by hydrolysing the phosphate esters of the host cell[8]. It remains an open question whether these enzymes are located on the outer surface of the sporozoite as in the case of ectoacid phosphatase of *L. donovani*[21,22,38] or whether they are embedded into the subcellular structures of the parasitic cell. To date the occurrence of lysosomes or lysosome-like structures in *E. tenella* has not yet been reported[39]. However, the secretory structures termed 'rhoptries' reportedly release their contents during penetration of embryonic bovine tracheal cells by *E. magna*[40,41]. Although the mechanisms involved in the penetration of host cells by *Eimeria* are not understood[42], available evidence from other related parasitic intracellular protozoans suggests that biochemical mechanisms should be carefully considered. In addition, the demonstration of hydrolytic enzymes in the various stages of *E. tenella* along with proteinases, in our opinion, enhances the possibility that cell recognition and penetration by these parasites may involve biochemical specificities at the molecular level. Additional work will be required to determine if this is true.

1 We are grateful to Professor Moises Agosin, Department of Zoology, for useful discussion. Cultures of *Eimeria tenella* (WIS) were provided by Drs L. McDougald and P. Long of this University. The authors were supported in part by grant No. 901-15-174 from the United States Department of Agriculture.

2 Trager, W., Science *183* (1974) 269.
3 Joyner, L.P., in: The Biology of the Coccidia, p.35. Ed. P.L. Long. University Park Press, Baltimore 1982.
4 Hammond, D.M., in: The Coccidia, p.45. Eds D.M. Hammond and P.L. Long. University Park Press, Baltimore 1973.
5 Ray, H.N., and Gill, B.S., Ann. trop. Med. Parasit. *48* (1954) 8.
6 Gill, B.S., and Ray, H.N., Indian J. vet. Sci. *24* (1954) 239.
7 Frandsen, J.C., Exp. Parasit. *23* (1968) 398.
8 Farooqui, A.A., and Hanson, W.L., Z. ParasitKde (1983) in press.
9 Patton, H.W., and Brigman, G.P., J. Parasit. *65* (1979) 526.
10 Wagenbach, G.E., J. Parasit. *55* (1969) 833.
11 Farooqui, A.A., and Srivastava, P.N., Biochem. J. *181* (1979) 331.
12 Farooqui, A.A., and Srivastava, P.N., Biochem. J. *191* (1980) 827.
13 Helwig, J.J., Farooqui, A.A., Bollack, C., and Mandel, P., Int. J. Biochem. *8* (1977) 323.
14 Farooqui, A.A., Adams, D.D., Hanson, W.L., and Prestwood, A.K., J. Parasit. (1983) in press.
15 Srivastava, P.N., and Farooqui, A.A., Biochem. J. *183* (1979) 531.
16 Srivastava, P.N., and Farooqui, A.A., Biol. Reprod. *22* (1980) 858.
17 Bradford, M.M., Analyt. Biochem. *72* (1976) 248.
18 De Duve, C., in: Lysosomes in Biology and Pathology, vol. 1, p.3. Ed. J.T. Dingle and H.B. Fell. North-Holland, Amsterdam 1969.
19 Novikoff, A.B., Essner, E., Goldfischer, S., and Heus, M., in: Interpretation of Ultrastructure, p.149. Ed. R.J.C. Harris. Academic Press, New York 1962.
20 Stefanovic, V., Mandel, P., and Rosenberg, A., J. biol. Chem. *251* (1976) 493.
21 Gottlieb, M., and Dwyer, D.M., Science *212* (1981a) 939.
22 Gottlieb, M., and Dwyer, D.M., Exp. Parasit. *52* (1981b) 117.
23 Brooker, B.E., Z. Zellforsch, *116* (1971) 532.
24 Spiro, R.G., Adv. Protein Chem. *27* (1973) 349.
25 Jeanloz, R.W., and Codington, J.F., in: Biological Roles of Sialic Acid, p.201. Eds A. Rosenberg and C.L. Schengrund. Plenum Press, New York 1976.
26 Kobata, A., Cell Struct. Funct. *4* (1979) 169.
27 Luft, J.H., Int. Rev. Cytol. *45* (1976) 291.
28 Davidson, E.A., in: Metabolic Conjugation and Metabolic Hydrolysis, vol. 1, p.327. Ed. W.H. Fishman, Academic Press, New York 1970.
29 Hayashi, S., J. Biochem., Tokyo *82* (1977) 1287.
30 Hayashi, S., J. Biochem., Tokyo *83* (1978) 149.
31 Kobata, A., Analyt. Biochem. *100* (1979) 1.
32 Lycke, E., Lund, E., and Strannegard, O., Br. J. exp. Path. *46* (1965) 189.
33 Lycke, E., and Norrby, R., Br. J. exp. Path. *47* (1966) 248.
34 Norrby, R., and Lycke, E., J. Bact. *93* (1967) 53.
35 Norrby, R., Londholm, L., and Lycke, E., J. Bact. *96* (1968) 916.
36 Norrby, R., Infect. Immun. *2* (1970) 250.
37 Kilejian, A., Trends biochem. Sci. *7* (1982) 5.
38 Gottlieb, M., and Dwyer, D.M., Molec. Cell Biol. *2* (1982) 76.
39 Aikawa, M., and Sterling, C.R., in: Intracellular Parasitic Protozoa, p.42. Eds M. Aikawa and C.R. Sterling. Academic Press, New York 1974.
40 Scholtyseck, E., and Chobotar, B., Z. ParasitKde *49* (1975) 91.
41 Jensen, J.B., and Edgar, S.A., J. Parasit. *62* (1978) 988.
42 Long, P.L., in: Cues that Influence Behavior of Internal Parasites, p.127. Ed. W.D.S. Bailey. Agricultural Research Service (Southern Region), U.S. Department of Agriculture, New Orleans 1982.

