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Contents of Carotenoids in Mammals III. Carotenoids in Specimens of Six Species of *Vespertilionidae*

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With 2 Figures

Examinations corried out on the incidence of carotenoids in several species of bats shortly before and after hibernation showed in some species the presence of carotenoids which are relatively rarely found in animals (CZECZUGA and RUPRECHT 1981). It concerns for instance the presence of rhodoxanthin which is rare carotenoid in animals. In connection with this we decided to examine other species of bats which we could catch. This article is a supplement of data about carotenoids in bats published in the previous paper (CZECZUGA and RUPRECHT 1982) and concerns the presence of these pigments in specimens collected in the middle of summer.

Material and methods

Animals

Examinations were carried out on 9 specimens of bats belonging to the following species: *Barbastella barbastellus* (Schreber, 1774) 1 ♂ ad.; *Myotis brandti* (Eversmann, 1845) 1 ♀ juv.; *Eptesicus serotinus* (Schreber, 1774) 1 ♂ juv., 1 ♂ ad. and 1 ♀ ad.; *Nyctalus noctula* (Schreber, 1774) 1 ♂ juv.; *Plecotus auritus* (Linné, 1758) 1 ♂ ad. and *Plecotus austriacus* (Fischer, 1829) 1 ♂ juv. and 1 ♀ juv.

The specimen of *Barbastella barbastellus* and 2 ♂♂ of *Eptesicus serotinus* were collected in Białowieża voivodship of Białystok on 27 August, 1981 and 28 September, 1981 respectively. The adult ♀ of *Eptesicus serotinus* and the young ♂ of *Nyctalus noctula* were collected in the garret of a forester's lodge in the place Kukawy voivodship of Włocławek on 12 August, 1981. And the ♀ of *Myotis brandti* was collected in the cave Raj near Chęciny, voievodship of Kielce on 8 August, 1981. The other specimens of the species *Plecotus* genus were collected in the garret of the church in Kowal, voivodship of Włocławek on 17–20 August, 1981.

The content of carotenoids was analyzed in the skin, muscles, liver and intestines. Till the moment of the chromatographic analysis the material was kept in the refrigerator.

Pigments

The carotenoid pigments were extracted by means of 95% acetone in a dark room. Saponification was carried out by means of 10% KOH in ethanol at temperature of about 20 °C for 24 hours in the dark in a nitrogen atmosphere.

Chromatography

Columnar and thin-layer chromatography, described in detail in our previous paper (CZECZUGA and CZERPAK 1976) were used for the separation of the various carotenoids. A glass column approximately 1 cm diam. and 15—20 cm in length, filled with Al_2O_3, was used in column chromatography. The extract was passed through the column after which the different fraction were eluated with the solvent.

Silicagel was used for the thin-layer chromatography, with the appropriate solvent systems, the R_f values being determined for each spot. For identification of β-carotene, canthaxanthin, lutein, zeaxanthin, adonixanthin, phoenicoxanthin and astaxanthin co-chromatography was applied using identical carotenoids (Hoffmann — La Roche, Basle).

Identification of Pigments

The pigments were identified by the following methods: a) behaviour on column chromatography; b) absorption spectra of the pigments in various solvents were recorded by a Beckman spectrophotometer model 2400 DU; c) the partition characteristic of the carotenoid between hexane and 95% methanol; d) comparison of R_f on thin-layer chromatography; e) the presence of allylic hydroxyl groups was determined by the acid chloroform tests; and f) the epoxide test.

Quantitative Determinations

Quantitative determinations of the concentrations of carotenoid solutions were made from the quantitative absorption spectra.

These determinations were based of the extinction co-efficient E 1%/cm at the wavelengths of maximal absorbance in petroleum ether or hexane.

Results

13 carotenoids were identified in the analyzed material (Table 1). Such carotenoids as β-cryptoxanthin, lutein epoxide and zeaxanthin were found in all of the examined species, the other carotenoids were found only in some species.

