5°C/min, a 2-min initial hold period, and a 30-min final hold period. Spectra were interpreted as described by Dunkleblum et al. (1985).

**Incorporation of Radioactive Arachidonic Acid into Phospholipid Classes.** Tritiated arachidonic acid (<sup>3</sup>H-5,6,8,9,11,12,14,15-20:4n-6, specific activity 8.0 TBq/mmol; New England Nuclear, Cambridge, Mass.) was made up in 100% EtOH to a specific activity of 3.7 × 10<sup>4</sup> Bq/μl. A 2-μl aliquot of this solution was injected into individual adults by inserting the needle (27 gauge, beveled) of a 10-μl Hamilton 701 syringe (Hamilton, Reno, Nev.) between the second and third dorsal abdominal sclerites. The needle was pushed into the abdomen about 3 mm, and the plunger depressed.

Malpighian tubules were isolated 18 h after injections, and radiolabel incorporation into individual phospholipid classes was measured. Phospholipid classes were chromatographed by TLC as described above to isolate individual phospholipid fractions. Individual TLC fractions were identified by comparison with the R<sub>f</sub> of authentic standards (Sigma), and 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.

**Immunohistochemical Localization of PGE<sub>2</sub> and PGF<sub>2α</sub>: Whole-Mount Preparation. Fixation and Rinsing.** Malpighian tubules were dissected from *T. molitor* late instars and immediately fixed in 4% paraformaldehyde for 30 min at room temperature in porcelain or glass-welled plates. The tubules were rinsed three times for 5 min

each in 2 mM phosphate buffered saline (PBS) (Nardi & Miklasz 1989).

**Primary Antibody Step.** Tubules were incubated overnight at 4°C with rabbit prostaglandin E<sub>2</sub> antisera (anti-PGE<sub>2</sub>) or with rabbit prostaglandin F<sub>2α</sub> antisera (anti-PGF<sub>2α</sub>) (Advanced Magnetics, Cambridge, Mass.) that had been diluted 1:1,000 with amended PBS (amended by adding 1 ml normal goat serum and 0.4 ml Triton X-100 to 100 ml PBS (Nardi & Miklasz 1989). After incubation, the organs were rinsed three times for 40 min each in amended PBS.

**Secondary Antibody Step (Biotinylation).** Secondary antibody was prepared by adding 50 μl of biotinylated goat antirabbit antibody stock (Rabbit IgC Vectastain Kit, Vector Laboratories, Burlingame, Calif.) to 10 ml PBS. The Malpighian tubules from the primary antibody step were incubated overnight at 4°C with the biotinylated goat antirabbit antibody solution, then rinsed three times for 2 min each with PBS that contained 5% Tween 20 (by vol).

**Avidin-Biotin-Horseradish Peroxidase Step.** Avidin-biotin-horseradish peroxidase complex was prepared according to instructions in the Vectastain Kit, let stand for 30 min, and the biotinylated Malpighian tubules were added and incubated for 2 h. The tubules were rinsed three times for 5 min each in PBS.

**Visualization and Mounting.** Horseradish peroxidase activity was visualized by suspending the tubules in 0.02% hydrogen peroxide, PBS, and the chromogen 3,3'-diaminobenzidine (Sigma Chemical, St. Louis, Mo.) (1:1:1, vol/vol/wt). Upon turning brown (≈1 min) the reaction was stopped by rinsing the tubules three times for 2 min each with PBS. Tubules were dehydrated and cleared using a stepwise gradient of ethanol and xylene (50-100% ethanol in 10% increments at 2-min intervals, and three rinses of 100% xylene for 2 min each). The fixed and labeled Malpighian tubules were then mounted on slides in Kleermount (Carolina Biological Supply) and examined with a light microscope.

**Controls.** Three types of controls were used. Omission of primary antiserum and evaluation with a dilution series showed that staining was a result of the primary antiserum and not from some other tissue component (method specificity). Similarly, omission of the secondary antibody showed that staining was caused by the secondary antibodies to host serum and not by some other tissue component. Antibody specificity was demonstrated by preincubating the antibody with either its appropriate antigen or with a nonantigenic prostaglandin. Preincubation with the natural antibody reduced staining, and preincubation with another prostaglandin did not reduce staining. Controls for the visualization reaction involved leaving out each of the three primary reagents, one at a time.

**Statistical Methods and Voucher Specimens.** Summary statistics and one-way analyses of variance (ANOVA) were conducted using the PC version 5.0 of Statgraphics (STSC, Rockville, Md.). ANOVA was conducted on arcsine square-root proportion transforms, but means and standard deviations are reported on the untransformed data. Significance tests were conducted at the  $\alpha = 0.05$  level. Voucher specimens (Lot No. 30) were deposited in the Research Collection of the Department of Entomology, Kansas State University, Manhattan, Kans.

### Results

The major fatty acids associated with the phosphatidylcholine fraction of the Malpighian tubules were 16:0, 18:0, 18:1, and 18:2 (Table 1). The carbon number distribution of fatty acids ranged from 14 to 22, and all carbon numbers were represented. All monoenes except 19:1 were mixtures of positional isomers. Relative abundances of the positional isomers were about equal for 16:1 and 20:1, and the other components had one major isomer and one or more minor isomers. No branched-chain fatty acids were detected. Six components showed significant differences between laboratory and feral populations. The most notable difference was the greater proportion of arachidonic acid (C20:4n-6) in the feral population (Table 1). Both laboratory and feral populations had very low abundances of 20:3n-6 in the phosphatidylcholine fraction.

