Future studies could assess whether females aggregate around male residues in *D. melanogaster* and *Z. tuberculatus* and avoid male and female residues in *D. funebris* in various laboratory paradigms. It would also be valuable to study these responses in the field where a natural range of behaviors could be expressed. There is a need to test more lines from each species and perhaps test additional species, as well as evaluate the possibility of cross-species responses to residual odors. Finally, observations at the whole organism level will lead to study of the chemical basis of these behaviors.

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*Present address: Department of Genetics and Human Variation, La Trobe University, Bundoora, Victoria 3083, Australia.

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Eicosapentaenoic acid in tissue lipids of *Pieris brassicae*

S. Pärnänen and S. Turunen*

Department of Zoology, Division of Physiology, University of Helsinki, SF-00100 Helsinki (Finland), 7 April 1986

Summary. Larvae of the cabbage white butterfly, *Pieris brassicae,* have a dietary requirement for linolenic acid (C18:3n3) and were found to accumulate two other members of the n-3 family, C20:3n3 and C20:5n3 (eicosapentaenoic acid) especially in testicular phospholipids. Arachidonic acid was observed in trace amounts only. During diapause the relative titer of eicosapentaenoic acid increased in testicular phospholipids to about 4.2% of the fatty acids. Eicosapentaenoic acid is a possible precursor of prostaglandins, suggesting that prostaglandins of the 3-series predominate in this insect.
Key words. Pieris brassicae; eicosapentaenoic acid; testis; diapause; prostaglandins.

The requirement for dietary C18 polyunsaturated fatty acids (PUFA), linoleic (C18:2n6) or linolenic (C18:3n3) or both, in insects suggests two different pathways of subsequent fatty acid elongation and desaturation to C20 PUFA for the possible synthesis of prostaglandins. In common with some vertebrate animals, e.g. several fish, many Lepidopteran species have been shown to require C18:3n3 as a major essential fatty acid. *Pieris brassicae* is one such species[1,2]. Dadd[3] discusses the PUFA requirement of insects and suggests that, with the exception of several species of mosquitoes which need dietary arachidonic acid (C20:4n6) or related PUFA, insects in general elongate and desaturate C18 PUFA to C20 and C22 acids, which are of common occurrence in insects just as they are in vertebrate animals.

If the C20 PUFA are converted to prostaglandins, they can be expected to be preferentially incorporated into membrane phospholipids. Data from some insect species support this[4]. Specifically, arachidonic acid is present in the reproductive tissues of the Australian field cricket *Teleogryllus commodus* and the American cockroach *Periplaneta americana*[5,6], and testicular phospholipids of *T. commodus* were recently shown to accumulate labeled arachidonic acid injected into the hemolymph[7]. Among the retinal phospholipids of the butterfly *Deilephila elpenor,* an unusually high concentration of eicosapentaenoic acid (C20:5n3) has been found[8].

The present study examines the possibility that *P. brassicae,* specifically requiring C18:3n3 in its diet for normal adult emergence, contains long-chain members of the n-3 family of fatty acids. If the essential C18:3n3 is used in part as a precursor of prostaglandins, C20 precursor fatty acids of the prostaglandin 3 series should be present in tissue lipids.

Material and methods. Our experimental stock of *P. brassicae* originated from insects obtained in 1984 from the Glasshouse Crops Research Institute, Littlehampton, England. Larvae were reared for these experiments at 23 °C, 16L:8D and about 65% relative humidity. Insects destined for diapause were transferred to 12–13 °C and 12L:12D at the beginning of the first larval instar and, upon pupation, to 2 °C in constant darkness. Larval food was *Brassica oleracea.*

Fat bodies, testes, heads and adult flight muscles were extirpated under a saline solution to rinse off any remaining hemolymph, and lipids were extracted with a modification of the procedure of Folch et al.[9] and separated into fractions in a column (1.5 × 8.0 cm) of activated silicic acid (Unisil)[10]. Lipids were transesterified for gas-liquid chromatography (GLC) using a methanolic-base (0.5 N) reagent (Supelco).

