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

Table I. Effect of REM sleep deprivation upon the concentrations of amino acids in different areas of cat brain

Amino acids (moles/ g wet. wt.)		Brain areas					
		Frontal cortex	Occipital cortex	Caudate nucleus	Thalamus	Hippocampus	Reticular formation (mesenc)
Aspartic acid	N	4.42 ± 0.13[a] (7)	4.69 ± 0.06 (6)	3.59 ± 0.71 (5)	3.24 ± 0.11 (7)	3.30 ± 0.20 (6)	2.69 ± 0.06 (7)
	D	5.57 ± 0.28 (7)	4.44 ± 0.70 (5)	3.15 ± 0.17 (5)	3.96 ± 0.12 (6)	4.22 ± 0.17[b] (6)	4.80 ± 1.72 (6)
	S	4.67 ± 0.38 (6)	4.78 ± 0.19 (6)	3.17 ± 0.40 (6)	3.23 ± 0.15 (6)	2.62 ± 0.27 (6)	2.53 ± 0.40 (6)
Threonine		0.72 ± 0.01 (7)	0.86 ± 0.04 (6)	0.68 ± 0.01 (7)	0.59 ± 0.007 (7)	0.52 ± 0.007 (7)	0.36 ± 0.07 (7)
		0.90 ± 0.07[b] (7)	0.94 ± 0.04[b] (7)	0.70 ± 0.004 (6)	0.74 ± 0.01[b] (6)	0.81 ± 0.01[a] (7)	0.63 ± 0.05[a] (6)
		1.08 ± 0.51 (6)	1.04 ± 0.18 (6)	1.32 ± 0.29[a] (6)	0.71 ± 0.11 (6)	0.78 ± 0.19 (6)	0.49 ± 0.09 (6)
GABA		4.05 ± 0.39 (5)	3.39 ± 0.03 (7)	5.25 ± 0.06 (6)	3.29 ± 0.11 (4)	3.56 ± 0.05 (6)	3.78 ± 0.02 (6)
		5.38 ± 0.12[b] (7)	3.30 ± 0.36 (4)	4.14 ± 0.02[b] (6)	4.32 ± 0.55[a] (4)	3.94 ± 0.81 (6)	4.08 ± 0.02[b] (5)
		4.52 ± 0.14 (6)	3.62 ± 0.22 (6)	5.98 ± 0.41 (6)	3.32 ± 0.35 (6)	3.67 ± 0.65 (6)	3.56 ± 0.30 (6)
Arginine		4.16 ± 0.20 (7)	3.68 ± 0.17 (6)	5.44 ± 1.11 (5)	3.76 ± 0.13 (7)	3.19 ± 0.12 (6)	2.82 ± 0.06 (6)
		5.22 ± 0.12[b] (5)	3.47 ± 0.28 (6)	6.98 ± 0.45[a] (5)	3.55 ± 0.23 (6)	3.64 ± 0.52 (6)	3.04 ± 0.05 (6)
		4.13 ± 0.21 (6)	4.40 ± 0.28 (6)	5.37 ± 0.14 (6)	3.85 ± 0.58 (6)	3.04 ± 0.45 (5)	2.57 ± 0.23 (6)
Glycine		4.23 ± 0.27 (7)	5.09 ± 0.58 (6)	2.99 ± 0.31 (6)	4.48 ± 0.36 (7)	2.71 ± 0.01 (6)	4.11 ± 0.03 (6)
		5.01 ± 0.48 (7)	4.82 ± 0.48 (7)	5.03 ± 0.58[b] (6)	4.32 ± 0.50 (6)	3.91 ± 0.02[b] (6)	7.38 ± 0.47[b] (4)
		4.03 ± 0.35 (6)	4.42 ± 0.14 (6)	2.93 ± 0.18 (6)	4.47 ± 1.70 (6)	2.72 ± 0.64 (6)	4.52 ± 0.17 (6)
Lysine		0.86 ± 0.003 (8)	0.96 ± 0.06 (7)	0.96 ± 0.04 (7)	0.88 ± 0.02 (6)	0.80 ± 0.01 (6)	0.81 ± 0.04 (6)
		1.11 ± 0.06[b] (6)	1.04 ± 0.06 (6)	0.74 ± 0.03[b] (6)	0.68 ± 0.02 (6)	0.77 ± 0.03 (7)	0.68 ± 0.04 (6)
		0.91 ± 0.61 (6)	0.98 ± 0.10 (6)	1.54 ± 0.18[a] (6)	1.00 ± 1.00 (6)	0.80 ± 0.25 (6)	1.32 ± 0.17 (6)
Cysteine		5.58 ± 1.04 (7)	4.80 ± 0.28 (5)	6.28 ± 1.41 (7)	2.95 ± 0.06 (4)	3.39 ± 0.80 (6)	2.87 ± 0.16 (5)
		5.01 ± 0.41 (6)	4.87 ± 2.87 (7)	6.17 ± 0.15 (6)	5.27 ± 0.71[a] (5)	3.72 ± 1.90 (7)	4.36 ± 0.21[a] (5)
		5.69 ± 0.16 (6)	4.80 ± 0.32 (6)	6.60 ± 0.16 (6)	2.75 ± 3.25 (6)	3.38 ± 0.69 (5)	3.05 ± 0.18 (6)

