THE CAROTENOIDS OF THE FRESH-WATER MUSSEL, *ANODONTA CYGNEA*

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The only report available concerning the distribution of carotenoids in fresh-water Lamellibranchs is that of COMFORT[1], who found that the pigments in the egg mass of the gastropod *Pila glauca* were carotenoids: they were not identified.

When specimens of the fresh-water mussel *Anodonta cygnea* became available, it was considered a good opportunity to investigate their carotenoids and thus help to fill one of the biggest gaps in our knowledge of the comparative distribution of these pigments in Nature.

EXPERIMENTAL

Materials. Specimens of *Anodonta cygnea* were obtained from a pond near Guilford (Surrey) and posted, alive, to Liverpool. Four batches were examined.

Extraction of carotenoids. The unsaponifiable matter of the organic tissues of the mussels was obtained according to the methods previously described[2,3,4].

Separation of pigments. The unsaponifiable matter was shaken with light petroleum (b.p. 40–60°). A part dissolved (Fraction A); the insoluble portion was filtered off, washed with a small volume of light petroleum (which was added to Fraction A) and dissolved in benzene (Fraction B).

Fraction A was chromatographed on a mixture of four parts of activated alumina (Spence grade "O") and one part of alumina deactivated with methanol[2]. Five bands separated when the chromatogram was developed with light petroleum containing 20% (w/v) ether. These pigments were provisionally identified as recorded in Table I.

TABLE I

The separation of the petrol-soluble fraction (A) of *Anodonta* unsaponifiable material on a mixture of 4 parts activated alumina and one part deactivated alumina. Developer, light petroleum (b.p. 40–60°) containing 20% (v/v) ether. The zones are numbered in order of increasing adsorptive power.

Fraction	*Description*	*Absorption spectrum maxima, mμ (in light petroleum)*	*Provisional Identification*
1	orange	450, 475	β-Carotene
2	orange brown	450, 475	Cryptoxanthin
3	reddish-brown	452	Echinenone
4	khaki	425	—
5	orange red	451, 475	Originally a mixture, major pigment zeaxanthin

References p. 116.

In different experiments Fraction B was separated on either $CaCO_3$ or $ZnCO_3$, using benzene containing between 1 and 5% (w/v) ethanol. As these adsorbents tend to vary in strength from batch to batch and as the concn. of ethanol is critical, it was found desirable to carry out a small pilot experiment in every case to determine the concn. of ethanol required. Table II indicates the pigment fractions obtained from Fraction B.

TABLE II

The separation of Fraction B of the unsaponifiable matter of *Anodonta* which is soluble in benzene but insoluble in light petroleum. Adsorbent $ZnCO_3$; developer benzene containing 5% (v/v) ethanol. The zones are lettered in order of increasing adsorptive power.

Fraction	*Description*	*Absorption spectrum maxima, (mμ). In benzene*	*Provisional Identification*
A	orange	462, 489	Zeaxanthin
B	yellow	456, 484	Violaxanthin
C	khaki	430	—
D	lemon-yellow	430, 459	Auroxanthin
E	traces of lemon-yellow	—	—

To confirm the provisional identifications suggested in Tables I and II, the various fractions were further purified chromatographically and then compared directly with authentic specimens of the suspected pigments. β-Carotene, cryptoxanthin, zeaxanthin and auroxanthin were obtained from the berries of *Lonicera japonica*[3]; echinenone from *Patella vulgata*[5]; an authentic specimen of violaxanthin was not available.

Quantitative experiments. In three of the batches examined, the relative amounts of the pigments present were determined. Each fraction was made up to a suitable volume in either light petroleum or ethanol and the E values measured at the wavelength of maximal absorption for the pigment under consideration. Assuming $E^{1\%}_{1\,cm}$ (452 mμ) for echinenone to be 2500 and $E^{1\%}_{1\,cm}$ (443 mμ) for violaxanthin to be 2520 and using the values previously recorded for the remaining pigments[2,3], the relative amounts of the constituent pigments could be calculated.

RESULTS

By comparison with authentic specimens from other sources the following carotenoids were found to be present in *Anodonta cygnea*: β-carotene, cryptoxanthin, echinenone, zeaxanthin and auroxanthin. Two pigments adsorbed on the columns as khaki bands (Tables I and II) and exhibiting absorption bands with maxima at 425–430 mμ were not identified. On further purification of Zone 5 (Table I) two pigments were found accompanying zeaxanthin in small amounts. The less tightly adsorbed pigment appeared to be lutein but this could not be confirmed, the other pigment was violaxanthin. Zone B (Table II) was almost certainly violaxanthin, for it had properties identical with those recorded for authentic violaxanthin[6].

TABLE III

The relative distribution of the constituent carotenoids of *Anodonta cygnea*

Pigment	*% of total pigments*
β-Carotene	5.1
Echinenone	3.1
Cryptoxanthin	3.0
Lutein	trace
Unknown	2.5
Zeaxanthin	40.9
Violaxanthin	14.2
Unknown	11.3
Auroxanthin	19.9

References p. 116.

Zone E (Table II), which was itself a small fraction could be further resolved into a series of very small bands all with absorption spectra very similar to auroxanthin; they were not identified.

The relative amounts of the pigments present in *Anodonta* tissues are given in Table III; the values are the means of three determinations. It will be seen that zeaxanthin is the major pigment and that xanthophylls far outweigh β-carotene.

DISCUSSION

The results now obtained on the identification of the carotenoids present in *Anodonta cygnea* show that this animal falls into line with marine lamellibranchs in that it accumulates much greater amounts of xanthophylls than carotenes. Especially does it resemble *Mytilus californicus*[7] in having zeaxanthin as its main pigment.

It differs from the other marine lamellibranchs which have been examined in two main ways (a) it does not synthesize from its alimentary carotenoids new and characteristic xanthophylls such as pectenoxanthin (from *Pecten maximus*[8]), glycymerin (from *Pectunculus glycymeris*[9]) or hopkinsiaxanthin (from *Hopkinsia rosacea*[10]) and (b) none of the marine lamellibranchs so far examined contains 5:8-epoxides such as auroxanthin.

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SUMMARY

The fresh-water mussel *Anodonta cygnea* contains the following caroténoids: β-carotene, echinenone, cryptoxanthin, zeaxanthin, violaxanthin, and auroxanthin. Lutein is also probably present in traces. Two unidentified carotenoid-like pigments were also present.

RÉSUMÉ

La moule d'eau douce (*Anodonta cygnea*) contient les caroténoïdes suivants: le β-carotène l'échinénone, la cryptoxanthine, la zéaxanthine, la violaxanthine et l'auroxanthine. On y trouve probablement aussi des traces de lutéine. Deux pigments de cette moule n'ont pas été identifiés.

ZUSAMMENFASSUNG

Die Süsswasser-Muschel *Anodonta cygnea* enthält die folgenden Carotinoide: β-Carotin, Echinenon, Kryptoxanthin, Zeaxanthin, Violaxanthin und Auroxanthin. Auch Lutein ist wahrscheinlich in Spuren vorhanden. Zwei nicht identifizierte Carotinoid-artige Pigmente wurden ebenfalls aufgefunden.

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