

Table II. RNA content and RNase activity in young and old cells prepared from the 18 mm roots of *Lens culinaris* seedlings

	Young cells	Old cells
RNA content (in µg)		
per 0.1 mg N-protein	93.4 ± 8.0	52.9 ± 4.7
per 10 <sup>7</sup> cells	456	223
RNase activity <sup>a</sup>	0.019	0.687

<sup>a</sup> In OD<sub>260</sub> nm per 0.5 mg N-protein.

auxin catabolism determine the endogenous auxin content: high enzymes activity, for the old cells, means low auxin level, and vice versa for the young cells.

Values as regards the RNA are presented in Table II from which one can conclude that the concentration of the total RNA is greater in young cells than in older ones, and the RNA biodegradation is higher in old cells than in younger ones. Thus, the young cells which have a high auxin content, have a high RNA level. A positive correlation between endogenous IAA and RNA is consequently confirmed<sup>12</sup>. It is well known that IAA can induce an increase of the RNA concentration by increasing the rate of RNA synthesis<sup>13</sup>, but it is also demonstrated that RNase activity is inhibited by IAA<sup>14</sup>.

Without asserting that only these interactions between auxin and RNA cause the cell aging, present data do suggest that enzymes, controlling the auxin biodegradation, are increasingly active as the cell grows older. Then the level of endogenous auxin is decreasing and therefore the RNase activity is enhancing, which consequently produces a decline of the RNA content as cell age increases.

**Résumé.** Sur des extraits de racine de *Lens culinaris*, il est observé qu'au cours du vieillissement cellulaire les enzymes qui contrôlent le catabolisme auxinique sont progressivement plus actives, ce qui a pour conséquence d'entraîner une diminution du taux en auxines endogènes. Par ailleurs, l'activité des systèmes RNasiques – inhibés par les auxines – va s'élever d'une façon significative dans les cellules âgées ce qui explique, partiellement du moins, la diminution de la teneur en RNA.

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<sup>12</sup> Y. MASUDA and S. WADA, *Physiologia plant.* 19, 1055 (1966).

<sup>13</sup> A. TREWAVAS, in *Progress in Phytochemistry* (Interscience Publishers Inc., New York 1968), vol. 1, p. 114.

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# The Presence of Carotenoids in Eggs Deposits of the *Chironomus annularius* Meig (Diptera: Chironomidae)

It is known that the eggs of mosquitoes are grouped together in so-called egg deposits of varying sizes. The deposit is enclosed in a transparent cover, the eggs being suspended in a colourless substance inside. The colour of the eggs and consequently the colour of the whole egg deposit depends on the colour of the yoke. The eggs are usually yellowish in colour, pinkish or sometimes greyish-green.

The purpose of these investigations was to determine which known carotenoids give this colour to the *Chironomus annularius* eggs.

The egg deposits were collected from the pond on the Bialystok aerodrome<sup>1</sup> and from a pond in Bialystok city. The columnar and thin-layer methods of chromatography were employed, various systems of solvents being used<sup>2</sup>. The identification of the various fractions of the columnar chromatogram was based on the absorption maxima and the spots of the thin-layer chromatogram on the R<sub>f</sub><sup>3-10</sup> values.

The absorption curves of extracts of the *Ch. annularius* eggs are given in Figure 1 and the absorption maxima of

these curves in Table I. As regards the columnar chromatography, by means of various solvent systems, the extract was divided into 6 fractions (Table II).

By means of thin-layer chromatography, employing A, B and C solvent systems (see Table III and Figure 2), the extract was separated into 5 spots, with solvent system D (benzene-ethyl ether-methanol in volumetric proportions of 17:2:1) only 4 spots were obtained.

On comparing the various absorption maxima and the R<sub>f</sub> values with the data given in literature, it was possible to identify most of the carotenoids. The R<sub>f</sub> value of the

Table I. Absorption maxima of extracts from eggs deposits of the *Ch. annularius* Meig

Absorption curve	Maximum absorption	Solvent
1	459–460; 480; 500; 520; 540; 550; 580; 600; 620; 660	Ethanol absolute
2	470; 500; 530; 580; 600; 620; 665	Acetone

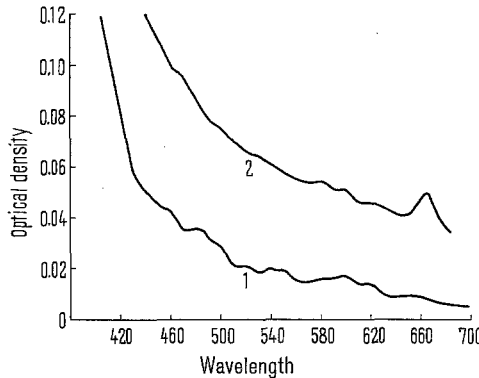


Fig.1. The absorption curves of extracts of the *Ch. annularius* Meig eggs.

<sup>1</sup> B. CZECZUGA, E. BOBIATYŃSKA-KSOK and E. NIEDŹWIECKI, *Zoolog. Pol.* 18, 317 (1968).

<sup>2</sup> B. CZECZUGA and R. CZERPAK, *Comp. Biochem. Physiol.* 24, 37 (1968).