0014-4754/83/121368-03$1.50 + 0.20/0
© Birkhäuser Verlag Basel, 1983

Metallothionein induced in the frog *Xenopus laevis*[1]

M. Yamamura and K.T. Suzuki

National Institute for Environmental Studies, Yatabe, Tsukuba, Ibaraki 305 (Japan), March 1, 1983

Summary. A low molecular weight cadmium- and copper-binding protein, induced in the liver of the frog, *Xenopus laevis*, by the administration of cadmium, was shown to consist of a single isoprotein and was characterized as an amphibian metallothionein based on its high cysteine and metal content, its low molecular weight, and the lack of aromatic amino acids.

Metallothionein is a low molecular weight, cysteine-rich protein (mol. wt = 6000–7000) binding heavy metals such as cadmium, zinc, copper and mercury. Metallothionein was first isolated as a cadmium- and zinc-binding protein from equine renal cortex by Margoshes and Vallee in 1957[2]. Thereafter, many investigators have reported the induction of metallothionein following the administration of heavy metals to a variety of organisms, both animals and plants. Although metallothionein has been well documented in vertebrates, especially in mammals, the protein in amphibia has been reported thus far only in the frog, *Xenopus laevis*[3,4]. Amphibia hold a phylogenetical position between

fish and reptiles, and are, hence, interesting species for studying the biological roles and the phylogenetical changes of metallothionein. Therefore, the present study was performed to characterize *Xenopus* metallothionein and to compare its properties with those of mammalian metallothionein.

Frogs (*Xenopus laevis;* 88–107 g b. wt) were injected i.m. with cadmium at a dose of 0.5 mg Cd/kg b. wt, 5 times weekly for 2 weeks. The livers were dissected 3 days after the final injection and homogenized in 4 vol. of 0.1 M Tris-HCl buffer (pH 7.4, 0.25 M glucose, bubbled through with 99.9999% N_2 gas before use) in an atmosphere of N_2 gas under ice-water cooling. The homogenate was centrifuged at 170,000 × g for 60 min at 4 °C.