The major fatty acids in the phosphatidylethanolamine phospholipid fraction were the same as in the phosphatidylcholine fraction (Table 2), and the carbon number distribution and monoenoic fatty acid patterns were also similar to the phosphatidylcholine patterns. Again, six fatty acids occurred in significantly different proportions between the laboratory and feral populations: 14:0, 15:0, 17:0, 17:1, and 18:3n-3 were in higher proportions in the feral population, and 18:0 was in higher proportion in the laboratory population (Table 2). As with the phosphatidylcholine fraction, 20:3n-6 occurred in the phosphatidylethanolamine fraction in trace quantities in the laboratory and feral populations. Arachidonic acid occurred in greater proportion, with a slightly greater apparent proportion in the feral population than in the laboratory population. The fatty acid profile of the phosphatidylserine-phosphatidylinositol fraction was different from the distribution in the phosphatidylcholine and phosphatidylethanolamine fractions. The major components were 16:0, 18:0, 18:1, 18:2, and 20:0 (Table 3). The 18:0 and 18:1 fatty acids occurred at  $\approx 25\%$  each. No branched acids were detected and the isomeric distribution of monoenoic acids was similar to that in the phosphatidylcholine and phosphatidyletha-

**Table 1. Proportion of fatty acids, as percentage of total fatty acids, in phosphatidylcholine isolated from the Malpighian tubules of laboratory and feral populations of *T. molitor***

Fatty acid	Laboratory	Feral
14:0	0.46 (0.20)	0.31 (0.04)
15:0	0.05 (0.02)	0.10 (0.01)*
16:0	7.42 (0.21)	5.48 (0.39)*
Z-7-16:1	0.37 (0.04)	0.15 (0.05)
Z-9-16:1	0.24 (0.11)	0.15 (0.10)
16:2	0.02 (0.02)	Tr
17:0	0.27 (0.22)	0.10 (0.02)
Z-8/9 <sup>a</sup> /10-17:1	0.03 (0.02)	0.05 (0.02)
18:0	13.16 (0.04)	14.24 (0.38)
Z-9-18:1	21.88 (1.15)	20.40 (0.79)
Z-11-18:1	0.22 (0.01)	0.20 (0.01)
Z,Z-6,9-18:2	55.17 (1.21)	57.95 (0.32)*
18:3n-3	0.13 (0.03)	0.25 (0.08)
19:0	0.27 (0.08)	0.08 (0.04)*
Z-9-19:1	0.03 (0.01)	0.01 (0.01)*
20:0	0.23 (0.19)	0.23 (0.06)
Z-9-20:1	0.02 (0.01)	0.03 (0.02)
Z-11-20:1	0.02 (0.01)	0.03 (0.02)
20:3n-6	Tr	Tr
20:4n-6	Tr	0.24 (0.04)
21:0	Tr	Tr
22:0	0.12 (0.01)	Tr

Reported values are means (SD) and  $n = 3$  separate analyses for each population. \*, significantly different row means ( $P \leq 0.05$ ). Tr, trace ( $<0.01\%$ ).

<sup>a</sup> Major positional isomer.

nolamine fractions. Only two components differed significantly between the laboratory and feral populations: 14:0 and 20:0. The relative abundance of 20:3n-6 is considerably greater in phosphatidylserine-phosphatidylinositol than in either phosphatidylcholine or phosphatidylethanolamine. 20:4n-6 was present in moderate abundance. Neither of these polyunsaturated fatty acids differed significantly between the laboratory and feral populations.

The major fatty acids of cardiolipin were 16:0, 18:0, 18:1, and 18:2 (Table 4). No branched acids were detected and the isomeric distribution of the monoenoic acids were similar to all the other phospholipid classes. Three compounds differed significantly between the laboratory and feral populations. The most striking difference was the very low proportion of 18:1 in the laboratory population. The major C20 polyunsaturated fatty acids (20:3n-6 and 20:4n-6) were present in moderate abundance.

The fatty acid composition of diet used to rear the laboratory population is presented in Table 5. Major components were 16:0, 18:1, and 18:2, with a small proportion of 18:3n-3. The diet contained no branched fatty acids, and only one 16:1 isomer.

A summary of data by phospholipid class and beetle population is presented in Table 6. Distinct differences occurred across most categories, but the most noteworthy from the context of eicosanoid metabolism are the significantly greater proportion of C20 polyunsaturated fatty acids in

**Table 2. Proportion of total fatty acids, as percentage of total fatty acids, from the Malpighian tubules of laboratory and feral populations of *T. molitor***

Fatty acid
14:0
15:0
16:0
Z-7-16:1
Z-9-16:1
16:2
17:0
Z-8/9 <sup>a</sup> /10-17:1
18:0
Z-9-18:1
Z-11-18:1
Z,Z-6,9-18:2
18:3n-3
19:0
Z-9-19:1
20:0
Z-9-20:1
Z-11-20:1
20:3n-6
20:4n-6
21:0
22:0

Reported values are for each population. \*, significantly different row means ( $P \leq 0.05$ ). Tr, trace ( $<0.01\%$ ).

<sup>a</sup> Major positional isomer.

the feral insects for both phosphatidylethanolamine. In phosphatidylcholine, phos-

**Table 3. Proportion of total fatty acids, as percentage of total fatty acids, in phosphatidylserine-phosphatidylinositol isolated from the Malpighian tubules of laboratory and feral populations of *T. molitor***

Fatty acid
14:0
15:0
16:0
Z-7-16:1
Z-9-16:1
16:2
17:0
Z-8/9 <sup>a</sup> /10-17:1
18:0
Z-9-18:1
Z-11-18:1
Z,Z-6,9-18:2
18:3n-3
19:0
Z-9-19:1
20:0
Z-9-20:1
Z-11-20:1
20:3n-6
20:4n-6
21:0
22:0

Reported values are for each population. \*, significantly different row means ( $P \leq 0.05$ ).