N, normal controls; D, REM sleep deprived animals; S, stress controls. Numbers in brackets are number of experiments. Mean ± standard error of the mean. [a] Significant at $p < 0.05$. [b] Significant at $p < 0.01$ (Student's t-test).

Table II. Concentrations of amino acids in different areas of cat brain which remained relatively constant in REM sleep deprived animals

Amino acids (moles/ g wet. wt.)		Brain areas					
		Frontal cortex	Occipital cortex	Caudate nucleus	Thalamus	Hippocampus	Reticular formation (mesenc)
Glutamic acid	N	12.37 ± 1.27 (7)	12.00 ± 0.53 (8)	11.22 ± 0.02 (6)	9.62 ± 0.35 (6)	9.85 ± 1.63 (7)	6.84 ± 0.90 (6)
	D	12.99 ± 1.54 (7)	12.39 ± 0.74 (7)	11.47 ± 2.84 (6)	10.22 ± 0.78 (4)	11.58 ± 5.57 (6)	6.18 ± 0.46 (5)
	S	12.20 ± 0.29 (6)	11.57 ± 0.18 (6)	10.96 ± 0.86 (6)	9.87 ± 0.35 (6)	9.08 ± 0.97 (5)	5.55 ± 0.26 (5)
Tyrosine		4.44 ± 0.44 (6)	3.57 ± 0.17 (7)	3.27 ± 0.27 (7)	2.70 ± 0.08 (7)	3.78 ± 0.04 (7)	2.09 ± 0.24 (6)
		4.03 ± 0.56 (7)	4.04 ± 0.57 (7)	3.69 ± 0.36 (6)	2.89 ± 0.18 (6)	3.97 ± 0.36 (7)	2.51 ± 0.20 (6)
		4.85 ± 0.19 (6)	3.58 ± 0.13 (6)	4.61 ± 0.14[b] (6)	3.22 ± 0.35 (6)	3.61 ± 0.66 (6)	2.15 ± 0.69 (6)
Histidine		0.85 ± 0.004 (6)	0.87 ± 0.02 (6)	0.78 ± 0.01 (6)	0.66 ± 0.02 (6)	0.61 ± 0.004 (6)	0.41 ± 0.004 (6)
		1.09 ± 0.03 (6)	0.94 ± 0.22 (6)	0.93 ± 0.09 (6)	0.48 ± 0.02 (6)	0.58 ± 0.01 (6)	0.57 ± 0.07 (6)
		1.06 ± 0.71 (6)	0.93 ± 0.16 (6)	1.44 ± 0.15[a] (6)	0.93 ± 0.20 (6)	0.71 ± 0.10 (5)	0.51 ± 0.19 (5)
Serine		2.37 ± 0.20 (7)	1.69 ± 0.03 (7)	1.94 ± 0.05 (8)	1.62 ± 0.09 (7)	1.86 ± 0.13 (7)	1.65 ± 0.09 (6)
		2.33 ± 0.09 (7)	2.40 ± 0.32 (7)	2.14 ± 0.11 (6)	1.57 ± 0.07 (6)	2.19 ± 0.20 (7)	1.98 ± 0.18 (6)
		2.38 ± 0.12 (6)	1.69 ± 0.24 (6)	1.70 ± 0.28 (6)	1.69 ± 3.75 (6)	1.77 ± 0.29 (6)	1.25 ± 0.11 (6)

N, normal controls; D, REM sleep deprived animals; S, stress controls. Numbers in brackets are number of experiments. Mean ± standard error of the mean. [a] Significant at $p < 0.05$. [b] Significant at $p < 0.01$ (Student's t-test).

3 groups: 1. normal animals, 2. REM sleep deprived animals and 3. control animal, to control the stress involved in the experimental technique of PS deprivation. 72 h long REM sleep deprivation was carried out according to the technique of JOUVET[8]. A control group of animals was maintained in the same conditions as the experimental one, but the water was removed for 8 h a day, at which time the cats could sleep and obtain REM sleep. This procedure was repeated for 5 successive days. Thus, the major difference between experimental (REM deprived) and stress control group is the amount of REM sleep, while stress is presumed to be equal. At

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the end of the experimental proceedings, the animals were killed by guillotine, the heads being dropped into liquid air and brains removed. After the fresh weight determination, the following regions of brain were dissected: frontal cortex, occipital cortex, hippocampus, caudate nucleus, thalamus and mesencephalic reticular formation. Quantitative analysis of amino acids was carried out chromatographically, using the technique described elsewhere[6,7]. No difference in body weight or in wet brain weight between REM sleep-deprived and control animals was discovered.