Cadmium from the supernatant fraction was eluted mainly together with the low molecular weight protein fraction (Ve/Vo = 1.8–2.2) on a Sephadex G-75 column. The cadmium peak was accompanied by a small amount of copper but not by zinc, and by strong absorption at 254 nm. Since zinc was not present in the low molecular weight protein fraction (Ve/Vo = 1.8–2.2), the supernatant was co-chromatographed with the isolated rat zinc-thionein on a Sephadex G-75 column for comparison of the apparent molecular weights as shown in figure 1. The cadmium peak of frog metallothionein was eluted one fraction earlier than the zinc peak of rat zinc-thionein.

The frog metallothionein isolated on a Sephadex column was further examined by co-chromatography with rat zinc-thionein on a DEAE Sephadex G-75 column (fig. 2). Zinc in rat zinc-thionein was separated into 2 peaks. The 2 peaks corresponded to metallothionein-I and -II, the former isoform being eluted earlier. On the other hand, although native frog metallothionein was eluted as a single peak on an SW column[3], cadmium was separated into four peaks in the DEAE Sephadex co-chromatogram (fig. 2). The main cadmium peak was attributed to the native *Xenopus* metallothionein, while the other three peaks were identified as follows: The 2 cadmium peaks were eluted at the elution volumes of the two 2 zinc-thioneins and, hence, they were attributed to the replacement of zinc in the 2 rat zinc-thioneins with the cadmium that was liberated from the *Xenopus* metallothionein during the preparative procedures. The 4 cadmium peak at fraction 62 was assigned to an intramolecular oxidation product of the *Xenopus* metallothionein[4]. Thus, as reported for chicken[5] and plaice[6] metallothioneins, native *Xenopus* metallothionein was shown to consist of only a single isoprotein on an anion exchange column. *Xenopus* metallothionein also has a lower isoelectric point than the 2 co-chromatographed rat isometallothioneins (fig. 2).

Apparent molecular weights of the native *Xenopus* metallothionein and the intramolecular oxidation product isolated on a DEAE Sephadex column were estimated by gel permeation chromatography with a TSKgel 3000 SW column at pH 7.0 in the presence of 0.2 M sodium chloride (fig. 3). Both of them were eluted at a retention time slightly earlier than cytochrome c (mol. wt = 12,400) and were

Amino acid composition and metal content of *Xenopus* metallothionein

Amino acid composition	Mol %[a]	Residues[b] / Molecule
Aspartic acid	6.7	3.9
Threonine	5.5	3.2
Serine	13.1	7.7
Glutamic acid	7.2	4.2
Proline	2.8	1.6
Glycine	6.6	3.9
Alanine	3.4	2.0
Valine	1.2	0.7
Half-cystine	38.9	22.9
Methionine	1.7	1
Isoleucine	–	–
Leucine	0.7	0.4
Tyrosine	–	–
Phenylalanine	–	–
Lysine	11.1	6.5
Histidine	1.1	0.6
Arginine	–	–

Metal content (mol%): Cd 93.3, Zn –, Cu 6.7
SH/metal ratio: 2.7

[a] *Xenopus* metallothionein (containing 37 µg Cd) was hydrolyzed in 0.5 ml of 6 N HCl at 110 °C for 20 h. Amino acid analysis was performed with an amino acid analyzer (Hitachi 835). Half-cystine and methionine were analyzed after performic acid oxidation. Values are expressed as percent of the total number of residues.

[b] Methionine was assumed to be one residue per molecule.