<sup>a</sup> Major positional isomer.

**Table 2. Proportion of fatty acids, as percentage of total fatty acids, in phosphatidylethanolamine isolated from the Malpighian tubules of laboratory and feral populations of *T. molitor***

Fatty acid	Laboratory	Feral
14:0	0.23 (0.04)	0.63 (0.19)*
15:0	0.04 (0.01)	0.12 (0.01)*
16:0	5.54 (0.79)	4.31 (0.60)
Z-7-16:1	0.11 (0.02)	0.10 (0.06)
Z-9-16:1	0.13 (0.01)	0.12 (0.04)
16:2	0.01 (0.01)	0.01 (0.01)
17:0	0.10 (0.02)	0.61 (0.05)*
Z-8/9 <sup>a</sup> /10-17:1	0.02 (0.01)	0.15 (0.06)*
18:0	15.69 (0.13)	12.70 (0.57)*
Z-9-18:1	26.97 (0.01)	28.47 (1.01)
Z-11-18:1	0.27 (0.01)	0.26 (0.02)
Z,Z-6,9-18:2	50.41 (0.64)	51.54 (1.28)
18:3n-3	0.08 (0.01)	0.23 (0.02)*
19:0	0.15 (0.02)	0.18 (0.01)
Z-9-19:1	0.02 (0.01)	0.02 (0.01)
20:0	0.21 (0.01)	0.27 (0.04)
Z-9-20:1	0.01 (0.01)	0.08 (0.03)
Z-11-20:1	0.01 (0.01)	0.08 (0.03)
20:3n-6	Tr	Tr
20:4n-6	0.01 (0.01)	0.13 (0.07)*
21:0	Tr	Tr
22:0	Tr	Tr

Reported values are means (SD) and *n* = 3 separate analyses for each population. \*, significantly different row means (*P* ≤ 0.05). Tr, trace (<0.01%).

<sup>a</sup> Major positional isomer.

the feral insects than in the laboratory population for both phosphatidylcholine and phosphatidylethanolamine. In both populations, phosphatidylcholine, phosphatidylserine-phosphatidylinos-

**Table 3. Proportion of fatty acids, as percentage of total fatty acids, in phosphatidylserine/phosphatidylinositol isolated from the Malpighian tubules of laboratory and feral populations of *T. molitor***

Fatty acid	Laboratory	Feral
14:0	0.01 (0.05)	0.43 (0.07)*
15:0	0.05 (0.02)	0.05 (0.01)
16:0	4.20 (0.69)	3.18 (1.82)
Z-7-16:1	0.18 (0.03)	0.22 (0.11)
Z-9-16:1	0.18 (0.04)	0.23 (0.12)
16:2	0.01 (0.01)	0.02 (0.01)
17:0	0.34 (0.19)	0.16 (0.10)
Z-8/9 <sup>a</sup> /10-17:1	0.03 (0.02)	0.05 (0.02)
18:0	23.61 (0.44)	26.39 (1.39)
Z-9-18:1	26.02 (1.24)	27.56 (1.31)
Z-11-18:1	0.26 (0.01)	0.27 (0.02)
Z,Z-6,9-18:2	37.07 (0.55)	38.44 (2.58)
18:3n-3	0.10 (0.05)	0.18 (0.12)
19:0	0.21 (0.08)	0.08 (0.05)
Z-9-19:1	0.02 (0.01)	0.02 (0.01)
20:0	6.14 (0.57)	2.10 (0.86)*
Z-9-20:1	0.22 (0.16)	0.23 (0.12)
Z-11-20:1	0.21 (0.16)	0.23 (0.12)
20:3n-6	0.03 (0.02)	0.02 (0.01)
20:4n-6	0.10 (0.05)	0.34 (0.33)
21:0	0.03 (0.02)	0.02 (0.01)
22:0	1.03 (0.36)	0.33 (0.28)

Reported values are means (SD) and *n* indicates the number of separate analyses. \*, significantly different row means (*P* ≤ 0.05).

<sup>a</sup> Major positional isomer.

**Table 4. Proportion of fatty acids, as percentage of total fatty acids, in cardiolipin isolated from the Malpighian tubules of laboratory and feral populations of *T. molitor***

Fatty acid	Laboratory	Feral
14:0	1.46 (0.10)	1.75 (0.92)
15:0	0.42 (0.03)	0.39 (0.20)
16:0	13.79 (3.17)	16.49 (6.49)
Z-7-16:1	2.75 (2.16)	1.66 (1.63)
Z-9-16:1	0.80 (0.21)	1.52 (0.44)
16:2	0.12 (0.03)	0.05 (0.02)
17:0	1.23 (0.32)	0.21 (0.11)
Z-8/9 <sup>a</sup> /10-17:1	0.06 (0.02)	0.02 (0.01)
18:0	7.68 (1.30)	8.68 (2.39)
Z-9-18:1	3.39 (0.44)	12.85 (1.75)*
Z-11-18:1	0.06 (0.02)	0.43 (0.42)
Z,Z-6,9-18:2	66.11 (4.32)	54.09 (5.65)
18:3n-3	0.11 (0.03)	0.03 (0.01)*
19:0	0.31 (0.08)	0.11 (0.05)*
Z-9-19:1	0.06 (0.02)	0.02 (0.01)
20:0	1.04 (0.13)	1.45 (1.57)
Z-9-20:1	0.06 (0.02)	0.06 (0.01)
Z-11-20:1	0.06 (0.02)	0.06 (0.01)
20:3n-6	0.06 (0.02)	0.02 (0.01)
20:4n-6	0.13 (0.05)	0.06 (0.01)
21:0	0.06 (0.02)	0.02 (0.01)
22:0	0.22 (0.14)	0.02 (0.01)

Reported values are means (SD) and *n* = 3 separate analyses for each population. \*, significantly different row means (*P* ≤ 0.05).