GLC of methyl esters was performed with a Hewlett-Packard 5890A instrument in a 50-m capillary column of 0.2 mm i.d. using CP SIL 88 and OV 275 as liquid phase and hydrogen or helium as carrier. Standard methyl esters used were C18:3n3, C18:3n6, C20:3n3, C20:4n6, C20:5n3 and C22:6n3 (Sigma) which were run individually between samples and subsequently each co-chromatographed with sample for peak identification. Peak areas were quantified with an electronic integrator (Hewlett-Packard 3392A). Mass spectra of testicular fatty acid methyl esters and of eicosapentaenoic acid methyl ester standard were determined with a Nermag R-10-10 C spectrometer (EI+ detection, 70 eV) and Varian 3400 gas chromatograph (30 m column, DB-1701, He as carrier).

Results. Only male insects were examined in this study. Rearing larvae at a low, diapause-inducing temperature and a short day-length results in an increased accumulation of the dietary

Table 1. Percentage fatty acid composition of phospholipids in tissues of *Pieris brassicae* reared under non-diapause (23 °C, 16L:8D) and diapause-inducing (13 °C, 12L:12D) conditions. Each sample consisted of the pooled tissues from ten insects (n = 3). A representative distribution is given

	16:0	16:1	18:0	18:1	18:2 n6	18:3 n3	18:3 n6	20:3 n3	20:5 n3	Other
Fat body										
Non-diapause larvae	10.7	3.9	16.6	13.2	16.6	34.9	0.3	0.2	0.3	3.3
Pre-diapause larvae	11.0	6.3	12.0	6.0	22.6	41.5	tr	0.4	tr	tr
Head										
Non-diapause larvae	8.4	1.9	12.5	14.6	19.7	40.9	–	0.9	0.3	0.8
Pre-diapause larvae	7.2	1.9	11.6	14.1	20.6	42.5	–	1.0	0.5	0.6
Flight muscle										
1-day adult	9.0	1.0	4.6	12.5	17.6	53.9	–	0.2	1.2	–

tr = less than 0.1%.

Table 2. Percentage fatty acid composition of testicular phospholipids of mature non-diapause and pre-diapause larvae and of pupae held in diapause (2 °C) for 65 days. Each sample consisted of 10 insects (n = 3). A representative distribution is given below

	16:0	16.1	18.0	18:1	18:2 n6	18:3 n3	18:3 n6	20:3 n3	20:5 n3	Other[a]
Non-diapause										
Phospholipids	11.9	1.8	13.6	23.3	11.2	35.3	–	1.0	1.7	0.2
Neutral lipids	15.1	2.9	2.3	24.9	10.8	41.1	–	–	–	2.9
Pre-diapause										
Phospholipids	9.8	2.6	12.1	23.8	14.3	34.2	–	1.1	1.7	0.4
Neutral lipids	16.2	5.0	2.3	16.3	14.2	43.1	–	–	–	2.9
Diapause pupae										
Phospholipids	7.7	0.9	12.5	29.7	12.5	29.4	0.1	1.0	4.2	2.0
Neutral lipids	15.4	0.3	3.7	29.8	10.9	36.2	0.3	0.3	1.1	2.0

[a] Including C14:0, C21:3 and C24:0.

linoleic (C18:2n6) and linolenic (C18:3n3) acids in fat body phospholipids of mature larvae (table 1). In contrast there is little or no change in the composition of fatty acids of head phospholipids between non-diapause and pre-diapause insects. The fat body, head and adult flight muscles all contained, albeit at a very small concentration, the long chain C20:3n3 and C20:5n3 (eicosapentaenoic acid), but significantly, we were unable to detect arachidonic acid (C20:4n6) in any of the tissues except in trace concentrations. Outside reproductive tissues, the highest concentration of eicosapentaenoic acid was observed in adult flight muscle phospholipids (1.2%). In none of these tissues did the neutral lipids contain detectable amounts of the C20 PUFA.