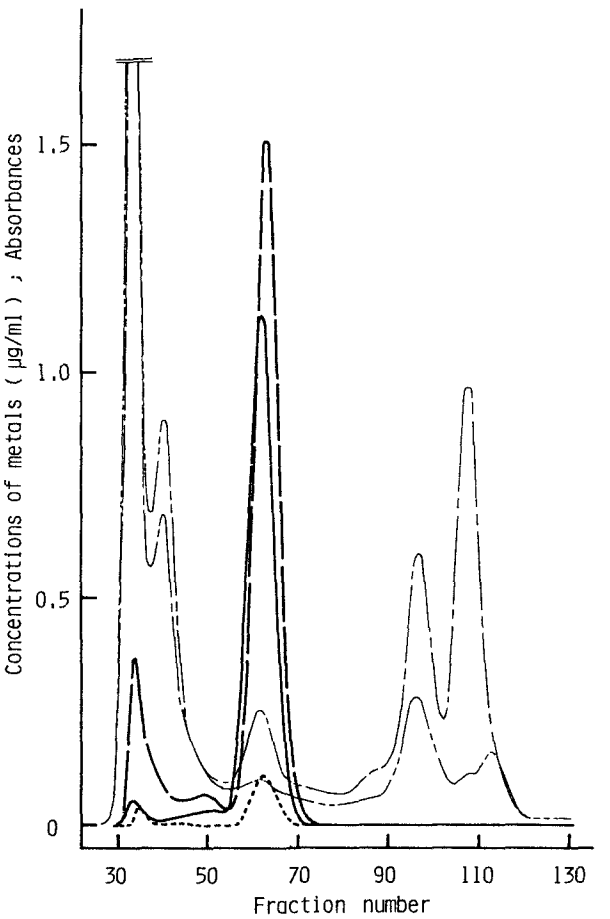


Figure 1. Co-chromatogram of the supernatant from cadmium-accumulated *Xenopus* liver with rat zinc-thionein on a Sephadex column. The frogs, *Xenopus laevis*, were injected i.m. with cadmium and the livers were homogenized in 4 vol. of 0.1 M Tris-HCl buffer (pH 7.4, 0.25 M glucose). The homogenate was centrifuged at 170,000 × g for 60 min. A 5-ml portion of the supernatant was mixed with a sample of rat zinc-thionein (containing 50 µg Zn) and applied to a Sephadex G-75 column (2.6 × 90 cm). The column was eluted with 10 mM Tris-HCl buffer solution (pH 8.6) and 5-ml fractions were collected. The concentrations of cadmium, zinc and copper in each fraction were determined by atomic absorption spectrophotometry (Hitachi 170-50A). The absorbances at 254 and 280 nm were monitored on a spectrophotometer (Hitachi 220). ———, Cd; —— ——, Zn; – – – – –, Cu; ——– ——, absorbance at 254 nm; ——– – – ——, absorbance at 280 nm.