<sup>a</sup> Major positional isomer.

itol, and phosphatidylethanolamine tended to have a greater proportion of C20 polyunsaturated fatty acids than the cardiolipin.

In vivo incorporation of <sup>3</sup>H-arachidonic acid into the Malpighian tubule phospholipids over

**Table 5. Proportion of fatty acids, as percentage of total fatty acids, in total lipids extracted from the standard diet used to raise the laboratory population of *T. molitor* used in this study**

Fatty acid	% Composition
14:0	0.41 (0.07)
15:0	0.12 (0.01)
16:0	19.81 (0.91)
Z-7-16:1	None detected
Z-9-16:1	0.76 (0.06)
16:2	None detected
17:0	0.15 (0.02)
Z-8/9 <sup>a</sup> /10-17:1	0.14 (0.04)
18:0	3.08 (0.02)
Z-9-18:1	19.75 (0.35)
Z-11-18:1	Tr
Z,Z-6,9-18:2	53.35 (0.98)
18:3n-3	2.39 (0.25)
19:0	Tr
Z-9-19:1	None detected
20:0	0.01 (0.01)
Z-9-20:1	Tr
Z-11-20:1	0.01 (0.01)
20:3n-6	None detected
20:4n-6	None detected
21:0	None detected
22:0	None detected

Reported values are means (SD) and *n* = 3 separate analyses. Tr, Trace (< 0.01%).

<sup>a</sup> Major positional isomer.

**Table 6. Mean (SD) percent distribution by degree of unsaturation of fatty acids in four phospholipid classes from Malpighian tubules of laboratory and feral populations of *T. molitor***

Fatty acid class <sup>a</sup>	Laboratory	Feral
Phosphatidylcholine		
Saturated	21.98 (0.97)	20.36 (0.94)
Monounsaturated	22.81 (1.36)	21.02 (1.02)
Diunsaturated	55.19 (1.23)	57.75 (0.32)*
C20 PUFA	Trace	0.24 (0.04)*
Phosphatidylethanolamine		
Saturated	21.96 (1.02)	18.82 (1.47)*
Monounsaturated	27.54 (0.10)	29.28 (1.26)*
Diunsaturated	50.42 (0.65)	51.55 (1.29)
C20 PUFA	0.01 (0.01)	0.13 (0.07)*
Phosphatidylserine- Phosphatidylinositol		
Saturated	35.62 (2.42)	32.74 (4.59)
Monounsaturated	27.12 (1.67)	28.81 (1.83)
Diunsaturated	37.08 (0.56)	38.46 (2.59)
C20 PUFA	0.13 (0.07)	0.36 (0.34)
Cardiolipin		
Saturated	26.21 (5.29)	29.12 (11.75)
Monounsaturated	7.24 (2.91)	16.62 (4.28)*
Diunsaturated	66.23 (4.35)	54.14 (5.67)*
C20 PUFA	0.19 (0.07)	0.08 (0.02)

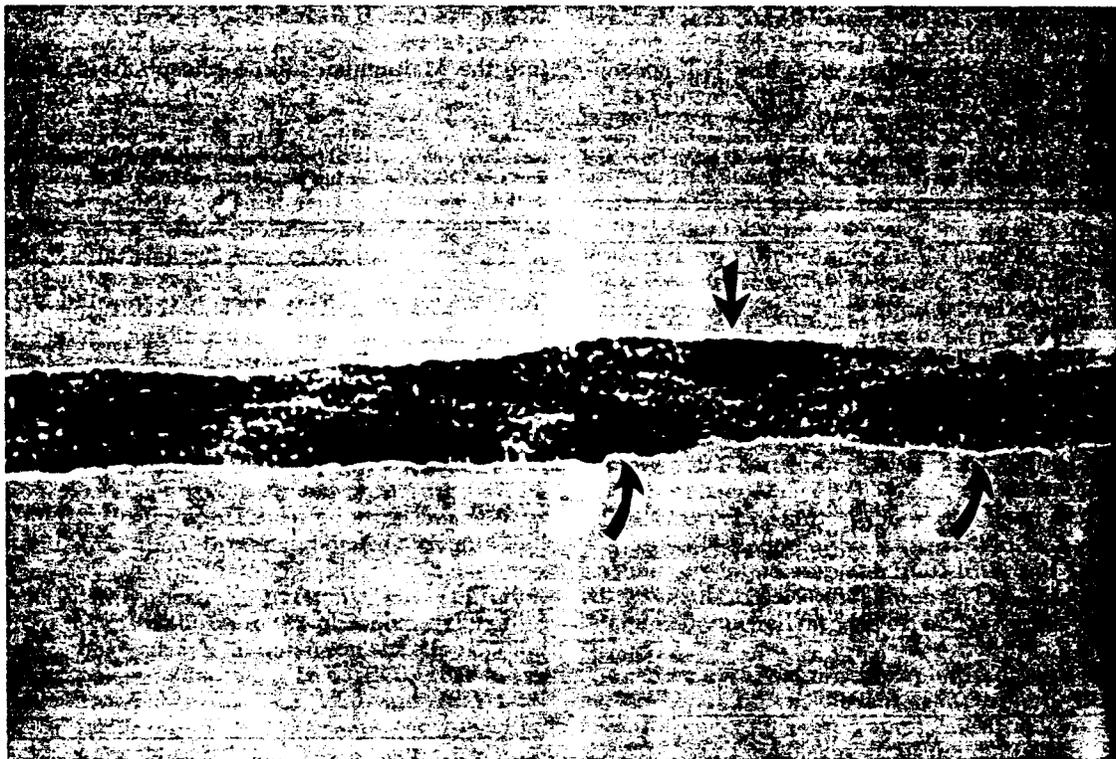
\*, significantly different row means ( $P \leq 0.05$ ).  
<sup>a</sup> PUFA, polyunsaturated fatty acids.

