TITLE: Antixerophthalmic effect of the esters of astxanthin
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While performing research on carotenoid pigments of penaeid shrimp (1), we specified the chromatographic behavior of astaxanthin and its esters: when filtered in an aluminum oxide column (2) of 20 cm height and 2 cm diameter, an etheropetrolic solution of a hepatopancreas oil or of a hypodermic extract of *Aristeomporpha foliacea* which contains free and esterified astaxanthin in variable proportions (3), the pigment is adsorbed in the upper portion of the column. After petrol ether processing, a chromatogram, having five zones from top to bottom, is obtained: the pigment in the upper zone is pink with 1 cm of thickness and is composed of free astaxanthin as its hypophasic nature confirms: the pigment in the remaining four zones below it is a mix of epiphasic esters. In the extracts which were studied, we were never able to detect vitamin A nor carotenes (4). We have, however, recently verified that the chromatographic behavior of these factors is completely different from that of astaxanthin and its esters (5): Free vitamin A is located on the aluminum oxide column below the astaxanthin esters, the vitamin A esters affix to the lower portion of the column, the carotenes slowly traverse the aluminum oxide and become part of the residue. These results, which are in agreement with Lederer (6) and those of Kon and colleagues (7), show that aluminum oxide chromatography alone separates the free and esterified astaxanthin from the free vitamin A, its esters and its carotenes if, despite our chemical and spectrophotometric controls, it is admitted that (8) the latter may be present in the extracts which we studied. On the other hand, since free vitamin A is hypophasic, as is free astaxanthin, by agitating their ethero-petrolic solution with ethanol at 85°, both vitamin and astaxanthin pass into the alcoholic phase.

We used all of these properties to prepare an oily solution of astaxanthin esters which we administered *per os* of vitamin A deficient rats. The preparation technique described below is such that, in all objectivity, it would be hard to invoke the possible intervention of free or esterified vitamin A and its carotenes to explain the antixerophthalmic activity of the studied extracts.

The 1 g of hypodermic ethero-petrolic solution (200 cm^3) of the pigment obtained while monitoring the previously- described operational method (1) was agitated with an equal volume of ethanol at 85°. This treatment, repeated five times, separates the free astaxanthin (at the same time as it eliminates the free vitamin A if the latter is present). The separated epiphasic solution was washed with water to eliminate the alcohol which was dissolved in the petrol ether. It is dried on anhydrous sodium sulfate and then chromatographed on an aluminum oxide column of 20 cm in height and 2 cm in diameter. The chromatogram is developed with 200 cm^3 of petrol ether and, after having separated the pigmented zone corresponding to the astaxanthin esters, it is then eluted by agitation with petrol ether diluted with 5 parts per 100 methanol. The eluate was washed with water, dried on anhydrous sodium sulfate then diluted with 1 g of devitaminized vegetable oil containing 27 mg of α-tocopherol as an anti-oxidant. While flushing the solvent with reduced-pressure distillation in an inert atmosphere, an oily solution of astaxanthin esters which cannot contain vitamin A (free or esterified) nor carotenes was obtained. It is this oily extract, which contains approximately 250 micrograms of astaxanthin per gram of oil, which, at the dose of 80 mg of such oil per animal per day, was administered to vitamin A deficient white rats.

Eleven Wistar rats, which we raised, were deficient when they were weaned. The signs of deficiency are manifested within the usual period of time (between 40 and 45 days of deficiency) by weight-stabilization soon followed by the appearance of xerophthalmia. The weight curve being on a plateau for 15 days and the xerophthalmia being clearly exhibited, the animals were distributed into 2 lots: seven of them (2 males and 5 females), received *pro die* in addition to the deficient regime, 80 mg of oily solution of astaxanthin esters; the other four served as controls (3 males, 1 female), received the deficient regime and 80 mg of devitaminized vegetable oil diluted with 28 mg of α-tocopherol per gram.

Among the control animals, the development of the deficiency was manifested by the growing intensity of xerophthalmia lesions, weight loss, and death between the 68^{th} and 87^{th} day of deficiency. At the same time, among the treated animals, the ocular lesions were regressing, the cornea were becoming perfectly healthy, healing was achieved between the 12^{th} and 15^{th} day of treatment. In parallel, among two of them, the weight curve was stable; in a third, which had an enormous abscess in the neck, a weight loss of 8 g was measured on the 23th day. Finally, among the other four, there was a slight gain in weight.

In summary, in the vitamin A deficient white rat, with a stable weight curve and a fully-developing xerophthalmia, the administration of an oily solution of astaxanthin esters led, within a few days, to the complete healing of the ocular lesions, the weight gain was minimal or non-existent. These results are in agreement with those which we published previously (9). The preparation method for the administered extract does not allow invocation of the participation of the vitamin A or carotenes to explain the observed vitaminic activity.