Gonadal Pigments of Sea-Cucumber *Stichopus japonicus* Selenka (Echinodermata)

In the previous paper[1], the isolation and identification of crystalline astaxanthin from the gonads (both sexes) of a sea-cucumber, *Holothuria leucospilota* Brandt, were reported by the authors.

The present paper deals with the occurrence of 5 carotenoids from the gonads of the sea-cucumber, *Stichopus japonicus* Selenka. The preliminary examination of an acetone extract of the pigments showed that the maximum absorption occurs within the range of 474 to 476 nm, thus indicating the presence of carotenoids. The ovaries (containing eggs) are deep orange coloured, while the testes milky pale orange. Gonadal pigments (both sexes) were extracted separately with acetone after dehydration with ethanol. The column chromatographic sequence of fraction on alumina (Woelm, neutral, activity grade 1), the solvent systems required for elution, and absorption maximum and relative amounts of each fraction were shown in the Table. The fraction 1 was entirely epiphasic when partitioned between light petroleum and 90% and 95% methanol. On thin layers of silica gel, the pigment was inseparable from authentic β-carotene when co-chromatographed with it. The fraction 2 had a single absorption maximum at 460 nm in light petroleum and the asymmetrical curve characteristic of echinenone. On reduction with sodium borohydride, this fraction was more hypophasic than the parent fraction and had a partition ratio of 9:1 between light petroleum and 95% methanol. The reduction product gave a positive allylic hydroxyl test. When compared on thin layers, the reduction product was inseparable from authentic isocryptoxanthin, so the fraction was identified as echinenone. Fraction 3 showed a single symmetrical curve with a maximum at 467 nm. On partition between light petroleum and 90% methanol, it was mainly epiphasic; but with 95% methanol, it was almost equally distributed between the 2 phases. On reduction with sodium borohydride, this fraction was more hypophasic than the parent fraction and had absorption maxima in light petroleum at 448 and 472 nm with a shoulder at 424 nm. The reduction product (isozeaxanthin) gave a positive allylic hydroxyl test. From the above data of the fraction and its reduction product, fraction 3 was shown to be canthaxanthin. Fraction 4 was a mixture but could not be any further purified. Fraction 5 and 6 were inseparable from authentic zeaxanthin and astaxanthin, respectively, when compared on thin layer plates. An authentic sample of zeaxanthin was isolated from *Cycas revoluta* Thunb.[2] and the one of astaxanthin from star fish (*Asterina pectinifera* Müller et Troschel[3]). The present investigation indicated the presence of such carotenoids as β-carotene, echinenone, canthaxanthin, zeaxanthin, astaxanthin and an unidentified pigment. The same carotenoids pattern described above was also revealed by the investigation of *H. leucospilota* Brandt[4].

Column chromatogram of carotenoids from the gonads of *Stichopus japonicus* Selenka

No. of fraction	System of solvents	Maximum absorption (nm)	Solvent	Identification	% of total	
					Testes	Ovaries
1	1.5% acetone in light petroleum	450, 477	Light petroleum	β-Carotene	3.2	2.4
2	3% acetone in l.p.	460	Light petroleum	Echinenone	8.7	7.3
3	5–8% acetone in l.p.	467	Light petroleum	Canthaxanthin	46.2	35.1
4	30% acetone in l.p.	455	Light petroleum	Unidentified	9.9	16.3
5	50% acetone in l.p.	449,478	Light petroleum	Zeaxanthin	22.9	17.7
6	5% acetic acid in ethyl ether	470	Light petroleum	Astaxanthin	9.1	21.2

Zusammenfassung. In den Pigmenten der Geschlechtsdrüsen von *Stichopus japonicus* Selenka wurden 5 Carotinoide, nämlich β-Carotin, Echinenon, Canthaxanthin, Zeaxanthin und Astaxanthin identifiziert.

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Alterations of Free Amino Acids Concentrations in Cat Brain Induced by Rapid Eye Movement Sleep Deprivation

It has been suggested that the characteristic effects of rapid eye movement (REM) sleep deprived state may be neurochemical in origin[1,2] and various investigations have brought to light certain chemical changes in the brain following such deprivation. These include a fall in acetylcholine level of rat telencephalon[3], a fall in the brain and blood potassium level[4], as well as a substantial fall in the total glycogen of subcortical structures and caudal brain stem[5]. Previously[6,7], amounts of some free amino acids (FAA) were shown to change in response to REM sleep deprivation. The present experiments further examine such response.

Methods and materials. Experiments were carried out on adult male cats. A total of 24 cats were divided into