an 18-h period occurred to the extent of  $1.20 \pm 0.57\%$  (mean  $\pm$  SD) of total injected radioactivity. The mean distribution of this incorporation

into the four phospholipid classes was  $76.87 \pm 4.72\%$  into phosphatidylcholine,  $11.87 \pm 3.29\%$  into phosphatidylserine-phosphatidylinositol,  $9.47 \pm 2.15\%$  into phosphatidylethanolamine, and  $1.80 \pm 0.65\%$  into cardiolipin.

Immunohistochemical examination of whole-mount preparations of Malpighian tubules from larval *T. molitor* showed that PGE<sub>2</sub> and PGF<sub>2 $\alpha$</sub>  staining occurred only in the free segments, which are deeply pigmented by brown granules. All other regions of the tubules and the nephric complex showed no evidence of prostaglandin staining. Virtually all (26/27) Malpighian tubules that were reacted with anti-PGE<sub>2</sub> at a dilution of 1:100 produced distinctive diamond-shaped reddish brown immunopositive patches in a repeating pattern (Fig. 1) along the entire length of the pigmented proximal segments. The proportion of such tubules displaying immunopositive results decreased as tubules were reacted with solutions that contained antibody at lower and lower concentrations (Table 7). Staining for PGE<sub>2</sub> was not detected when the antibody was diluted to 1:10<sup>8</sup>.

Malpighian tubules reacted with anti-PGF<sub>2 $\alpha$</sub>  produced immunopositive results in the same anatomical portions of the tubules as was found for the anti-PGE<sub>2</sub> treatment. Examination of 81 such tubule preparations stained with an anti-



**Fig. 1.** Photograph of the diamond staining pattern observed when the Malpighian tubules of *T. molitor* were reacted with anti-PGE<sub>2</sub>.

**Table**  
**on horse**  
**three sep**  
**molitor**

Dilution:

Anti-PGE <sub>2</sub>	1:10 <sup>2</sup>
	1:10 <sup>3</sup>
	1:10 <sup>4</sup>
	1:10 <sup>6</sup>
	1:10 <sup>8</sup>
Anti-PGF <sub>2<math>\alpha</math></sub>	1:10 <sup>2</sup>
	1:10 <sup>3</sup>
	1:10 <sup>4</sup>
	1:10 <sup>6</sup>
	1:10 <sup>8</sup>

Values of  
of tubules  
<sup>a</sup> Not te

PGF<sub>2 $\alpha$</sub>  c  
tions wi  
(Fig. 2).  
fewer ar  
staining  
1:10<sup>8</sup> (T  
and anti  
duced 1



**Fig. 2.**  
reacted w

**Table 7. Effect of diluting anti-PGE<sub>2</sub> and anti-PGF<sub>2α</sub> on horseradish peroxidase staining for PGE<sub>2</sub> and PGF<sub>2α</sub> in three segments of Malpighian tubules from larval *T. molitor***

Dilutions	Proximal segment	Mid-segment	Distal segment
Anti-PGE <sub>2</sub>			
1:10 <sup>2</sup>	26/27	0/3	0/6
1:10 <sup>3</sup>	87/99	0/17	0/22
1:10 <sup>4</sup>	3/6	0/1	0/1
1:10 <sup>6</sup>	2/3	— <sup>a</sup>	—
1:10 <sup>8</sup>	0/4	—	—
Anti-PGF <sub>2α</sub>			
1:10 <sup>2</sup>	20/21	0/2	0/3
1:10 <sup>3</sup>	78/81	0/7	0/12
1:10 <sup>4</sup>	0/2	0/1	0/1
1:10 <sup>6</sup>	1/4	0/1	0/1
1:10 <sup>8</sup>	0/3	0/1	0/1

Values are number of positive staining tubules/total number of tubules.

<sup>a</sup> Not tested.

PGF<sub>2α</sub> dilution of 1:1000 resulted in 78 preparations with staining in a distinctive mosaic pattern (Fig. 2). Dilution series assays again resulted in fewer and fewer immunopositive results, and no staining occurred when antibody was diluted 1:10<sup>8</sup> (Table 7). Control assays with anti-PGE<sub>2</sub> and anti-PGF<sub>2α</sub> indicated that the methods produced 17% false positives, a value typical for this

type of assay (Hsu et al. 1981, Bensen & Cohen 1970).

### Discussion

Although a vast number of fatty acid analyses have been conducted on insect tissues (Fast 1970, Stanley-Samuelson et al. 1988), relatively little of this effort was aimed at understanding physiological roles of fatty acids in specific tissues. However, it has become increasingly clear from the mammalian literature that phospholipids are intimately involved in signal transduction processes that regulate a multitude of homeostatic processes (Ansell & Spanner 1982, Braquet et al. 1985), in addition to their well-known structural and energy storage roles. A similar situation undoubtedly exists in insects. Advances in understanding how particular phospholipids and eicosanoids regulate the metabolic functions of any given cell system will require a knowledge of which phospholipids (and their fatty acid composition) and which eicosanoids are present. Such information is only beginning to emerge in the insect literature (Bridges 1983, Stanley-Samuelson et al. 1988).

