Metabolism of cryptoxanthin in freshwater fish*

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1. In search of other provitamins A, the metabolism of cryptoxanthin was studied in several species of freshwater fish, i.e. *Channa gachua*, *Labeo boga* (retinol-rich) and *Heteropneustes fossilis* (dehydroretinol-rich). The fish were either allowed to starve for 20–25 d to make their intestines free from carotenoids and vitamin A or kept on a vitamin-A-deficient diet for 140–150 d to deplete the initial reserve of vitamin A in the livers.

2. Retinol-rich freshwater fish such as *C. gachua* and *L. boga* converted cryptoxanthin into retinol and no 3-dehydroretinol or 3-hydroxyretinol could be isolated from those fish that received cryptoxanthin.

3. 3-Hydroxyretinol and 3-dehydroretinol were isolated from the vitamin-A-deficient *H. fossilis*, a 3-dehydroretinol-rich freshwater siluroid, after the administration of cryptoxanthin.

Several carotenoids capable of acting as provitamin A are known (Moore, 1957) and it has been conclusively established that a number of carotenoids with at least one unsubstituted β-ionone ring and a fully conjugated isoprenoid side chain can serve as the precursor of retinol in mammalian and avian species. Earlier reports indicated that both vitamins A_1 and A_2 can be formed from a common precursor such as β-carotene (Morton & Creed, 1939) or astaxanthin (Grangaud & Massonet, 1955; Grangaud & Moatti, 1958; Grangaud *et al.* 1958, 1962). Barua and Goswami with their co-workers (Barua *et al.* 1973, 1979; Barua & Das, 1975; Barua & Goswami, 1977; Goswami & Barua, 1981*b*; Goswami & Bhattacharjee, 1982; Goswami, 1983, 1984) showed that 3-dehydroretinol can be formed from lutein via anhydrolutein in several species of freshwater fish. Thus it was established that lutein acted as one of the best precursors of 3-dehydroretinol. We have recently reported that *Channa gachua*, a retinol-rich (Goswami & Barua, 1979) freshwater fish can convert β-carotene into retinol (Goswami & Barua, 1981*a*). It is possible that other carotenoids such as cryptoxanthin can act as precursors of retinol or dehydroretinol. It has previously been shown that cryptoxanthin is converted into retinol in rats (Patel *et al.* 1951). Cama and co-workers (Jacob John *et al.* 1970) further studied the metabolism and biological activity of cryptoxanthin in the rat; they reported that it is converted into retinol and its biopotency is 22% that of β-carotene. Hence, it was considered worthwhile to study the metabolism of cryptoxanthin in freshwater fish and we report here that retinol-rich fish can convert cryptoxanthin into retinol, whereas dehydroretinol-rich fish can convert cryptoxanthin into 3-dehydroretinol and 3-hydroxyretinol.

MATERIALS AND METHODS

The sources of different chemicals and solvents used in the present study have been described previously (Barua & Goswami, 1977). An authentic sample of cryptoxanthin was provided by Hoffman–La Roche, Basel, Switzerland. Further quantities of cryptoxanthin were isolated from ripe *Carica papaya* by following methods described by Jacob John *et al.* (1970).

Carr–Price reagent was prepared as described by Barua *et al.* (1973). Lipid extraction and column chromatography methods have been described by Barua & Goswami (1977) and Barua *et al.* (1979). Thin-layer chromatography was performed using silica gel G plates

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(200 mm × 100 mm, 0·25 mm thickness) for purification and co-chromatography with vitamin A derivatives using light petroleum (b.p. 60–80°) and acetone.

Fish

Channa gachua, *Labeo boga* and *Heteropneustes fossilis* were obtained from the local market in live condition. In the intestinal conversion study, the fish were starved for 20–25 d, when evidence from u.v.-visible spectra and antimony trichloride reaction of the lipid extracts indicated that the intestines of the fish were free from any vitamin A or carotenoids.