Zeaxanthin was the prevailing carotenoid in the specimens of *Plecotus auritus* whereas lutein in specimens of *Plecotus austriacus*. Concerning the remaining species β-cryptoxanthin prevailed in *Barbastella barbastellus* and *Eptesicus serotinus* and phoenicoxanthin in *Myotis brandti*, and *Nyctalus noctula*. Lutein epoxide amounted to 33.7% of all carotenoids in adult ♀♀ of *Eptesicus serotinus*.

The total content of carotenoids in the examined material ranged from 1.412 µg/g (*Eptesicus serotinus* ♂ juv.) to 10.062 µg/g fresh weight (*Myotis brandti* ♀ juv.). A distinct difference in the total content of carotenoids was observed in specimens of different sex (Fig. 1). Both the young ♀♀ of *Plecotus austriacus* and the mature ♀♀ of *Eptesicus serotinus* included much more carotenoids than the ♂♂ of the same species.

Table 1. Carotenoids content in some species of bats (in % of the total content)

Carotenoids	*Plecotus auritus* ♂ ad.	*Plecotus austriacus* ♀ juv.	*Plecotus austriacus* ♂ juv.	*Barbastella barbastellus* ♂ ad.	*Myotis brandti* ♀ juv.	*Nyctalus noctula* ♂ juv.	*Eptesicus serotinus* ♂ ad.	*Eptesicus serotinus* ♀ ad.	*Eptesicus serotinus* ♂ juv.
1. β-carotene					5.4	9.4	8.0		
2. β-carotene epoxide	4.9								
3. β-cryptoxanthin	5.8	7.1	8.4	33.4	8.6	27.7	30.6	32.6	46.0
4. canthaxanthin	2.3	2.7		6.5		10.5			17.1
5. lutein	9.0	10.1			5.5	2.7	16.0	5.1	
6. lutein epoxide	13.9	66.5	57.6	18.4	18.2	2.5	21.1	33.7	24.8
7. zeaxanthin	48.9	6.2	2.5	25.3	20.0	7.2	trace	3.2	trace
8. adonixanthin			10.2				3.8	3.4	2.4
9. α-doradexanthin	11.4	2.6		16.4	20.0	7.9			3.2
10. phoenicoxanthin					22.3	32.1		15.7	
11. astaxanthin ester		4.8	21.3				15.3	6.3	6.5
12. mutatoxanthin							5.2		
13. β-apo-2′-carotenal	3.8								
Total content in μg/g fresh weight	3.858	4.174	2.633	2.567	10.062	5.417	1.832	2.492	1.412

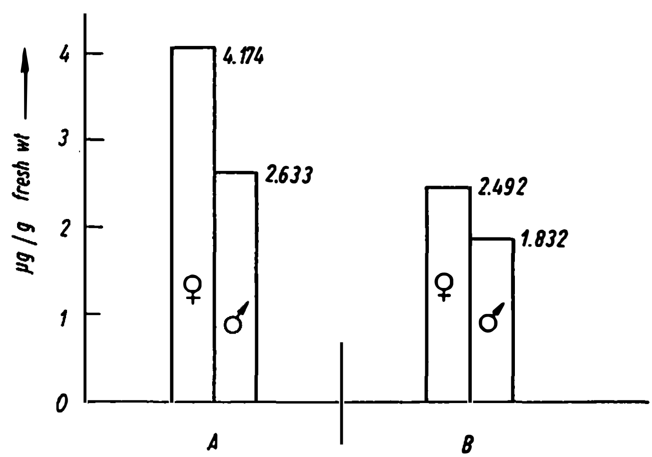

Fig. 1. Carotenoids content in both sexes of *Plecotus austriacus* (A) and *Eptesicus serotinus* (B)

Discussion

Carotenoids found in the examined species of bats except β-apo-2′-carotenal were shown in insects on which bats feed (FELTWELL 1979, CZECZUGA 1976, 1980 a and b). Concerning β-apo-2′-carotenal it was shown up to now in birds (CZECZUGA et al. 1983) and other species of bats (CZECZUGA and RUPRECHT 1982) and arises in process of conversion of β-carotene into vitamin A (BAUERNFEIND 1972).