There are no reports of fatty acid compositions of individual phospholipids in the Malpighian tubules of any insect, although such work is



**Fig. 2.** Photograph of the mosaic staining pattern observed when the Malpighian tubules of *T. molitor* were reacted with anti-PGF<sub>2α</sub>.

forthcoming for mosquitos (D. H. Petzel & D.W.S., unpublished). Fatty acid compositions of phospholipid fractions have been reported for other insect tissues. Zinkler (1975) reported on the fatty acid composition of phosphatidylcholine and phosphatidylethanolamine fractions derived from the retinal membranes of three insect species. Parnova (1982) reported on distribution of polyunsaturated fatty acids in three phospholipids from the nervous system of four species of cockroaches; Stanley-Samuelson & Loher (1983) described the distribution of phospholipid fatty acids in the spermatophores of the cricket *Teleogryllus commodus* Walker; and Ogg et al. (1991) described the distribution of fatty acids in phospholipids of hemocytes of *Manduca sexta* (L). In all these cases, the major observation was that individual phospholipid fractions have their own fatty acid profiles, which suggests that phospholipid fatty acid incorporation and turnover are regulated at the level of phospholipid fractions. The results are consistent with this hypothesis.

In this paper, we have addressed four basic hypotheses regarding the biochemistry of the Malpighian tubules of *T. molitor*: (1) specific phospholipid classes will have a greater relative proportion of polyunsaturated fatty acid than are found in total phospholipids; i.e., C20 polyunsaturated fatty acids are sequestered into phospholipid fractions for special purposes as seen in other tissues; (2) feral, free-roaming *T. molitor* have different Malpighian tubule phospholipid fatty acid compositions than highly inbred laboratory populations constrained to a single diet; (3) <sup>3</sup>H-Arachidonic acid is incorporated into the major Malpighian tubule phospholipids; and (4) the presence of PGE<sub>2</sub> and PGF<sub>2α</sub> can be demonstrated in intact Malpighian tubule preparations by immunohistochemical techniques. Our data support these ideas.

In an earlier examination of total phospholipids from Malpighian tubules of laboratory cultures of *T. molitor*, we could not find more than trace quantities of C20 polyunsaturated fatty acids (Howard & Stanley-Samuelson 1990). By comparison, each of the individual phospholipid fractions in laboratory and feral populations examined in this study contained readily measurable levels of C20 polyunsaturated fatty acids (Table 6). Although the differences obtained are perhaps a result of differing dietary regimes for the two studies, they probably also represent an example of specific cellular fractions with greater abundance of a given fatty acid than that present in the tissue as a whole. The relative abundance of these components varied among the phospholipids, with phosphatidylcholine, phosphatidylethanolamine and phosphatidylserine-phosphatidylinositol having somewhat greater proportions than cardiolipin. With access to a variety of food resources, the feral population tended to have

greater proportions of C20 polyunsaturated fatty acids than did the laboratory population. A comparison of the other fatty acid classes listed in Table 6 indicates that each phospholipid fraction has different relative proportions of saturated, mono-, and diunsaturated fatty acids. Although we are not aware of any insect literature relating to the localization of particular phospholipid classes with regard to membrane surfaces, such a stratification is known from the mammalian literature. For example, erythrocyte phosphatidylethanolamine and phosphatidylserine are predominantly located on the inside of the cell membrane, whereas phosphatidylcholine and sphingomyelin are primarily located on the outer surface of the cell membrane (Zwaal et al. 1973). Similar asymmetries may occur in insect membranes, and differences observed in fatty acid composition among the phospholipid subclasses may be a reflection of these asymmetries.

As observed by Howard & Stanley-Samuelson (1990), in vivo incorporation of <sup>3</sup>H-arachidonic acid into the Malpighian tubules of *T. molitor* readily occurred. In that study, 75% of the incorporated radioactivity occurred in the phospholipids, but the distribution of the label among phospholipid fractions was not determined. We have now done so, and as expected from similar studies with other insects (Stanley-Samuelson et al. 1988) most radioactive arachidonic acid was incorporated into phosphatidylcholine (≈77%), with phosphatidylethanolamine and phosphatidylserine-phosphatidylinositol each incorporating ≈10% and cardiolipin incorporating only ≈2%. These values do not match the relative abundances of phosphatidylcholine and the other phospholipid fractions in cells (Bridges 1983). These data provide evidence that arachidonic acid is preferentially incorporated into phosphatidylcholine. Incorporation of radioactive arachidonic acid also differs from the proportions of endogenous C20 polyunsaturated fatty acids. These differences are probably related to differences in phospholipid turnover within Malpighian tubules, because exogenous arachidonic acid does not occur in the diets of these beetles (Table 5).

Finally, although we demonstrated by using chromatographic techniques (Howard & Stanley-Samuelson 1990) that injected <sup>3</sup>H-arachidonic acid was converted into PGE<sub>2</sub>, PGF<sub>2α</sub>, and PGD<sub>2</sub>, and other unidentified presumed lipoxigenase products, these eicosanoids were obtained from whole body extracts and not from specific tissues. Chromatographic identifications are not without uncertainties and additional verification of the identity of these eicosanoids in *T. molitor* would be highly desirable. We used an immunohistochemical technique widely reported in the mammalian literature (Beltz & Burd 1989), which apparently was not reported before in the insect literature to detect ei-

cosanoids. technique antibodies antibody, signal and The avid here is cu able. As s appear to s free portio the Malpig different p taglandins ferent cell bule. No s clear comm result is c tions of th with ion c branes. In: ducted or preparation were intact: tibodies. T of the pres the distal would be v also contain

We have that prosta: renal func *T. molitor*. suggest th may also c many othe Samuelson cosanoid b Malpighiar stantially 1 The phosph tetraynoic tor, indome basal fluid inhibitor, S tor, esculer dose deper reversed w clooxygena moieties in though the *molitor* is n it is likely pathways w preparation