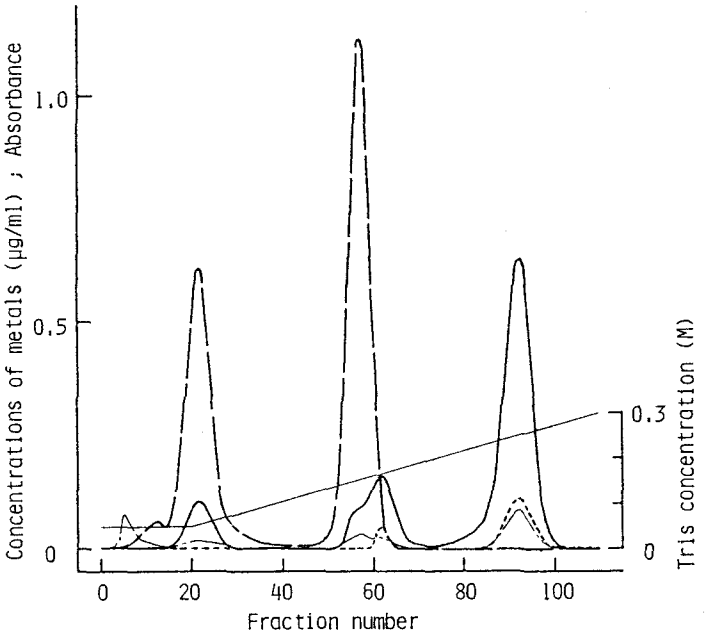


Figure 2. Co-chromatogram of frog metallothionein and rat zinc-thionein on a DEAE Sephadex column. Frog cadmium, copper-thionein and rat zinc-thionein isolated by Sephadex G-75 chromatography were applied to a DEAE Sephadex A-25 column (1.6×20 cm). The proteins were eluted with a linear concentration gradient of Tris-HCl buffer (pH 8.6) from 75 to 300 mM and 3-ml fractions were collected. The concentrations of cadmium, zinc and copper were determined by atomic absorption spectrophotometry. The absorbance at 254 nm was monitored on a spectrophotometer. ———, Cd; — — —, Zn; - - - - - -, Cu; ——·——, absorbance at 254 nm.

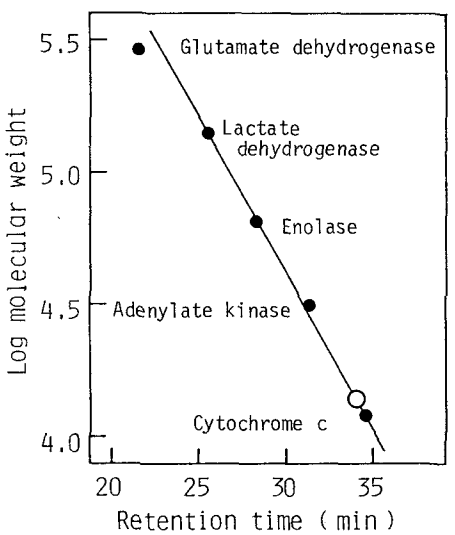


Figure 3. Molecular weight estimation of *Xenopus* metallothionein. *Xenopus* metallothionein and its intramolecular oxidation product were applied on a TSKgel 3000 SW column (7.5×600 mm with a precolumn of 7.5×75) with marker proteins. The column was eluted with 0.1 M phosphate buffer solution (pH 7.0, 0.2 M NaCl) at a flow rate of 0.7 ml/min. The circle indicates *Xenopus* metallothionein. Its intramolecular oxidation product was eluted with the native protein, so that it was not shown.

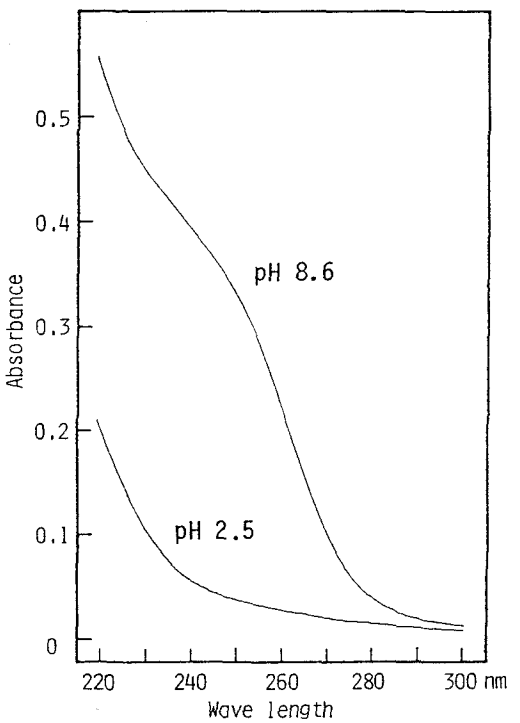


Figure 4. pH-Dependence of absorption spectrum of *Xenopus* metallothionein. UV spectra of purified *Xenopus* metallothionein (5.7 μg Cd/2 ml of 10 mM Tris-HCl buffer, pH 8.6) were determined on a Hitachi 220 spectrophotometer. pH 2.5 was adjusted by adding 1 N HCl solution.

estimated to have an apparent molecular weight of 14000 from the retention time.