The initial observation presented in the previous paper (CZECZUGA and RUPRECHT 1982) concerning the total content of carotenoids depending on the sex is confirmed in this paper. It concerns both the young specimens of *Plecotus austriacus* and the adult specimens of *Eptesicus serotinus* as well. The content of carotenoids in adult ♀♀ of *Eptesicus serotinus* is markedly bigger than in ♂♂. Comparing the total content of carotenoids found in the specimens of *Eptesicus serotinus* during spring and autumn (CZECZUGA and RUPRECHT 1982) with results obtained for the same species during summer (the data included in this paper) it should be said that the biggest amounts of carotenoids were found in the specimens from the autumn period and the variety of carotenoids was then the highest. Surely it should be explained by the fact that during the period of activity of bats from spring to autumn the variety of species of insects being their food is changeable. Carotenoids taken with the food accumulate in particular species of bats with the time of the activity and the total amount of carotenoids and their variety also increase. Besides the total content of carotenoids in bats increases with the age e. g. the adult ♂♂ of *Eptesicus serotinus* (Fig. 2) include more carotenoids than

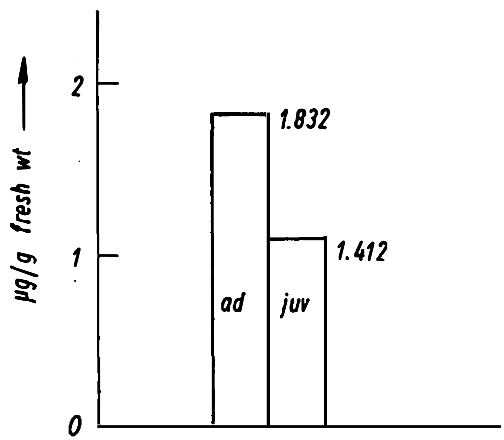

Fig. 2. Carotenoids content in the young and the mature ♂♂ of *Eptesicus serotinus*

the young specimens. A similar phenomenon was observed in some species of fish (CZECZUGA 1982). Perhaps this is the phenomenon of wider importance for species of different systems. That is why the sex, age and season should be taken into consideration when determining the total contents of carotenoids in specimens of different species.

Summary

By means of column and thin-layer chromatography the authors investigated the occurrence of carotenoids in individuals of six following bats species: *Plecotus auritus, Plecotus austriacus, Barbastella barbastellus, Myotis brandti, Nyctalus noctula* and *Eptesicus serotinus.*

The presence of the following carotenoids were noted: β-carotene, β-carotene epoxide, β-cryptoxanthin, canthaxanthin, lutein, lutein epoxide, zeaxanthin, adonixanthin, α-doradexanthin, phoenicoxanthin, astaxanthin ester, mutatoxanthin and β-apo-2'-carotenal.

The total content of carotenoid varied from 1.412—10.062 µg/g fresh weight and the ♀♀ included more carotenoids than the ♂♂.

Zusammenfassung

Unter Anwendung der Säulen- und Dünnschichtchromatographie untersuchten die Verfasser das Vorkommen der einzelnen Karotinoide bei einzelnen Vertretern von folgenden 6 Fledermausarten: *Plecotus auritus*, *P. austriacus*, *Barbastella barbastellus*, *Myotis brandti*, *Nyctalus noctula* sowie *Eptesicus serotinus*.

Anhand der Untersuchungen wurde die Anwesenheit folgender Karotinoide festgestellt: β-Karotin, β-Karotin epoxid, β-Cryptoxanthin, Canthaxanthin, Lutein, Lutein epoxid, Zeaxanthin, Adonixanthin, α-Doradexanthin, Phoenicoxanthin, Astaxanthinester, Mutatoxanthin and β-apo-2'-Karotinal.

Der Gesamtgehalt an Karotinoiden schwankte von 1,412—10,062 µg/g frischer Masse und war bei den ♀♀ höher als bei den ♂♂.

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