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CAROTENOIDS AND THEIR IMPORTANCE IN THE NUTRITION OF FISH AND CRUSTACEANS

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Carotenoids are quantitatively very prominent among the substances which render colour - Biochrome. There are an immense array of carotenoids in the animal kingdom and so also in the plants. They are present predominantly in the eye spot of the protozoans, in the body of coelentrates, sponges and other organisms. The only clear cut physiological function they perform is as precursor for vitamin A. Apart from this though many a varied roles ascribed to them in the physiology of the organism are highly speculative in nature. We are at the dawn of revolutionary findings pertaining to the function of carotenoids.

BIOCHROMES

The following group of biochemics are collectively known as biochromes - 1. Carotenoids, 2. Quinones, 3. Flavonoids, 4. Flavins, 5. Tetrapyrroles, 6. Pterins and 7. Indole pigments. The first four are synthesised *de novo* only by plants of which the first three contain only carbon, hydrogen and oxygen while the fourth in addition nitrogen. Fifth and sixth also contain nitrogen and could be synthesised by both plants and animals. The last viz., indole pigments are melanins and indigoids which are synthesised by the animals by catabolic degradation of essential amino acids tyrosine and tryptophan. Of these carotenoids are the most diverse and occur in very high quantity.

CAROTENOIDS

Carotenoids are fat soluble lipochromes and can be divided into two groups. 1. Carotenes, which are carotenoid hydrocarbons lacking in oxygen ie. are made of carbon and hydrogen only. 2. Xanthophylls, which are oxygen containing alcohol soluble, non-acidic, non-saponifiable carotene derivatives. The xanthophylls are classified as follows (Fox, 1976).

1.	Carotenoid alcohols	-	carotenols
2.	" ketones	-	carotenones
3.	" alcohol ketones	-	carotenolones
4.	aldehydes	-	carotenols
5.	ethers	-	caroten - ethers
6.	Esters of carotenoid alcohols	-	carotenol esters
7.	Carotenoid acids and their esters.		

In Xanthophylls suffix 'Xanthin' is added while for carotenes 'carotene'. Eg: Astaxanthin, β- carotene, tunicarotene.

Carotenoids are present in the organisms in two types of complexes, viz., carotenoproteins - i) pigment is bound to the protein in stoichiometric amounts in non-covalent linkages and ii) dissolved in lipoprotein or lipoglycoprotein component.

The central dogma of carotenoid biochemistry is that animals cannot synthesise the pigments <u>de novo</u> but can only alter the molecules by oxidation, as in the conversion of β-carotene into astaxanthin, or, if the structure is appropriate, by central fission to form vitamin A. Though the first part of the dogma still holds for the latter part 2 new metabolic activities have come to our knowledge as exceptions. i) Conversion of β-rings into ε-rings in birds and ii) a change of chirality as in the formation of 3' - epilutein from lutein in fishes (Goodwin, 1984).

Though animals lack the capacity to synthesis carotenoids from protozoa to mammalia they contain varieties of carotenoid molecules acquired through food web. It is of interest that both animals and plants put together have more xanthophylls, while the marine and freshwater mud contain higher amounts of carotein than xanthophylls. Fox (1979) divides the organisms into the following 5 categories based on their carotenoid nutrition.

1. Carotene selectors: These animals when provided with both forms of carotenoids in diet are able to assimilate hydrocarbon type of carotenes, while defaecate xanthophylls completely. Typical examples are horse and cattle. The marine detritus eating polychaete Euzonus mucronata assimilate and store β-carotene only. The parasitic crustacean Sacculina carcina too assimilate and store in considerable quantity only β- carotene (Fox, 1979).

2. Carotenoid rejectors: There are also animals which assimilate little of carotenoids except for visual pigments from carotenoid rich diets. The examples are sheep, goat, swine and also most strains of rabbits. The hag fish (cyclostome) Eptatretus stoutii, the mako shark Isurus glaucus and the chimaerid Hydrolagus sp. are also carotenoid rejectors.

Xanthophyll accumulators: These organisms store no carotenes but assimilate alcoholic, hydroxylated and ketonic carotenoids. They convert only a little carotene ingested into vitamin A, just sufficient for their needs. Otherwise dispose off all carotenes in faeces. The examples are domestic fowl, most of the fishes and many invertebrates.