In the liver-storage experiment, three groups of *C. gachua* and *H. fossilis* were kept on a vitamin-A-free diet (Barua *et al.* 1973) and within 140–150 d they became vitamin-A-deficient. When the intestines or the livers were found to be depleted of any vitamin A or carotenoids, the fish were given cryptoxanthin mixed with moist goat meat treated with groundnut oil (Barua *et al.* 1973).

Isolation of 3-dehydroretinol and 3-hydroxyretinol from Wallago attu

3-Dehydroretinol and 3-hydroxyretinol were isolated from the liver oil of *Wallago attu*, a freshwater siluroid, by column chromatography on an alumina (5% water deactivated) column (10 g, 15 × 55 mm), as described by Barua *et al.* (1977, 1979). The vitamin A derivatives were eluted with light petroleum (b.p. 60–80°) or light petroleum containing increasing amounts of diethyl ether. The fractions containing esterified 3-dehydroretinol and 3-hydroxyretinol were identified, estimated and purified as described by Barua *et al.* (1977).

Saponification of vitamin A compounds

A known quantity of vitamin A compound was saponified under reflux for 20 min with methanolic potassium hydroxide (100 g/l). The different vitamin A compounds were extracted three times with peroxide-free diethyl ether. The diethyl ether extract was freed from alkali, dried over anhydrous sodium sulphate and the solvent removed by distillation under reduced pressure. The unsaponifiable material was dissolved in a known volume of light petroleum (b.p. 60–80°) for further analysis.

RESULTS AND DISCUSSION

An aqueous suspension of cryptoxanthin (80 μg/fish) in groundnut oil was administered orally by stomach tube and the formation of any metabolites in the intestines of *C. gachua*, *L. boga* and *H. fossilis* was examined within 2–8 h. It was found that in most of the experiments the conversion of cryptoxanthin took place within 4·5–6 h. The lipids were extracted with light petroleum (b.p. 40–60°) and the u.v.-visible absorption spectrum of the extract was recorded. Whenever there was any indication of formation of vitamin A, the extract was chromatographed on a water-deactivated (5%) alumina column (10 g, 15 mm × 55 mm). The different metabolites were eluted with light petroleum containing increasing amounts of diethyl ether.

From the intestinal extracts of *L. boga* and *C. gachua* after the administration of cryptoxanthin, retinyl ester was separated from the first part of the collected fractions and identified from its chromatographic behaviour, u.v. absorption spectrum showing its absorption maximum at 325 nm and $SbCl_3$ colour maximum at 620 nm. After increasing diethyl ether to 150 ml/l, the material eluted by diethyl ether was evaporated to dryness and the residue dissolved in 5 ml light petroleum (b.p. 40–60°). The fraction had a u.v. absorption maximum at 325 nm and a $SbCl_3$ colour reaction with an absorption maximum at 620 nm, both characteristic of retinol. Results are shown in Table 1. It may be mentioned that in the lipid extracts of *C. gachua* and *L. boga* there was complete absence of any

Table 1. *Metabolism of cryptoxanthin in the intestines of* Channa gachua *and* Labeo boga

(An aqueous suspension of cryptoxanthin (80 μg/fish) in groundnut oil was administered orally by stomach tube to *C. gachua* and *L. boga* which had previously been starved for 20–25 d to free the intestines from any vitamin A or carotenoids. Not all of the cryptoxanthin solution administered was retained by the fish; a part was always released either orally or through opercular openings)

Fish	No. of fish used	Period between administration of cryptoxanthin and killing (h)	Amount of cryptoxanthin administered (μg)	Amount of metabolites found in intestines (μg)		Amount of unconverted cryptoxanthin (μg)
				Esterified retinol	Retinol	
C. gachua	4	4·5	320	27	—	197
	6		480	31	—	137
	6		480	44	26	161
	6	5	480	53	16	181
	5		400	32	12	191
	5		400	46	—	127
	4	5·5	320	34	—	167
	7		560	51	—	131
	5		400	86	26	155
	6		480	55	—	194
	6	6	480	62	—	242
	7		560	52	—	88
	6		480	49	—	137
L. boga	3	4·5	240	19	23	86
	5		480	32	—	102
	6		480	32	—	123
	5		400	26	—	143
	5	5	400	35	27	96
	3		240	16	—	139
	4		320	28	—	206
	5	5·5	400	22	—	—
	6		480	40	—	151
	7	6	560	52	14	212
	7		560	38	—	191
	4		320	27	—	123

detectable metabolites of vitamin A or carotenoids between the retinyl ester and retinol fractions.

