TITLE: Antixerophthalmic Activity of the Carotenoid Pigment of the Aristeomorpha foliacea (Penæidæ)
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CHEMICAL BIOLOGY – Antixerophthalmic Activity of the Carotenoid Pigment of the Aristeomorpha foliacea (Penæidæ). Note by Mr. René Grangaud and Ms. Renée Massonet, presented by Mr. Maurice Javillier.

Astaxanthin, present in the oil of *Aristeomorpha foliacea*, behaves like vitamin A whose antixerophthalmic activity is much more marked than the action on growth.

We have shown ([1]) that the antixerophthalmic activity of oil from the shrimp, *Aristeomorpha foliacea*, is much more acute than the action on the deficient white rat's growth can predict. The dissociation between these two characteristics, whose coexistence is considered to be specific to vitamin A activity, led us to admit that "*It is, therefore, likely that, in this oil, there is a constituent, other than the carotenes and vitamin A, which play a role in the antixerophthalmic activity.*"([2]). The study of summer and winter oils also show that the first, deep red, are active, the second, light red with more yellow, were practically devoid of activity ([3]). These results suggest that the antixerophthalmic principle could be identified in the red carotenoid pigment of the summer oil. We isolated this pigment in the astacine state (β-carotene-3,3',4,4'-tetrone)([4]). Nevertheless, the differences between the chromatograms obtained before and after saponification and the fact that the addition of potassium to the oil solution in ethanol caused the appearance of a violet tint which turned to orange, showed that the pre-existing pigment is probably not astacine but its precursor, astaxanthin (3,3'-dihydroxy-ß-carotene-4,4'-dione)([5]) which exists in many crustaceans in the form of esters or in association with proteins([6]).

In a first test, the oil was saponified by 15% alcoholic potassium for four hours at 20°C. With the addition of water and petrol ether and storage in the refrigerator, the major portion of pigment gathered at the surface of separation of the two liquids in the form of red flakes. After separation and washing with 50% alcohol, the latter was mixed with a de-vitaminized vegetable oil equal to the volume of saponified oil. The preparation was administered to vitamin A deficient rats: even at a dose of 90 mg per

(1) *Comptes rendus,* 227, 1948, p. 568.
(2) 5yn. : *Penoeus foliaeeus,* Risso.
(3) R. GRANGAUD, C. CHÉCHAN et Mlle R. MASSONET, C. *R. Soc. Biol., 143,* 1949, p. *1179.*
(4) R. GRANGAUD, C. CHÉCHAN et Mlle R. MASSONET, *C. R. Soc. Biol.* (Alger, séance du 16 mars 1950).
(5) P. KARRER et E. JUCKER, *Carotino;;de, Verl. Birehauser* (Basel, 1948), p. 244.
(G) G. WALD, *Vitamins and Hormones (Academie Press,* New-York, 1, 1943 p. 213).

animal per day, with the exception of a slight, transitory improvement in the ocular infection in the two subjects, there was no detectable action on the development of the signs of deficiency. This result is in agreement with the traditional data: astacine does not present any vitamin A activity.

Under the assumption that natural carotenoid might behave differently, a new test was conducted by administering separated pigment to the deficient animal not by oil saponification but by aluminum oxide chromatography. 2 g of oil were dissolved in 200 ml of petrol ether. The solution was slowly poured and ingested into an aluminum oxide column of 20 cm in height and 2 cm in diameter. The pigment was kept in the upper part of the column. After development using petrol ether (200 ml), elution was performed by agitation of the colored aluminum oxide with petrol ether diluted with 1% methanol. The eluate was separated, then diluted with 1 ml of de-vitaminized oil containing 2 mg of α-tocopherol then the solvent was flushed in an inert atmosphere under reduced pressure. The residual oil ([7]) was administered under the same conditions as previous experiments ([1]) to vitamin A deficient white rats. At a daily dose of 40 mg, healing from xerophthalmic lesions was obtained in less than ten days; the improvement was undisputable on the third day. The action on the weight gain was, at that dose, practically null and the animals died within the three to four weeks after start of treatment.

Although chromatography was not able to obtain the pigment in its pure state, it is unlikely that the antixerophthalmic factor was distinct: in fact, it must be admitted that it involved a *colorless* substance present in the summer oil at the same time as the pigment, absent in that form in the winter oil, absorbed in the aluminum oxide at the same level and eluated by the same solvent. On the other hand, the disappearance of the activity after saponification indicates the nature of the active principle by confirming that, in the Aristeomorpha oil, the pigment is not astacine but astaxanthin in the form of esters ([8]). Our results are to be compared with those of Wald and Zussman ([9]) who observed the presence of these carotenoids in the retinas of certain birds, thus providing indication of its role in the visual process ([10]).

Consideration of this data leads to the conclusion of astaxanthin as

(7) Pigment concentration is substantially equal to that of the initial oil.
(8) The administration to deficient rats of Aristeomorpha eggs (where astaxanthin is protein-bound) crushed and suspended in devitaminized vegetable oil is followed by no attenuation of xerophthalmic lesions: the ease with which non-esterified astaxanthin is oxidized to astacin probably provides the explanation for this failure.
(9) *J. biol. Chem.,* 122, 1938, p. 449.
(10) R. A. MORTON, *The Application of Absorption Spectra to the Study of Vitamins, Hormones and Coenzymes,* London, 1942.

a new Group A vitamin, being distinguished from other factors in this group by the predominance of the antixerophthalmic effect on the growth rate.