We thank E. Toolson (USDA-ARS-University of this manus

cosanoids. This three-step immunoenzymatic technique results in several labeled secondary antibodies binding to each molecule of primary antibody, resulting in a marked amplification of signal and minimization of background noise. The avidin-biotin-peroxidase complex used here is currently the most sensitive one available. As shown in Fig. 1 and 2, these antibodies appear to stain PGE<sub>2</sub> and PGF<sub>2α</sub> contained in the free portions (the deeply pigmented sections) of the Malpighian tubule of larval *T. molitor*. The different patterns observed for these two prostaglandins suggest that they are localized in different cellular sites within the Malpighian tubule. No staining was observed in either of the clear common trunk portions of the tubules. This result is consistent with the idea that these portions of the tubules are not actively involved with ion or water movement across cell membranes. Inasmuch as the experiments were conducted on intact Malpighian tubule-hindgut preparations, the cryptonephric membranes were intact and possibly impermeable to the antibodies. The data do not permit determination of the presence or absence of prostaglandins in the distal portions of the tubules. However, we would be very surprised if these regions did not also contain prostaglandins.

We have not conducted experiments to show that prostaglandins or other eicosanoids regulate renal function in the Malpighian tubules of *T. molitor*. However, our studies with *A. aegypti* suggest that such physiological regulation may also occur in *T. molitor*, and probably in many other insect species. Petzel & Stanley-Samuels (1992) showed that inhibition of eicosanoid biosynthesis in *in vitro* preparations of Malpighian tubules from adult *A. aegypti* substantially reduced basal fluid secretion rates. The phospholipase A<sub>2</sub> inhibitor, 5,8,11,14-eicosatetraenoic acid, and the cyclooxygenase inhibitor, indomethacin, far more efficiently reduced basal fluid secretion than did the epoxygenase inhibitor, SKF-525A, or the lipoxigenase inhibitor, esculetin. The effect of indomethacin was dose dependent, and the inhibitor effects were reversed with cAMP. These data argue that cyclooxygenase products are important signaling moieties in renal function in this insect. Although the renal biology of an insect such as *T. molitor* is much different from that of *A. aegypti*, it is likely that similar eicosanoid regulatory pathways will be involved in Malpighian tubule preparations from both insects.

#### Acknowledgment

We thank J. Dillwith (Oklahoma State University), E. Toolson (University of New Mexico), K. Kramer (USDA-ARS-USGMRL), and C. Ogg and B. Siegfried (University of Nebraska) for reviewing an earlier draft of this manuscript. We also thank C. Ogg for technical

assistance with the radioisotopic experiments. This article is paper no. 9777, Journal Series, Nebraska Agricultural Research Division, and Contribution no. 784 of the Department of Entomology, University of Nebraska, Lincoln. Investigations were supported by USDA-ARS, Department of Entomology, and Institute Agriculture and Natural Resources, University of Nebraska-Lincoln.

#### References Cited

- Ansell, G. B. & S. Spanner. 1982. Phosphatidylserine, phosphatidylethanolamine and phosphatidylcholine, pp. 1-49. In J. N. Hawthorne & G. B. Ansell [eds.], Phospholipids, vol. 4. New comprehensive biochemistry, A. Neuberger & L. L. M. van Deenen [gen. eds.], Elsevier Biomedical, Amsterdam.
- Beltz, B. S. & G. D. Burd. 1989. Immunocytochemical techniques. Blackwell Scientific, Cambridge, Mass.
- Bensen, M. D. & A. S. Cohen. 1970. Antinuclear antibodies in systemic lupus erythematosus (detection with horseradish-peroxidase-conjugated antibody). *Ann. Intern. Med.* 73: 943-949.
- Bligh, E. G. & W. J. Dyer. 1959. A rapid method of total lipid extraction and purification. *Can. J. Biochem.* 37: 911-917.
- Braquet, P., R. P. Garay, J. C. Frölich & S. Nicosia [eds.]. 1985. Prostaglandins and membrane ion transport: advances in ion transport regulation. Raven, New York.
- Bridges, R. G. 1983. Insect phospholipids, pp. 159-181. In T. E. Mittler & R. H. Dadd [eds.], *Metabolic aspects of lipid nutrition in insects*, Westview, Boulder, Colo.
- DeStephano, D. B., U. E. Brady, & R. E. Lovins. 1974. Synthesis of prostaglandin by reproductive tissue of the male house cricket, *Acheta domesticus*. *Prostaglandins* 6: 71-79.
- Dunkelblum, E., S. H. Tam & P. J. Silk. 1985. Double-bond location in monounsaturated fatty acids by dimethyl disulfide derivatization and mass spectrometry: application to analysis of fatty acids in pheromone glands of four Lepidoptera. *J. Chem. Ecol.* 11: 265-277.
- Fast, P. G. 1970. Insect lipids. *Prog. Chem. Fats Other Lipids* 11: 181-214.
- Francis, G. W. & K. Veland. 1981. Alkylthiolation for the determination of double bond positions in linear alkenes. *J. Chromatogr.* 219: 379-384.
- Hawthorne, J. N. & G. B. Ansell [eds.]. 1982. Phospholipids, vol. 4. New Comprehensive biochemistry, A. Neuberger & L.L.M. van Deenen, [gen. eds.], Elsevier Biomedical, Amsterdam.
- Howard, Ralph W. & David W. Stanley-Samuels. 1990. Phospholipid fatty acid composition and arachidonic acid metabolism in selected tissues of adult *Tenebrio molitor* (Coleoptera: Tenebrionidae). *Ann. Entomol. Soc. Am.* 83: 975-981.
- Hsu, S.-Ming, L. Raine & H. Fanger. 1981. Use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase techniques: a comparison between ABC and unlabelled antibody (PAP) procedures. *J. Histochem. Cytochem.* 29: 577-580.
- Hurst, J. S., S. Flatman & R. G. McDonald-Gibson. 1987. Thin-layer chromatography (including radio thin-layer chromatography and autoradiography) of