The intestinal lipid extracts of *H. fossilis* showed the presence of esterified 3-hydroxyretinol and 3-dehydroretinol after the administration of cryptoxanthin (Table 2). Esterified 3-hydroxyretinol and 3-dehydroretinol were characterized by their chromatographic behaviour, gross u.v. absorption maxima at 330 nm and 350 nm and $SbCl_3$ colour reaction as described previously (Barua *et al.* 1977).

In the liver-storage test three groups of *C. gachua* and *H. fossilis* were maintained on a vitamin-A-deficient diet and within 140–150 d they became vitamin-A-deficient. Cryptoxanthin (80 μg/fish) was given along with slightly moist goat meat treated with groundnut oil for 10 d and the fish were killed 24 h after the last feed. Liver lipids were extracted with light petroleum (b.p. 40–60°) and u.v.-visible spectra were recorded. As in the intestinal conversion test, whenever there was any indication of formation of vitamin A, the whole

Table 2. *Metabolism of cryptoxanthin in the intestines of* Heteropneustes fossilis

(An aqueous suspension of cryptoxanthin (80 μg/fish) in groundnut oil was administered orally by stomach tube to *H. fossilis* which had previously been starved for 20–25 d to free the intestines from any vitamin A or carotenoids. Not all of the cryptoxanthin solution administered was retained by the fish; a part was always released either orally or through opercular openings)

No. of batches	No. of fish in each batch	Period between administration of cryptoxanthin and killing (h)	Amount of cryptoxanthin administered/ batch (μg)	Amount of metabolites found in intestines (μg)						Amount of unconverted cryptoxanthin (μg)	
				Esterified retinol	Retinol	Esterified dehydroretinol		Diesterified 3-hydroxyretinol			
						Mean	SD	Mean	SD	Mean	SD
13	5	4·5–6	400	0	0	23	12	54	12	154	20

Table 3. *Metabolism of cryptoxanthin in the livers of* Channa gachua *and* Heteropneustes fossilis

(An aqueous suspension of cryptoxanthin (80 μg/fish per d) in groundnut oil, mixed with slightly moist goat meat, was given for 10 d to *C. gachua* and *H. fossilis* which had previously been given a vitamin-A-free diet for 140–150 d to produce vitamin A deficiency. The fish were killed 24 h after the last meal)

Fish	No. of fish	No. of days of feeding	Amount of cryptoxanthin administered (μg)	Amount of metabolites found in liver (μg)				Amount of unconverted cryptoxanthin deposited in liver (μg)
				Esterified retinol	Retinol	Esterified dehydroretinol	Diesterified 3-hydroxyretinol	
C. gachua	4	10	3200	131	59	—	—	59
	6	10	4800	210	27	—	—	27
	4	10	3200	150	57	—	—	57
H. fossilis	6	10	4800	—	—	185	82	37
	6	10	4800	—	—	168	61	45
	6	10	4800	—	—	155	49	—

extract was subjected to column chromatography. It was possible to isolate different vitamin A compounds, i.e. retinyl ester and retinol from *C. gachua* and esterified 3-hydroxyretinol and esterified 3-dehydroretinol from *H. fossilis* (Table 3).

As a control, a similar type of liver-storage experiment was performed with three batches of fish (five, four and five fish) for both vitamin-A-deficient *C. gachua* and *H. fossilis*, using only groundnut oil and goat meat. It was found that after 10 d of feeding, liver-lipid extracts from the three control groups failed to show any vitamin A or carotenoids as assessed from their u.v. and visible absorption maxima. As the extracts did not exhibit u.v. and visible absorption maxima of any vitamin A derivatives or carotenoids, chromatographic analysis was not attempted.