Both the native protein and the intramolecular oxidation product isolated on a DEAE Sephadex column were purified further with a TSK SW column by elution with a 50 mM Tris-HCl buffer solution (pH 8.0)[7]. They were shown to be pure by 7.5% polyacrylamide gel electrophoresis at pH 8.3, and were characterized by amino acid analysis. The amino acid compositions of the 2 proteins were identical and, hence, only the results obtained for the native protein are shown in the table. Half-cystine (38.9 mol%) was most abundant, followed by serine (13.1 mol%) and lysine (11.1 mol%). However, aromatic amino acids were absent, as in the case of the mammalian metallothioneins[8]. The amino acid composition of *Xenopus* metallothionein is similar to those of the mammalian metallothioneins[8]. The major metal bound to native *Xenopus* metallothionein was cadmium (93.3 mol%). Copper was present only as a minor metal (6.7 mol%). In contrast to the mammalian metallothioneins[8], zinc was absent from the *Xenopus* metallothionein. The ratio of cysteinyl residues to metals (SH/metal) was 2.7, nearly identical to that in the mammalian metallothioneins[8]. The molecular weight of frog metallothionein inferred from amino acid analysis was

slightly smaller than that of the mammalian metallothionein. This estimate conflicts with the result in figure 1.
The discrepancy can be explained as follows: Since a Sephadex column has cation exchange properties at alkaline pH due to dissociation of residual carboxyl and/or hydroxyl groups of the gel material, proteins with lower isoelectric points are eluted earlier on the Sephadex column than those with higher ones[9]. The isoelectric point of the *Xenopus* metallothionein is lower than those of the 2 rat isometallothioneins (fig. 2). Hence, *Xenopus* metallothionein elutes faster than rat metallothionein (fig. 1). In accordance with the lack of aromatic amino acids, the UV spectrum of the *Xenopus* metallothionein did not show appreciable absorption at 280 nm. The shoulder of 250 nm was confirmed to arise from the cadmium-mercaptide complex since it was completely abolished upon the loss of the metal following adjustment to pH 2.5 (fig. 4).
The present study revealed the amino acid composition and some properties characteristic of *Xenopus* metallothionein. Aside from properties similar to those of mammalian metallothioneins[8], the frog metallothionein can be characterized as follows a) it consists of a single isoprotein, b) it has an isoelectric point lower than rat isometallothioneins and c) it is less stable to air oxidation than the rat isometallothioneins.

1 The authors thank Dr K. Kubota for his encouragement.
2 Margoshes, M., and Vallee, B.L., J. Am. chem. Soc. *79* (1957) 4813.
3 Suzuki, K.T., and Tanaka, Y., Comp. Biochem. Physiol. *74C* (1983) 311.
4 Suzuki, K.T., Tanaka, Y., and Kawamura, R., Comp. Biochem. Physiol. *75C* (1983) 33.
5 Weser, U., Rupp, H., Donay, F., Linnemann, F., Voelter, W., Voetsch, W., and Jung, G., Eur. J. Biochem. *39* (1973) 127.
6 Overnell, J., and Coombs, T.L., Biochem. J. *183* (1979) 277.
7 Suzuki K.T., Analyt. Biochem. *102* (1980) 31.
8 Kägi, J.H.R., and Nordberg, M., eds, Metallothionein. Birkhäuser Basel 1979.
9 Suzuki, K.T., Motomura, T., Tsuchiya, Y., and Yamamura, M., Analyt. Biochem. *107* (1980) 75.