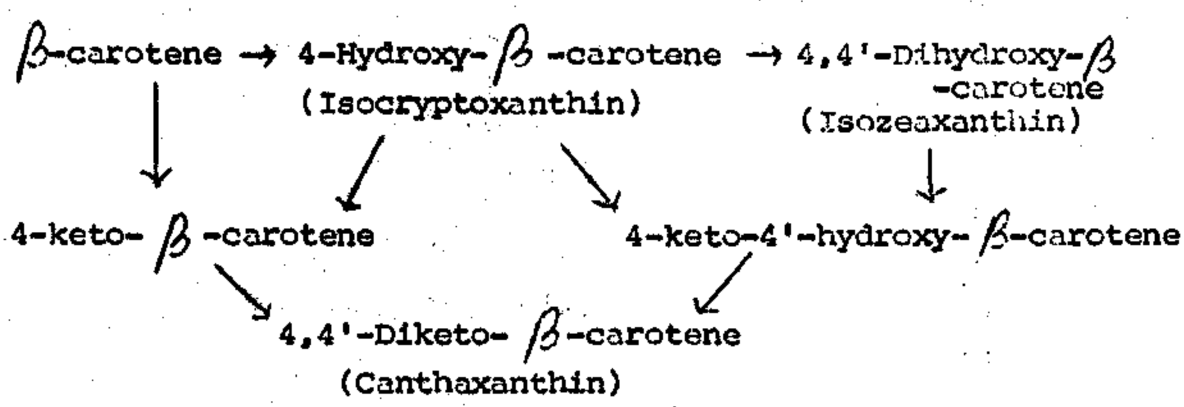
<u>Non-selectors</u>: These organisms readily assimilate and deposit both carotenes and xanthophylls in the body tissues. Examples are man, frog and octopus.

<u>Carotenoid innovators</u>: These assimilate varied kind of carotenoids, oxidises them to yield derivatives. Thus β-carotene could be converted into mono - or dihydroxy-alcohols (Eg: cryptoxanthin, zeaxanthin) or into mono - or dihydroxy - diketo compounds (Eg: astaxanthin).

Many echinoderms, crustaceans and a few fishes come under this category.

<u>Crustaceans</u>:

<u>Artemia salina</u>, the brine shrimp can convert β- carotene into echinenone and canthaxanthin. <u>Penaeus japonicus</u> and <u>Metapenaeus barbata</u> oxidise β-carotene to astaxanthin (Fox, 1979). In most crustaceans free astaxanthin forms the bulk of the pigment in carapace. The pigments are transferred from epidermis during moulting to the carapace (Goodwin, 1984). It is observed that during intermoult stages the carotenoid content in the hepatopancreas is very high (unpublished data). In hepatopancreas the conversion of carotenoids take place. Some crustaceans - <u>Penaeus japonicus</u> - can metabolise β-carotene to astaxanthin while still others - <u>Panulirus japonicus</u>, <u>Portunus trituberculatus</u> and <u>Pagurus prideauxi</u> - can metabolise upto exhinenone (Fig. 1 and 2). The pigments are transported from hepatopancreas to epidermis through haemolymph while the transfer from gut to hepatopancreas is mediated by a carrier protein, structurally similar to apoprotein of α- crustacyanin (Goodwin, 1984).



<u>Figure - 1</u>: β carotene to echinone and canthaxanthin synthesis is crustaceans (Goodwin, 1934).

The carotenoid concentrations within the crustacean can vary very much. In <u>Sergestes</u> corniculum carotenoids are very high in the carapace and hypodermis while very little is present in the eyes. In some euphausids about 94% of the total carotenoids occur in the eyes. In <u>Oplophorous spinosus</u> the eggs contain 2447 µg/g carotenoids while the adults only 244 µg/g. In <u>Pandalus borealis</u> and <u>Penaeus japonicus</u> β carotene is high in the hepatopancreas while astaxanthin is abundant in the carapace.

<u>Amino acids</u>: It is of interest that Otazu-Abrill, <u>et al</u>. (1982) have found that purified amino acids in the diet especially methionine (at 8% level) can elevate carotenoid levels in <u>Palaemon serratus</u>. Methionine and isoleucine pain produced stricking increase in carotenoids. However too higher addition of free amino acids is the food reduced the pigment content.