The male reproductive tissues contained somewhat more eicosapentaenoic acid and C20:3n3 than other tissues examined (table 2). In both non-diapause and pre-diapause larvae about 1.7% of testicular phospholipid fatty acids were eicosapentaenoic acid. To examine whether diapause at +2 °C affects the relative proportion of PUFA, we analyzed the testes of pupae maintained 65 days in diapause. The fat body of these insects has undergone histolysis and cannot be studied separately. The data show a substantial increase in the relative proportion of eicosapentaenoic acid in testicular phospholipids (4.2%). Analysis of the food of *Pieris* larvae revealed that plant leaves *(Brassica oleracea)* contain small amounts of both C20:3n3 and eicosapentaenoic acid. Photosynthetic tissue phospholipids of several plants are known to contain both arachidonic and eicosapentaenoic acids[11]. Our data indicate that about 0.5% of the fatty acids of leaf glycolipids and about 1.0% of those of leaf phospholipids was eicosapentaenoic acid. The larvae ingest about 5–6 mg lipids during the final 5th instar and both glyco- and phospholipids are efficiently utilized[10]. A portion of tissue eicosapentaenoic acid most likely originates from the diet.

GC/MS analysis confirmed the presence of C20:3n3 and C20:5n3 in testicular phospholipids but, significantly, showed no C20:4n6 (arachidonic acid) in this tissue.

Discussion. Synthesis of eicosapentaenoic acid from dietary linolenic acid occurs in mammals and it has been shown that eicosapentaenoic acid is converted to prostaglandin I_3 in humans[12]. Synthesis of tissue eicosapentaenoic acid from linolenic acid is suggested by data from the waxmoth *Galleria mellonella,* for which species the n-3 fatty acids are required for normal adult emergence[13]. Our data suggest the possibility of such synthesis in *P. brassicae:* the species has a requirement for dietary linolenic acid, a portion of which could be converted to eicosapentaenoic acid and incorporated into testicular phospholipids. The increase in testicular phospholipid eicosapentaenoic acid during 65 days of pupal diapause, when no feeding occurs, is of the same order as the decrease in testicular phospholipid linolenic acid. Another tissue accumulating eicosapentaenoic acid in *B. brassicae* were adult flight muscles. The function of eicosapentaenoic acid in these tissues remains to be studied. In view of the finding of prostaglandins of the 2-series in some insect tissues[14–17], these results suggest that in *P. brassicae* the n-3 fatty acids are potential precursor molecules of prostaglandins. We find little or no evidence of the n-6 fatty acids being used in a similar role in the testis of this insect.

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*To whom correspondence should be addressed.

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The autooxidation of 2,3,5,6-tetrahydroxy-2,5-cyclohexadiene-1,4-dione under physiological conditions

M. E. Hoffmann, D. B. Ciampi and N. Duran*

*Instituto de Biologia, Departamento de Bioquimica, *Instituto de Quimica, Laboratorio de Quimica Biologica, Universidade Estadual de Campinas, C.P. 6154, Campinas, CEP 13.100, S.P. (Brasil), 20 February 1986*

Summary. A radical anion of 2,3,5,6-tetrahydroxy-2,5-cyclohexadiene-1,4-dione was detected using the EPR technique by complexing with Zn ions. Hydrogen peroxide, superoxide radical anion and hydroxyl radical were also detected in the reaction mixture. Kinetic study and product distribution indicated a probable mixed type one- and two-electron transfer mechanism. A possible relationship between the autooxidation process and the biological activity of substituted quinones was suggested.
Key words. Oxygen activated species; chemiluminescence; quinones; autooxidation; radicals; EPR.

The biological properties of several substances which contain carbonyl groups, such as alloxane, ninhydrin, dihydroascorbate, glyoxal and cyclic ketones are related to the strong reductant activities of these classes of compounds[1,2]. Recently, it has been shown that α-hydroxyketones autooxidize under physiological conditions via the ene-diol tautomer[3,4]. One condition which favors the formation of the ene-diol is the presence of a vicinal carbonyl group (scheme 1).

The equilibrium is in general displaced in favor of the thermodynamically more stable ketol tautomer. The autooxidation of such ene-diols, as well as of ascorbate and dihydrofumarate, has been shown to involve the generation of reactive intermediates such as carbon-centered free radicals, superoxide radical anion (O_2^-), hydroxyl radical (OH·) and hydrogen peroxide[5,6].