- prostaglandins and related compounds, pp. 53-73. In C. Benedetto [ed.], Prostaglandins and related substances, a practical approach. IRL, Oxford, England.
- Loher, W., I. Ganjian, I. Kubo, D. Stanley-Samuelson & S. S. Tobe. 1981. Prostaglandins: their role in egg-laying of the cricket *Teleogryllus commodus*. Proc. Natl. Acad. Sci. U.S.A. 78: 7835-7838.
- McCloskey, J. M. 1970. Mass spectrometry of fatty acid derivatives, pp. 369-440. In F. E. Gunstone [ed.], Topics in lipid chemistry, vol. 1. Wiley, New York.
- Moore, P. K. 1985. Prostanoids: pharmacological, physiological and clinical relevance, Cambridge University Press, Cambridge, England.
- Nardi, J. B. & S. D. Miklasz. 1989. Hemocytes contribute to both the formation and breakdown of the basal lamina in developing wings of *Manduca sexta*. Tissue Cell 21: 559-567.
- Ogg, C. L., R. W. Howard & D. W. Stanley-Samuelson. 1991. Fatty acid composition and incorporation of arachidonic acid into phospholipids of hemocytes from the tobacco hornworm, *Manduca sexta*. Insect Biochem. 21: 809-814.
- Parnova, R. G. 1982. Polyunsaturated fatty acids in phospholipids of the nervous system of cockroaches. Evol. Biochem. Physiol. 6: 611-614 (in Russian).
- Petzel, David H. & David W. Stanley-Samuelson. 1992. Inhibition of eicosanoid biosynthesis modulates basal fluid secretion in the Malpighian tubules of the yellow fever mosquito (*Aedes aegypti*). J. Insect Physiol. 38: 1-8.
- Ragab, A., C. Bitsch, J.M.F. Thomas, J. Bitsch & H. Chap. 1987. Lipoygenase conversion of arachidonic acid in males and inseminated females of the firebrat, *Thermobia domestica* (Thysanura). Insect Biochem. 17: 863-870.
- Ragab, A., J. Duran, C. Bitsch, H. Chap & M. Rigaud. 1991. The lipoygenase pathway of arachidonic acid metabolism in reproductive tissues of the firebrat, *Thermobia domestica* (Thysanura). Insect Biochem. 21: 321-326.
- Ryhage, R. & E. Stenhagen. 1963. Mass spectrometry of long-chain esters, pp. 399-452. In F. W. McLafferty [ed.], Mass spectrometry of organic ions. Academic, New York.
- Stanley-Samuelson, D. W. 1987. Physiological roles of prostaglandins and other eicosanoids in invertebrates. Biol. Bull. 172: 92-109.
1991. Comparative eicosanoid physiology in invertebrate animals. Am. J. Physiol. 260 (Regulatory Integrative Comp. Physiol.) 29: R849-R853.
- Stanley-Samuelson, D. W. & R. H. Dadd. 1983. Long-chain polyunsaturated fatty acids: patterns of occurrence in insects. Insect Biochem. 13: 549-558.
- Stanley-Samuelson, D. W. & W. Loher. 1983. Arachidonic and other long-chain polyunsaturated fatty acids in spermatophores and spermathecae of *Teleogryllus commodus*: significance in prostaglandin-mediated reproductive behavior. J. Insect Physiol. 29: 41-45.
- Stanley-Samuelson, D. W., R. A. Jurenka, C. Cripps, G. J. Blomquist & M. deRenobales. 1988. Fatty acids in insects: composition, metabolism and biological significance. Arch. Insect Biochem. Physiol. 9: 1-33.
- Stanley-Samuelson, D. W., R. W. Howard & E. C. Toolson. 1990. Phospholipid fatty acid composition and arachidonic acid uptake and metabolism by the cicada *Tibicen dealbatus* (Homoptera: Cicadidae). Comp. Biochem. Physiol. 97B: 285-289.
- Stanley-Samuelson, D. W., E. Jensen, K. W. Nickerson, K. Tiebel, C. L. Ogg & R. W. Howard. 1991. Insect immune response to bacterial infection is mediated by eicosanoids. Proc. Natl. Acad. Sci. U.S.A. 88: 1064-1068.
- Yamaja Setty, B. N. & T. R. Ramaiah. 1980. Effect of prostaglandins and inhibitors of prostaglandin biosynthesis on oviposition in the silkworm *Bombyx mori* L. Indian J. Exp. Biol. 13: 539-541.
- Zinkler, D. 1975. Zum Lipidmuster der Photorezeptoren von Insekten. Verh. Dtsch. Zool. Ges. 28: 28-32.
- Zwaal, R.F.A., B. Roelofsen & C. M. Colley. 1973. Localization of red cell membrane constituents. Biochem. Biophys. Acta 300: 159-182.

Received for publication 9 December 1991; accepted 24 February 1992.

M

WE HA  
and po  
terase  
cessory  
rootwo:  
Conte.  
locus (  
charact  
and a  
≈75,00  
of Est-1  
accesso  
vation t  
tion (R  
function  
reprodu  
tems su  
product  
is to de  
pects of  
ical lev  
recently  
clonal a  
probes f  
enzyme

This art  
proprietar  
recommen  
<sup>1</sup> Depart  
search Ce  
<sup>2</sup> Depart  
North Dak