<u>Eyestalk secretions</u>: Ablation of eye-stalks in <u>Rithoropanopeus harrisii</u> during development of ovaries resulted in increase in ovarian weight with accumulation of carotenoids,while if ablated during the resting period, [illegible] ovarian weight increased but not the carotenoid content [illegible]. Removal of eyestalk also seems to have a bearing on chromatophores

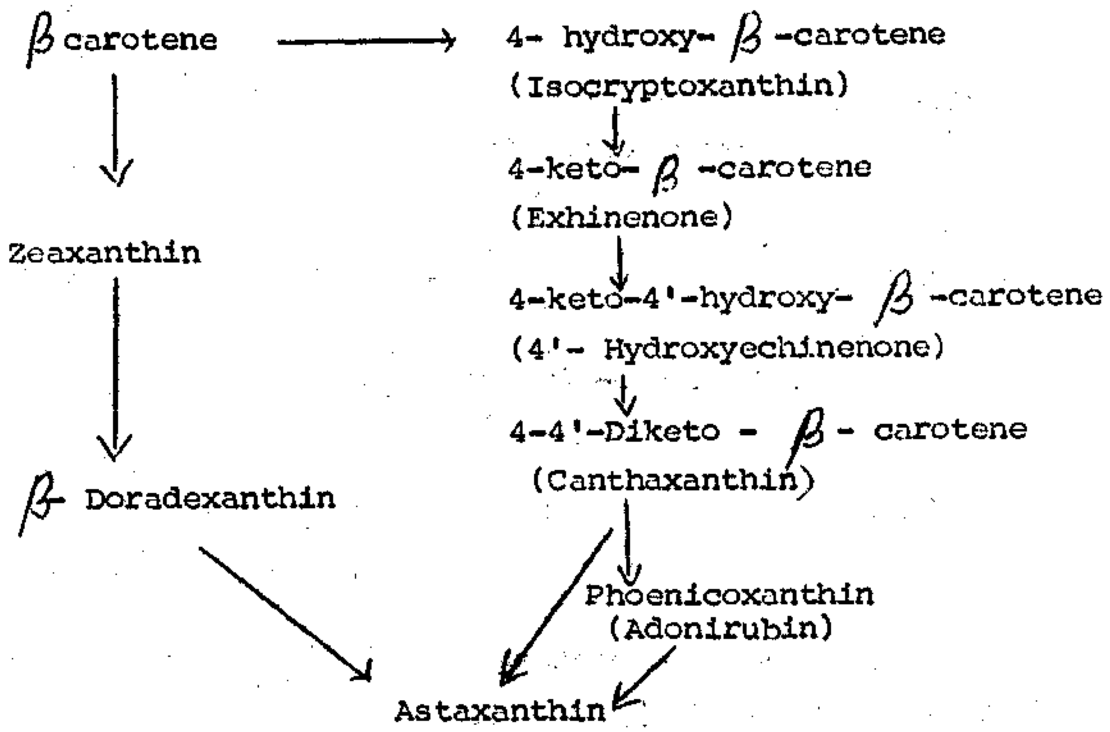


Figure- 2: Metabolic pathway from β-carotene to Astaxanthin in crustaceans (Goodwinn, 1984)

and colour change. In *Palaemon serratus* pigment granules are reduced with the loss of xanthophyll metabolism. In *Uca pugilator* bilateral removal results in loss of mobilisation of carotenoids from the hepatopancreas. Bilateral removal lowered the amounts of esterified axtaxanthin in *Dardanus arrosor* while in *Macrobrachium rosenbergii* increase in both free and esterified astaxanthin was observed.

FISHES

Carotenoids have been located in skin, muscle, liver, egg, sperm and in buccal mucosa of fishes. It is difficult to give authoritative values for carotenoids in fishes, since carotenoid content is influenced by dietary availability. In salmon *Oncorhynchus nerka* is the male during spawning 75% of the carotenoids are in the skin while during pre-spawning 98% is concentrated in the muscle. In the testis it is nil. In the female during spawning 85% are in the ovaries, 14% in the skin and 5% in the muscle. During prespawning 90% are in the muscle, 1% in the skin and little in the ovaries. Thus mobilisation of carotenoids vary between sexes and with reproductive cycle. In fishes too as given in the figures 1 & 2 both metabolic pathways are evident. Much of the information on this is based on reasonable evidences, yet intermediary links and mediating enzymes are to be studied (Goodwin, 1984).

FUNCTIONS ASCRIBED

The only major function known is the role of carotenoids in the synthesis of vitamin A. β-carotene by breaking at 15-carbon can give rise to two molecules of vitamin A. α-carotene, β-cryptoxanthin and β-apocarotene - 8'-al are active retinol precursors. Thus the two essentials to be a precursor for vitamin A seems i) an unsubstituted β-ring and ii) a side chain of conjugated double bonds at least as long as that in retinol. Zeaxanthin, lutein and β-carotene are all inactive (Goodwin, 1984). In freshwater fishes desaturation at C-3 does not destroy activity which is the characteristic of vitamin A_2 (dehydroretinol). But saturation of a double bond in the side chain or removal of a methyl group (eg. at C-13) entirely eliminated the vitamin activity. The usual form of natural carotenoids is all - trans form, though some Cis-isomers do exist. The pro- γ-carotene though being a cis-isomer has potency equal to all-trans- γ-carotene, because one half contain all the structural requirements of vitamin A precursor (Goodwin, 1984).