<sup>3</sup> B. CZECZUGA and R. CZERPAK, *Comp. Biochem. Physiol.* 14, 523 (1966).

<sup>4</sup> B. CZECZUGA and R. CZERPAK, *Comp. Biochem. Physiol.* 24, 37 (1968).

<sup>5</sup> B. CZECZUGA and R. CZERPAK, *Experientia* 24, 218 (1968).

<sup>6</sup> B. CZECZUGA and R. CZERPAK, *Comp. Biochem. Physiol.* 25, 547 (1968).

<sup>7</sup> B. CZECZUGA and R. CZERPAK, *Comp. Biochem. Physiol.* 26, 101 (1968).

<sup>8</sup> B. CZECZUGA and R. CZERPAK, *Europ. J. Biochem.* 5, 429 (1968).

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<sup>10</sup> R. CZERPAK and B. CZECZUGA, *Marine Biol.* 4, 122 (1969).

Table II. Column chromatogram of carotenoids from eggs deposits of the *Ch. annularius* Meig

No. of fraction	System of solvents	Maximum absorption	Solvent	Identification
I	Petroleum ether (b.p. 45–65 °C)	445	Petroleum ether	$\alpha$ -Carotene
II	7% acetone in petroleum ether	460	Petroleum ether	Echine- none
III	15% acetone in petroleum ether	425 451	Hexane	Crypto- xanthin
IV	25% acetone in petroleum ether	410; 435	Hexane	Unknown
V	50% acetone in petroleum ether	445; 475	Hexane	Lutein
VI	5% cold acetic acid in ethyl ether	445 497–498	Hexane	Unknown

Table III. Thin-layer chromatograms of carotenoids from eggs deposits of the *Ch. annularius* Meig

No. of spots	Rf	Figure	System of solvents	Identification
A				
1	0.98	2a	Benzene-ethyl acetic-ethanol (16:4:1)	$\alpha$ -Carotene
2	0.88			Echinenone
3	0.69			Cryptoxanthin
4	0.55			Lutein
5	0.28			Unknown
B				
1	0.99	2b	Benzene petroleum ether-acetone (10:2.5:2)	$\alpha$ -Carotene
2	0.62			Cryptoxanthin
3	0.39			Lutein
4	0.25			Unknown
5	0.03			Unknown
C				
1	0.61	2c	Petroleum ether-benzene methanol (40:15:3.6)	
2	0.36			
3	0.22			
4	0.18			
5	0.05			
D				
1	0.98	2d	Benzene ethyl ether (17:2:1)	
2	0.53			
3	0.35			
4	0.18			

1st spot of the thin-layer chromatogram in solvent systems A and B and the absorption maxima of the 1st fraction of the columnar chromatogram indicated the presence of  $\alpha$ -carotene in the eggs of the *Ch. annularius*. As we know<sup>11–14</sup>, the maximum absorption of  $\alpha$ -carotene in petroleum ether occurs at a wavelength of 445 nm. The maximum absorption of the 2nd fraction of the columnar chromatogram occurred at a wavelength of 460 nm. This would, according to data given in literature<sup>15–18</sup>, indicate the presence of echinenon. The Rf value of the 2nd spot in solvent system of the thin-layer chromatogram also indicates the presence of echinenon in the *Ch. annularius*.

The 3rd fraction of the columnar chromatogram gave a maximum absorption in hexane at a wavelength of 425 and 451 nm which is characteristic of cryptoxanthin<sup>19–22</sup>. The Rf value in solvent system A was 0.69 and in solvent system B 0.62.

In addition, the 5th fraction of the columnar chromatogram was identified from its maximum absorption in hexane. The maximum values were 445 and 475 nm. This would indicate the presence of lutein<sup>23–26</sup> in the eggs of the *Ch. annularius*.

The 4th and 6th fractions of the columnar chromatogram were not identified.

The findings of other authors, usually in investigations on the eggs of Crustacea<sup>27–30</sup>, indicate the presence of carotene and astaxanthin. These carotenoids were not found in the eggs of the mosquito, *Ch. annularius*. It should here be mentioned that Gross and Budowski<sup>31</sup> found cryptoxanthin, ester and  $\beta$ -carotene in the larvae of the *Chironomus* genus.

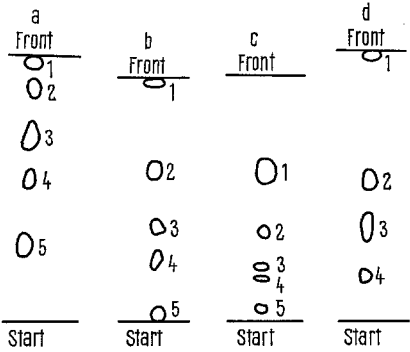


Fig. 2. Thin-layer chromatograms of earotenoids from *Ch. annularius* Meig eggs in systems of solvents A, B, C and D.

*Résumé.* A l'aide de la chromatographie sur colonne et sur couche fine, les auteurs ont effectué la séparation des caroténoides des œufs de *Chironomus annularius* Meig (Diptera: Chironomidae). Ces recherches ont attesté la présence du  $\alpha$ -carotene, de l'échinénone, de la cryptoxanthine et de la lutéine.

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