thus indicated to occur 6 min after sr and 16 min before ss. Its highest level seems to occur 1-1.5 h after sr and 29 min before ss. The coincidences of absorption bands of the Fraunhofer lines and of phytochrome are noted in this context: The  $P_{FR}$  absorption at 660 nm compared with the

Fraunhofer water vapor line at 650 nm and the C-line at 656 nm and also the absorption of  $P_R$  at 730 nm against the Fraunhofer water vapor absorption band also at 730 nm. Whether these coincidences are accidental or evolutionary in nature is an unresolved question.

- H. Jonas, Z. Pflphys. 80, 395 (1976).
- 2 G. Roblin, J. interdiscipl. Cycle Res. 8, 89 (1977)
- 3 M.J.-C. Fondeville, Congrès Socs savantes 87, 977 (1962).
- 4 M.G. Holmes and H. Smith, Photochem. Photobiol. 25, 533 (1977).
- 5 M.G. Holmes and H. Smith, Photochem. Photobiol. 25, 539 (1977).
- 6 H. Smith and M.G. Holmes, Photochem. Photobiol. 25, 547 (1977).
- 7 M.G. Holmes and H. Smith, Photochem. Photobiol. 25, 551 (1977).
- 8 S. Setty and M. J. Jaffe, Planta 108, 121 (1972).
- 9 H. Jonas, J. interdiscipl. Cycle Res. 1, 335 (1970).
- 10 G.L. Knestrick and J.A. Curcio, Measurment of Spectral Radiance of the Horizon Sky, Naval Research Laboratory Report 6615, Naval Research Laboratory, Washington, D.C. 1967.

## Time-course of changes in lipofuscin-like pigments in rat liver homogenate and mitochondria after whole body gamma irradiation

## J. Wilhelm and J. Šonka

Laboratory for Endocrinology and Metabolism, Faculty of Medicine, Charles University, CS-12821 Prague (Czechoslovakia), 7 March 1980

Summary. An increased level of lipofuscin-like pigments in rat liver homogenate was observed 18 days after whole body gamma irradiation, while in mitochondria they decreased below the control value.

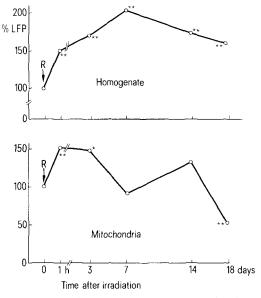
Ionizing radiation leads to the formation of lipid peroxides<sup>1</sup>. The product of their decomposition, malondialdehyde<sup>2</sup>, reacts readily to form very stable lipofuscin-like pigments<sup>3</sup>. These are formed both after ionizing irradiation and during aging as the so called age pigment<sup>4</sup>. The chromophore is probably a Schiff base with a characteristic fluorescence<sup>5</sup>. We studied in the present work the changes in the content of lipofuscin-like pigments (LFP) in rat liver homogenate and mitochondria in the course of 18 days after whole body gamma irradiation.

Materials and methods. Male white rats, Velaz breeding, were divided into 6 groups of 6 animals, weight 150-180 g. One group served as a control, the other groups were gamma irradiated by 60Co (3.83 Gy with a dose rate 4.5 mGy/sec). The irradiation was performed in a turning cage to assure field homogeneity. The animals were decapitated without narcosis after 1 h, and on the 3rd, 7th, 14th and 18th days after irradiation. A 10% liver homogenate was made in sucrose (0.25 M sucrose, 0.001 M EDTA, 0.02 M Tris · HCl pH 7.4). After separation of nuclei, mitochondria were sedimented at 7000 g and washed twice. All operations were carried out at 0-4 °C. A standard solution of quinine sulfate was used for the estimation of the LFP fluorescence (0.05 mg quinine sulfate per ml of 0.1 N H<sub>2</sub>SO<sub>4</sub> represented 550 relative units).

2 ml of homogenate or of mitochondrial suspension were added to 8 ml of a chloroform-methanol mixture (2:1, v/v) and extracted under argone 1 h on a motor-driven shaker. The fluorescence was measured at 435 nm after excitation at 365 nm on the Hitachi-Perkin Elmer MPF 2A spectro-fluorimeter. The values were expressed as relative fluorescence units per mg of protein, determined according to Miller<sup>6</sup>. For statistical analysis Student's t-test was used.

Results. The content of LFP in homogenate or mitochondria during the time course of 18 days after irradiation is summarized in the table. The ratio of LFP content in the homogenate to that in mitochondria increased steadily up to the 7th post-irradiation day. On the 14th day it

decreased, and increased again on day 18. While the LFP content in both homogenate and mitochondria increased equally up to the 1st post-irradiation h, it differed afterwards (figure). The LFP content of the homogenate rose on the 7th post-irradiation day up to 205% and then steadily decreased. Nevertheless, the LFP value was still 60% above the controls on day 18.



The time course of changes in LFP content in rat liver homogenate and mitochondria after irradiation. The control value is taken as 100%. The statistical significance of the difference between the particular and the control value is given by 1 asterisk (p < 0.05) and 2 asterisks (p < 0.01).

In mitochondria, the LFP content was almost the same on the 3rd post-irradiation day as it was 1 h after irradiation. Nonsignificant tendencies to a decrease (92%) and to an increase (135%) followed. However, a significant decrease of mitochondrial LFP was recorded on day 18 (54% of initial value)

Discussion. LFP formation is associated with lipid peroxide formation. As a stable product, LFP exert a deleterious effect for a long time and their accumulation can lead to cell death<sup>7</sup>. Thus the mechanisms which prevent their accumulation are of vital importance for the cell. Up to now, centrophenoxine is the only drug known to eliminate the LFP from cells<sup>8</sup>, but the mechanism of this effect has not been elucidated yet.

