Title: Transformation of astaxanthin to vitamin A in the albino rat: neoformation in vivo and in vitro.

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PHYSIOLOGY – Transformation of Astaxanthin into Vitamin A in the Albino Rat: Neo-formation in vivo and in vitro. Note (*) by Mr. René Grangaud, Ms. Renée Massonet, Madams Thérèse Conquy and Jacqueline Ridolfo, presented by Mr. Robert Courrier.

In deficient rats, the *per os* administration of astaxanthin diacetate causes neoformation of vitamin A which is detectable in the eye. *In vitro* experimentation can be used to specify that this organ is the seat of the enzymatic transformation which converts astaxanthin to retinol.

In vitamin A deficient albino rats, astaxanthin (3,3'-dihydroxy-4,4'-diketo-β-carotene) manifests a decidedly antixerophthalmic activity. In fact, the daily administration of 10 to 15 μg of pigment heals ocular lesions rapidly without resulting in weight increase which is only observed with much greater dosages [(1) to (4)]. In fish, however, astaxanthin is an authentic provitamin A because, in *Gambusia holbrooki*, it leads to a neoformation of retinol which is particularly detectable in the intestinal mucous [(3), (6), (7)]. Confrontation of these facts suggests that the rat might have preserved the restricted ability, in the eye, of converting astaxanthin into vitamin A. This is, at least, the hypothesis which was subjected to experimental examination.

In a first experiment, some astaxanthin diacetate was administered to vitamin A deficient rats which were then sacrificed at the same times as control rats and vitamin A was administered, in parallel, in the eyes of each category of subjects. In a second experiment, the eyes of deficient animals were divided into 2 lots: the first lot was incubated in the presence of astaxanthin diacetate, the second was treated in the same manner but without any addition of pigment; the respective concentrations of retinol were then determined.

ASTAXANTHIN DIACETATE PREPARATION – The inner walls of the stomach pockets of *Aristeomorpha foliacea,* Risso and *Aristeus antennatus*, Risso (jumbo red penaeid shrimp) contain a blue chromoprotein whose prosthetic group is astaxanthin [(4)]. After dissection, the pockets were emptied of their contents and agitated with distilled water in which chromoprotein was dissolved. The solution was then diluted with four times its volume of acetone: the astaxanthin was detached from its protein copula and the solution turned an orange-red. With the addition of petrol ether and water, the pigment passed into the light phase which was separated and then dried on anhydrous sodium sulfate. The solution was filtered in a magnesium oxide column which was then washed with petrol ether, then with hexane diluted with 2% volume acetone (elimination of vitamin A carotenes and

esters) and, finally, with a mix of 92 ml of hexane and 8 ml of ethanol (elimination of the retinol). The column was then sectioned, the pigmented zone was immersed in pyridine which, by elution, turned red. The concentrated solution under reduced pressure and under nitrogen was diluted with acetic anhydride (XX drops/ml). After 9 hours at ambient temperature, the diacetate was then treated by petrol ether. The solution was washed, dried and chromatographed on aluminum oxide. The column was processed similar to the magnesium oxide. The pyridinic solution which was obtained was diluted with petrol ether and water. The light phase was separated, dried and concentrated. The residue was treated with hot pyridine, diluted by a third volume of water and left for 24 hours at 0°C. The crystalline precipitate was purified by two re-crystallizations.

PROTOCOL AND RESULTS. – a. In vivo experiment – 18 Wistar rats, weighing 32 g, were weaned and subjected to the base regime which was free of all vitamin A factors. After 40 days, the signs of deficiency were manifest, the animals were divided into 3 lots which received the following daily doses:

Lot A: traces; lot B: 1 µg; lot C: 2.1 µg.

b. In vitro experiments. – 12 deficient rats, under the same conditions as the preceding, were decapitated on the 40th day. 1 ml of blood serum and 1 mmole of α-tocopherol dispersed in 1 ml of water was placed into two small colloidion bags. The right eyes of 6 subjects and the left eyes of the other 6 subjects were placed into one of the bags (Lot I).; The 12 remaining eyes and 1 mg of astaxanthin diacetate in dispersion in 1 ml of Tween 80 were placed into the other bag (Lot II). The bags were immersed into a buffered Krebs-Ringer solution and kept in an oven at 37°C for 12 hours. The dosage of vitamin A produced in the unsaponifiable [tissues] of all the eyes of each lot provided the following values:

Lot I: 0.75 µg; Lot II: 1.65µg.

Discussion. – A spectrophotometric study of the pigment solution before chromatography did not reveal any vitamin A nor carotene. If traces of these substances had, however, escaped examination, they would have been eliminated by the washings of the columns performed for that purpose in accordance with (10). Re-crystallization of the astaxanthin diacetate constitutes a final purification. The following control excludes the interference of impurities: under the same conditions as that in the *in vitro* experiment, two lots of deficient rat intestinal fragments were incubated, 1 lot in the presence of β-carotene and the other in the presence of astaxanthin diacetate. Neoformation of vitamin A was only detected in the first lot.

Thus, in the experimental conditions described, *in vivo* as well as for *in vitro*, neoformed and detected vitamin A in the eye can only be due to astaxanthin transformation. Additional experiments will try to determine whether, since it is likely, it is the retina which performs the enzymatic conversion.

Conclusion. – The recorded results verify the working hypothesis which dictated these experiments: in vitamin A deficient albino rats, the administration of astaxanthin diacetate causes neoformation of retinol in the eye. The *in vitro* experimentation led to the same results and also localized the reaction seat to the ocular tissue.

(*) Meeting of 13 March 1961.
(1) R.Grangaud and R. Massonet, C.R.Acad.Sc., 1948, v.227, p.568.
(2) R.Grangaud and R. Massonet, C. R. Acad. Sc., 1950, v.230, p.1319.
(3) R.Grangaud, Doctoral Thesis in Physical Sciences, Lyon, 1950.
(4) Mlle R. Massonet, Doctoral Thesis in Natural Sciences, Lyon, 1958.
(5) R.Grangaud and R. Massonet, C.R.Acad.Sc., v.241, 1955, p.1087.
(6) R.Grangaud, P.Vignais, Mlle R. Massonet, and J.P.Moatti, C.R.Acad.Sc., v.243, 1956, p.1170.
(7) J.P.Moatti, State Doctoral Thesis (Pharmacy), Algiers, 1959.
(8) 2% d.α-tocopherol is added to a solution of devitaminized vegetable oil.
(9) J.M.Lewis, O.Bodansky, K.G.Falk and G. McGuire, *J. Nutrition, v.*23, 1941, p.351.
(10) S.Y.Thompson, J.Ganouly and S.K.KON, *Brit. J. Nutr.,*v.3, no.1, 1949, p. 57.

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