0014-4754/83/121370-04$1.50+0.20/0
©Birkhäuser Verlag Basel, 1983

The effect of methyl jasmonate on lycopene and β-carotene accumulation in ripening red tomatoes

M. Saniewski[1] and J. Czapski

Department of Plant Physiology, Research Institute of Pomology, Pomologiczna 18, PL-96-100 Skierniewice (Poland), and Department of Food Science, Institute of Vegetable Crops, 22 Lipca, PL-96-100 Skierniewice (Poland), February 14, 1983

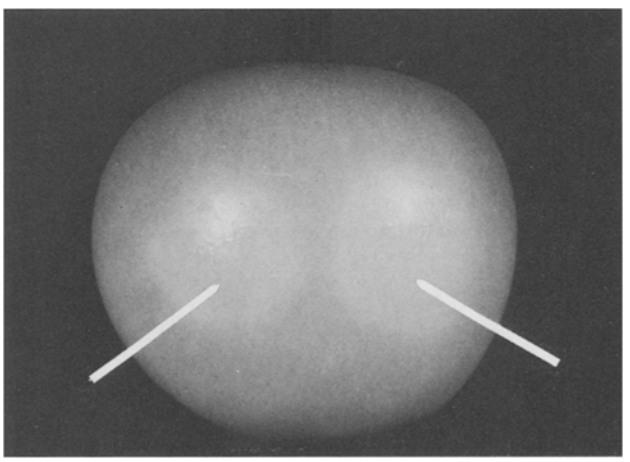
Summary. Methyl jasmonate at a concentration of 0.5% in lanolin paste was applied to detached mature green tomatoes cv. Venture. It caused the formation of a yellow colored epidermis and parenchyma at a depth of 2 mm on the place of treatment. Untreated areas, and areas treated with lanolin paste alone, developed a normal red color at the fully ripened stage. Analyses of carotenoid compositions showed that methyl jasmonate almost totally inhibited lycopene accumulation and stimulated β-carotene accumulation in the ripening of tomatoes.

Methyl jasmonate, methyl 3-oxo-2-(2′-cis-pentenyl)-cyclopentane-1-acetate, and jasmonic acid, were recently found to be powerful promotors of leaf senescence[2-4]. JA-Me and its related compounds were also found to eliminate the senescence-retarding action of kinetin when used in the dark[4]. Methyl jasmonate and/or jasmonic acid are endogenous substances which have been identified in many plants[2,5-10].
The aim of this work was to study the effect of methyl jasmonate on the ripening and carotenoid composition of tomatoes.
Materials and methods. Mature green tomatoes, *Lycopersicon esculentum* Mill. cv. Venture, grown in a greenhouse and picked on September 10, 1982 were used. Ten fruits were treated with (±)-methyl jasmonate at a concentration of 0.5% (w/w) in lanolin paste (prepared by mixing lanolin with $^1/_3$ part of distilled water). This was applied on one side on an area of about 2.5 cm^2. The other side of every tomato was treated with lanolin paste alone as a control. During the course of the experiment, fruits were kept at room temperature (about 18 °C) under natural light conditions.

The effect of JA-Me treatment on the content of lycopene and β-carotene in tomato fruit tissue

Carotenoids	Control $\mu g \cdot g^{-1}$ fresh weights	% of total determined carotenoids	JA-Me $\mu g \cdot g^{-1}$ fresh weight	% of total determined carotenoids
Lycopene	84.30	97.6	1.43	19.6
β-Carotene	2.08	2.4	5.87	80.4

After 7-10 days, when the tomato fruits were ripe, the samples of treated and untreated tissue were cut off at a depth of 2 mm for lycopene and β-carotene analysis. Carotenoids from tomato tissues (about 2 g fresh weight) were isolated by extraction with acetone[11]. In later steps of the procedure the acetone extract was mixed with n-hexane instead of diethyl ether. Then the ether-acetone mixture



The effect of JA-Me on carotenoid formation in ripe tomatoes; arrows indicate yellow color of tissue on the place treated with JA-Me; both untreated areas and those treated with lanolin paste alone are red.