One compound which possesses the ene-diol structural element is 2,3,5,6-tetrahydroxy-2,5-cyclohexadiene-1,4-dione (tetrahydroxy-1,4-quinone) (**I**), which bears several structural similarities to coenzyme Q (**II**) (fig. 1) and exhibits a series of biological activities, such as antitrypanosomal[7] and antiviral activities[8,9], and inhibition of glyoxylase I[10], whose selective inhibition could explain carcinostatic activity by preventing metabolism of α-ketoaldehydes in tumor cells. Presumably, all of these effects are related to redox processes[1] and can be reasonably rationalized on the basis of MNDO calculations[11].

These properties led us to study the autooxidation of substituted hydroquinones and seek to establish the relationship between that process and the biological effects induced by these substances. In the present work we report the general features and probable mechanism of quinone **I** autooxidation under physiological conditions.

We have observed that the acyl-ene-diol moiety of quinone **I** does indeed undergo autooxidation under physiological conditions with the generation of the intermediate ene-diol radical, superoxide radical anion and hydroxyl radical, the final products being hydrogen peroxide and the corresponding tetrone (**III**) (2,3-dihydroxy-5-cyclohexene-1,2,3,4-tetrone)[12].

Quinone **I** autooxidizes under physiological conditions, as shown by oxygen consumption (fig. 2), with concomitant disappearance of tetrone **III** and appearance of H_2O_2 followed by chemiluminescence method[14] (fig. 3). Superoxide dismutase slightly increased the oxygen uptake, indicating that either oxygen probably reacts very rapidly with one or more of the autooxidation intermediates or an H_2O_2-mediated step in the sequence or the rapid removal of O_2^- displaces the equilibrium of the reaction with a concomitant accelerated O_2 uptake (fig. 3). There is a good correlation between the rate of disappearence of quinone **I** and appearance of the superoxide radical anion as measured by the nitroblue tetrazolium chloride test. The control experiments showed that quinone **I** in the absence of oxygen did not reduce NBT. Presumably the presence of O_2^- and the H_2O_2 appearance simultaneously in this reaction is indicative of a mixed one- and two-electron transfer mechanism[13]. The initial concentration of O_2^- formed in the autooxidation of quinone **I** is low, due to a rapid reaction with the semiquinone to generate H_2O_2 after oxygen consumption (see Eq. 3 in scheme 2). From the data of figure 2, a total of 47 μM of oxygen are consumed by an initial concentration of quinone **I** of 95 μM, indicating an overall stoichiometry of 1:0.5 for the autooxidation (see Eq. 6). The observation of O_2^- and H_2O_2 is indicative of the presence of OH· radicals, and probably singlet oxygen which are part of the Haber-Weiss reaction[15]. The table exhibits data for the influence of various agents on the photon emission during autooxidation of quinone **I** following the method of Durán et al.[16-18]. The

Influence of various agents upon the emission from autooxidation of tetrahydroxy-1,4-quinone[a]

Compounds	Max. intensity counts (× 1000)	Ratio	Remarks
Control	8.7	1.0	—
+ CO_3^{--} (10 mM)	52.9	6.0	Enhancement of the emission when the species are CO_2^-
+ CO_3^{--} (20 mM)	84.7	9.7	Enhancement of the emission when the species are CO_2^-
+ Mannitol (30 mM)	7.3	0.8	OH trap
+ Mannitol (50 mM)	5.2	0.6	OH trap
+ Benzoate (10 mM)	7.0	0.8	OH trap
+ DABCO (10 mM)	39.1	4.5	Enhancement of $^1O_2$ emission
+ Catalase (150 units)	2.0	0.2	H_2O_2 scavenger
+ Catalase denatured (150 units)	5.5	0.6	—
+ SOD (132 units)	2.2	0.3	O_2^- scavenger
+ SOD denatured (132 units)	8.9	1.0	—
+ 1,10-Phenanthroline (10 mM)	1.7	0.2	Complex with iron salts
+ Fe^{++} (10 mM)	57.0	6.6	Free radicals initiator

[a] Tetrahydroxy-1,4 quinone of 1.9 mM in PBS buffer at pH 7.2.