Results and discussion. As shown in Tables I and II, the FAA examined were present in different concentrations, lysine threonine and histidine having the lowest levels, while aspartic and glutamic acids the highest. Individual FAA varies to a lesser degree from one region to another. Our results on GABA concentration in cat are higher than those obtained by FAHN and CÔTÈ[9] in Rhesus monkeys. However only 4 regions could be compared: frontal and occipital cortices, thalamus and nucleus caudatus. The difference in results in GABA levels can be explained probably on the basis of species differences rather than assay methods.

From the 11 amino acids observed, 7 changed significantly in certain brain regions under the effect of REM sleep deprivation. The aspartic acid concentrations were significantly elevated in the hippocampus as well as threonine in the frontal cortex and thalamus, arginine in the frontal cortex, glycine in the nucleus caudatus, hippocampus and mesencephalic reticular formation, and lysine in the frontal cortex and nucleus caudatus. The concentration of glutamic acid, tyrosine, serine and histidine underwent no changes in brain structures examined (Table II). The largest changes occurring in GABA showed significant decrease in nucleus caudatus and increase in the frontal cortex and mesencephalic reticular formation. The relatively short REM sleep deprivation produced alterations in the concentrations of several amino acids in various brain regions. The most frequent changes were observed in GABA and threonine concentrations. The fact that the amount of glutamic acid, as precursor of GABA in brain, remained unchanged in the structures where the amount of GABA was increased, or decreased, suggests that, inspite of the metabolic relations between those two amino acids, REM sleep deprivation may create a condition where they are independantly regulated. The involvement of GABA and glutamic acid in the control of states of vigilance has been shown by JASPER, KHAN and ELLIOT[10]. It is of interest to emphasize that 2 acids which showed significant changes in concentrations, i.e. glycine and GABA, were considered as the inhibitory transmitter agents[11]. The fall of GABA concentrations within nucleus caudatus, and aspartic acid increase in frontal cortex and hippocampus may be involved in increased neural excitability associated with REM sleep deprived state[12]. The present results could only suggest the possible amino acids participation in neurochemical mechanisms governing the states of vigilance.

Résumé. La concentration de certains acides aminés libres au niveau du cerveau chez le chat change sous l'influence de la privation élective du sommeil paradoxal. Ces changements pourraient s'expliquer par l'augmentation d'excitabilité nerveuse associée à la privation élective du sommeil paradoxal.

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Transport of Cortisol by Cultured Chronic Lymphocytic Leukemic Lymphocytes

It was reported recently that certain cultured mammalian cells have the ability to actively extrude cortisol[1]. Human lymphocytes do not concentrate cortisol above the external concentration[2], and, since these cells have specific cortisol receptors[3], the possibility that lymphocytes too, have a similar energy-dependent cortisol extrusion process, seemed worthy of investigation. Furthermore, since results from our previous studies[4,5] indicate that the presence of plasma was required in order for cortisol to have an inhibitory effect on the synthesis of lymphocyte protein, the possible influence of plasma on the uptake of cortisol was also studied.

Materials and methods. The preparation of lymphocytes suspended in autologous plasma or saline has been previously described[4-6]. 0.6 ml of lymphocytes (10^7 cells) were cultured at 4 or 37 °C in 2 ml of TC 199 medium containing 1 Ci of ^{3}H-Hydrocortisone (New England Nuclear Corp., 9 C/m*M*) for the periods of time stated in the text. At the end of each incubation period 10 ml of TC 199 medium whose temperature corresponded to that of the culture, were added and the cultures centrifuged at $1500 \times g$ for 4 min. The culture tubes were quickly inverted, drained carefully with the aid of cotton swabs, the cell button suspended in 0.5 ml of saline, the contents transferred to scintillation vials, the tubes washed 5 times with 3 ml of Brays scintillant and the washings transferred to the vials. The activities of each vial were determined with the aid of a Picker Ansitron II Liquid Scintillation Counter (efficiency of 53% for tritium) and each activity was corrected for background by subtracting the CPM of identically treated blank cultures. Quenching was found to be negligible and hence no further corrections were necessary.

Results. The uptake of cortisol by chronic lymphocytic leukemic lymphocytes (CLL) cells cultured at 4 and 37 °C

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