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THE STUDY OF ASTAXANTHIN TRANSFORMATION INTO VITAMIN A IN THE ALBINO RAT:

IN VITRO EXPERIMENTS

by

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It is traditionally thought that the only substances in mammals which display vitamin A properties are those with an axerophthol group formed by the union of a β-ionion nucleus and an iso-prenic chain, i.e. a potent molecule of vitamin A. Carotenoid pigments, with 40 atoms of carbon in which two nuclei are oxygenated, such as zeaxanthin, xanthophyll etc. are deprived of vitamin A activity.

However, astaxanthin, 3.3'-dihydroxy 4.4'-diceto-β-carotene, the main carotenoid pigment in crustaceans, constitutes a singular exception in this regard. Administered to the vitamin A deficient white rat, this pigment manifests a notable antixerophthalmic activity, however, it is distinct from other vitamin A factors since significant weight gain is only observed with doses much higher than those which can heal ocular lesions (2) (3) (1). In addition, regardless of the administered pigment dose, serious disorders in the reproduction functions are noticed: males become sterile and, while females can still be impregnated, gestation is profoundly disturbed (miscarriages, still-births [9]).

Nevertheless, research conducted at the same time in fish, should show that the latter are able to use astaxanthin as a vitamin A precursor. In fact, the administration of the pigment to *Gambusia holbrooki Grd*. leads to a neoformation of retinol which is detectable in the intestinal mucosa, the liver and the eyes (5) (4) (7) (12).

Examination of this data set suggests that the rat may have the same ability but only in a localized manner and in a quantitatively-limited manner. This hypothesis, subjected to experimentation, received an initial confirmation (6) (10): after administration of astaxanthin diacetate, the rat was incapable of creating pigment reserves but the content of retinol in the retina was notably increased. In vitro experimentation shows that the ocular tissue performs the astaxanthin to vitamin A conversion.

This research was pursued in order to specify both the chemical and tissue specificity. Comparatively, astaxanthin acetate, β-carotene and xanthophyll dipalmitate were incubated with retinal, intestinal mucous and adrenal tissue, successively, from deficient rats. The extraction of astaxanthin, the preparation of diacetate and the purity controls were previously described as well as the preparation of animals and the incubation techniques (10).

Animals were sacrificed by decapitation, three days after the appearance of the deficiency symptoms (weight stabilization and beginning of xerophtalmy). The organs were immediately removed.

The eyes (except for the first experiment which was performed on the intact ocular globe) were vertically sectioned in order to only preserve the retinal region which was immediately immersed in the incubation liquid. In order to eliminate any error due to individual variations, two lots were created by combing the right eye segments of half of the subjects (seven) with the left eye segments of the remaining animals and vice versa, the incubation liquid of the first lot being the only lot diluted with carotenoid.

For the creation of the adrenal lots, the same precautions were observed.

The intestines were washed using 9% physiological serum and then cut into 1 cm sections. Each of the two lots was composed of six alternate sections.

In all the experiments, the incubation time at 37C was uniformly kept to 3 ½ hours except for the first (whole eyes) which were kept for 12 hours.

At the end of this time, the tissue fragments from the various lots were saponified in accordance with the Lewis, Bodansky, Falk and MacGuire technique (8). The unsaponifiable [tissues] were treated with petrol ether. After washing and evaporation in a nitrogen atmosphere under reduced pressure, the residue was dissolved in chloroform. The search and dose of vitamin A were performed using the Carr and Price reaction in accordance with the Meunier and Raoul technique (11).

The results are recorded in the following table:

Incubated Organ	Carotenoid Addition	Vitamin A in µg
Whole eyes	Astaxanthin diacetate	1.65
	0	0.75
Retinal region of eyes	Astaxanthin diacetate	1.0
	0	0.3
Retinal region of eyes	β-carotene	0.8
	0	Traces (*)
Retinal region of eyes	Xanthophyll dipalmitate	Traces (*)
	0	Traces (*)
Adrenal [tissue]	Astaxanthin diacetate	0
	0	0
Adrenal [tissue]	β -carotene	Traces (*)
	0	0
Adrenal [tissue]	Xanthophyll dipalmitate	0
	0	0
Intestines	Astaxanthin diacetate	0
	β -carotene	Traces (*)
	0	0
* Non-dosable		

It clearly appears that, in the rat, it's the retinal tissue which alone has the ability to convert astaxanthin into retinol. The absence of any xanthophyll transformation also highlights the fact that, in accordance with the traditional data, this property does not extend to all carotenoids whose two cycles are oxygenated.

The limitation of this ability to convert astaxanthin in the retinal tissue indicates the essentially antixerophthalmic activity which this carotenoid manifests in the rat. Vitamin A forms in local quantities which are not sufficient to allow its passage into circulation, the action on the general status is, at least partially abolished, from where the dissociation observed in the pigment's vitamin activity.

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DISCUSSION

Mr. Raoul. – I observed that the retina is added to already-known areas where a double-type carotenoid is transformed into a single-type: intestinal mucosa and liver where the marked β-carotene was used for this trial. Recent work with marked β-carotene has confirmed the effectiveness of the hepatic parenchyma. There are, therefore, many possibilities.

Mr. Matet. – 1. Did the author find alcohol vitamin A or retinal vitamin A in the eyes of the rats which received astaxanthin?

Ms. Massonet. – In the examined animals, it was retinol which was found.

Mr. Matet. – 2. In the same animals, was unaltered astaxanthin found, especially in the liver?

Ms. Massonet. – In the animals treated with astaxanthin, the pigment was found, in trace amounts, in the eyes and thyroids. The pigment was missing in the other organs examined and, in particular, it was never detected in the liver.

Mr. Grangaud. – The 1948 publication of the observation of the exclusively antixerophthalmic properties of penaeid oils was generally and naturally received with skepticism. However, Paul Meunier agreed with our results and encouraged us to continue. Also, the completion of this work which has just been presented to you is, in my opinion, an homage to his memory and I am happy to declare it here in front of many of his closest friends and collaborators.