All other functions proposed are just hypotheses only. Goodwin (1984) catalogues the following as the supposed functions of carotenoids in crustaceans, perception of light, electron acceptor, protective-chromatic adaptation, protect eggs from solar radiation, high temperature, reducing reflectivity, masking luminescence of prey in stomach, and protecting gut wall against digestive enzymes - stabilisation of proteins, stabilisation of chitin, transfer of carotenoprotein pigments, reproduction, and chemo-reception in antennae.

Tacon (1981) speculate the following as the possible function of carotenoids in fishes. 1. Antioxidant - β carotene is 50 times faster as oxygen quencher than α- tocopherol. 2. Fishes are capable of storing and modifying carotenoids to suit their requirements, whereby indicating some vital necessity for carotenoids other than vitamin-A precursor. 3. Egg astaxanthin in <u>Salmo gairdneri</u> enhances chemotaxis of spermatozoa. 4. In <u>Cyclopterus lumpus</u> there is intensive expenditure of carotenoids in yolk sac also in <u>Salmo trutta</u>. 5. When canthaxanthin is supplemented in the diet of <u>Salmo gairdneri</u> resulted in enhancement of growth and maturation rate. 6. Degree of pigmentation is related to tolerance to various stringent environmental conditions - low oxygen level, elevated temperature and ammonia levels. 7. Carotenoids may be respiratory in cellular level under adverse oxygen conditions. Eggs deposited in places of low oxygen and animals which live in low oxygen localities are more highly pigmented. 8. Though the role of carotene in gametogenesis is not clearly known in animals, in certain filamentous fungi (phycomycetes) β-carotene serves as a precursor to sex hormone trisporic acid which controls gametogenesis. 9. In fishes it is most likely they are mainly antioxidents since their association with high lipid content site make one to speculate so.

QUINONES

Though the carotenoids are the subject of discussion it is better that a very brief mention is made of other biochromes. Of the quinones, ubiquinones (Q - coenzymes) belonging to benzoquinone series serve as biocatalysts in cellular respiration. Eventhough they occur almost in all the animals, always present in very low quantities.

In the skeleton of the echinoderms naphthoquinones pigments are present but their physiological role is not established. Vitamins K_1 and K_2, ... prothrombin and anti-haemorrhagic factors are also related to naphthoquinones.

Anthraquinones are predominently present in the scale insects. (Fox, 1979)

FLAVONOIDS

Flavonoides are less common than the former. These could have a role similar to that of vitamin P. Rutin has been found to accelerate the healing from severe X-ray burns and prevents capillary fragility. In the animals subjects to irradiation calcium flavonoids decreased haemorrhagic lessions and mortality (Fox, 1979).

FLAVINS

Riboflavin is the most stricking example under this group of biochromes. Riboflavin could be sunthesised by the plants and specially young and tender parts are rich. Riboflavin is greenish - fluorescing, water soluble, yellow (when oxidised), heterocyclic compound and serves as a reversible hydrogen acceptor in the redox systems. (Fox, 1979)

TETRAPYRROLES

Porphyrins and Bilichromes belong to this group. Chlorophylls and haem are porphyrins. Cyanonocobalamin (B_{12}) is a tetrapyrrole. Bilirubin and the allied bile salts and the blood pigment biliverdin are the examples of bilichromes. (Fox, 1979)

PTERINS

These are polycylic nitrogenous compounds. Fluorescyanin present in fish scales are found to have physiological properties like accelerating oxygen consumption like those of vitamin B_1. Xanthopterin is found to stimulate normal cell division but suppresses tumorous growth and also functions as anti-anaemic. (Fox, 1979)

INDOLE

The melanins, which are black or brown coloured substances common on the skin, fur, scale and feathers. The ink of cephalopods are melanins. Melanins are synthesised by the organism from the amino acid tyrosine while indigoids from oxidation of tryptophan. (Fox, 1979)

In aquaculture, by tactful addition of specific biochromes the shell/skin/meat colour of the cultured organism can be modified to suit the aesthetic taste of the consumer market.

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