Mitochondria are well protected against lipid peroxidation

by gluthathione peroxidase which inhibits malondialdehyde formation<sup>9,10</sup>. This action of glutathione peroxidase could explain why after the initial burst of LFP formation a further increase of LFP was observed in the homogenate, but not in mitochondria. A diffusion of malondialdehyde from cytoplasm<sup>11</sup> might participate in the slight LFP increase observed in mitochondria from the 7th to the 14th post-irradiation day. It is suggested that the terminal LFP decrease in the mitochondria of irradiated rats is due to an enhanced elimination of LFP from mitochondria and/or the cessation of malondialdehyde production in the cytoplasm. The observed decrease of LFP during the time after irradiation deserves further study, as it could bring more understanding of the mechanisms leading to LFP elimination.

LFP content in rat liver homogenate and mitochondria during the time after irradiation

Time after irradiation	LFP ( $\bar{x} \pm SEM$ ), relative units		Homogenate
	Homogenate	Mitochondria	Mitochondria
Control	$5.76 \pm 0.33$	$2.83 \pm 0.41$	2.04
1 h	$8.57 \pm 0.27**$	$4.30 \pm 0.10**$	2.00
3rd day	$9.84 \pm 0.30 **$	$4.23 \pm 0.15*$	2.33
7th day	$11.81 \pm 0.72**$	$2.61 \pm 0.18$	4,52
14th day	$9.99 \pm 0.53**$	$3.81 \pm 0.32$	2.62
18th day	$9.19\pm0.82**$	$1.52 \pm 0.04**$	6.05

 $\bar{x}$ , Mean value of 6 animals. \*p<0.05; \*\*p<0.01 (related to the control group).

- E.D. Wills and A.E. Wilkinson, Radiat. Res. 31, 372 (1967).
- W.G. Niehaus, Jr, and B. Samuelson, Eur. J. Biochem. 6, 126
- K.S. Chio, U. Reiss, B. Fletcher and A.L. Tappel, Science 166, 1535 (1969).
- J. Miquel, P.R. Lundgren and J.E. Johnson, Jr, J. Geront. 33, 5 (1978).
- A.S. Csallany and K.L. Ayaz, Lipids 11, 412 (1976). G.L. Miller, Analyt. Chem. 31, 964 (1959).
- D. M. Mann and P.O. Yates, Brain 97, 481 (1974).
- B.O. Christophersen, Biochem. J. 106, 515 (1968).
- L. Flohé and R. Zimmermann, Biochim. biophys. Acta 223, 210 (1970).
- K. Nandy and G. H. Bourne, Nature 210, 313 (1966).
- A. Benedetti, A.F. Casini, M. Ferrali and M. Comporti, Biochem. J. 180, 303 (1979).

## Inhibition of insect larval growth by phenolics in glandular trichomes of tomato leaves

S. S. Duffey and M. B. Isman<sup>1</sup>

Department of Entomology, University of California, Davis (Ca. 95616, USA), 5 November 1980

Summary. We have found that the foliar tetracellular glandular trichomes (tetrads) of the tomato plant, Lycopersicon esculentum Mill., contribute significantly to the antibiotic effect of the leaf against the fruitworm Heliothis zea (Boddie), as measured by reduction in larval growth. This effect is attributable to phenolic compounds localized within the tetrads. We have found that the cellular fluid of the tetrads is particularly rich in the flavonol glycoside rutin, accompanied by lesser amounts of other phenolics.

In both natural and agro-ecosystems, the trichomes of several plant species are known to contribute to defense against herbivorous insects<sup>2</sup>. It is known that high densities of 4-lobed glandular trichomes on certain commercial cultivars of tomatoes and related wild species of Lycopersicon, can enhance resistance to several pests<sup>3</sup>. Often leaf hairs function as a physical barrier to insects' movement, feeding, and/or oviposition, or as an enmeshing trap in which small insects die from dessication or starvation<sup>4</sup>. The physical trapping of small insects on tomato foliage (whiteflies, flea beetles, aphids, or mites) is often accomplished via a sticky exudate released on breakage of the fragile trichomes<sup>5</sup>. Glandular trichomes of a similar type have been shown to protect commercial cultivars and related wild species of the potato, Solanum tuberosum<sup>6</sup> against some insect pests. However, many plants' glandular tri-chomes contain secondary compounds<sup>7</sup> that are potentially toxic or deterrent to phytophagous insects. Our analyses of tomato leaf tetrads quantify the toxic effects of phenolics on a larval insect pest.

The chemical composition of the tomato leaf tetrads was determined by rubbing (without crushing) leaves and stems of field-grown L. esculentum (vars. VF 145, Ace 55, VF 198, UC 134, VF 315, VFN Bush, Royal Flush) with tissue papers. The fluid from the shattered tetrads was subsequently eluted with 70% methanol, and evaporated in vacuo, yielding a bright yellow precipitate. TLC on cellulose or polyamide8 or column chromatography with Sephadex LH-209 revealed a mixture of about 10 phenolics. Rutin (quercetin-3-rutinoside), by comparison with TLC mobilites and UV-spectra of a standard, was identified as the major phenolic (80-90%) admixed with a lesser amount of an unidentified catechin (5%) and, traces of chlorogenic acid, other conjugates of caffeic acid, and several uncharacterized flavonoid glycosides.

We estimated the density of tetrads on the upper and lower (adaxial and abaxial) surfaces of tomato leaves of several cultivars by replicated direct counting of tetrads using an ocular guide on a dissecting microscope. Amongst the 6 cultivars, the density of adaxial tetrads ranged from 1200/