As it is difficult to separate vitamin A derivatives (retinyl ester and dehydroretinyl ester or retinol and dehydroretinol) by column chromatography, each of the metabolites isolated after alumina chromatography was studied further in order to establish whether crypto-

xanthin forms dehydroretinol or 3-hydroxyretinol in retinol-rich fish and retinyl ester or retinol in dehydroretinol-rich fish.

The $SbCl_3$ colour reactions were carried out with the metabolites after saponification with methanolic KOH (100 g/l) of esterified compounds isolated after the administration of cryptoxanthin to *C. gachua*, *L. boga* and *H. fossilis*. It was found that there were no absorption maxima characteristic of (1) dehydroretinyl ester or dehydroretinol in the fractions of retinyl ester or retinol, (2) retinyl ester in the fractions of 3-dehydroretinyl ester.

It was found in earlier studies (Goswami, 1978; Goswami & Barua, 1979, 1981*b*) that even a trace amount of dehydroretinyl ester in retinol or retinyl ester fractions was associated with the appearance of the characteristic absorption peak at 287–286 nm along with the main absorption maximum at 325 nm. This indicates the presence of trace amounts of 3-dehydroretinol or 3-dehydroretinyl ester along with the retinol or retinyl ester. In the present investigation there was no absorption maximum at 287–286 nm in the retinyl ester or retinol fractions collected from *C. gachua* or *L. boga* after the administration of cryptoxanthin. This finding suggests that cryptoxanthin fails to form even trace amounts of 3-dehydroretinol or 3-hydroxyretinol in retinol-rich fish like *C. gachua* and *L. boga*.

After administration of cryptoxanthin, the following fractions were obtained from intestines and liver: retinyl esters from *C. gachua* and *L. boga*, 3-dehydroretinyl esters and 3-hydroxyretinol diesters from *H. fossilis*. They were separately saponified and the products chromatographed on thin layers of silica gel using light petroleum and acetone (9:1, v/v), along with 3-dehydroretinol and 3-hydroxyretinol obtained by saponification of fractions from the liver oil of *W. attu*, and two spots of retinol from *C. gachua* and *L. boga*. No 3-dehydroretinol (R_f, 0·76) or 3-hydroxyretinol (R_f, 0·54) was found in the unsaponifiable residues of fractions from the intestines and livers of *C. gachua* and *L. boga*, and no retinol (R_f, 0·71) in the unsaponifiable residues of the fractions from *H. fossilis*. Thus it can be seen that there was no retinyl ester in the dehydroretinyl fractions isolated from the intestines and livers of *H. fossilis* and no esterified 3-dehydroretinyl or diesterified 3-hydroxyretinol in the retinyl ester fractions of *C. gachua* and *L. boga*.

It was found that *C. gachua* and *L. boga* could convert cryptoxanthin into retinol. This observation is in agreement with earlier findings (Patel *et al.* 1951; Jacob John *et al.* 1970). Gross & Budowski (1966) reported *Chironomus* larvae as a source of cryptoxanthin in the food of freshwater fish; chironomid larvae are a favourite food of *C. gachua* and *L. boga* (Goswami & Deka, 1976).

The possible formation of 3-dehydroretinol from cryptoxanthin in *C. gachua* and *L. boga* was investigated in the present study and it was found that cryptoxanthin cannot serve as a precursor for 3-dehydroretinol in these fish. 3-Hydroxyretinol, which is a metabolite of lutein in several species of freshwater fish, was not detected in fish of these species given cryptoxanthin. The experiments involving co-chromatography of lipid extracts from intestines and livers with 3-dehydroretinol and 3-hydroxyretinol also failed to show the presence of either 3-dehydroretinol or 3-hydroxyretinol after administration of cryptoxanthin. Cama and co-workers (Jacob John *et al.* 1970) were unable to show the formation of 3-dehydroretinol or 3-hydroxyretinol in mammals given cryptoxanthin. In the present experiment, it was shown that the dehydroretinol-rich freshwater fish *H. fossilis* converted cryptoxanthin into 3-dehydroretinol and 3-hydroxyretinol in their intestines. The liver storage test also indicated bioconversion and storage of 3-dehydroretinol and 3-hydroxyretinol after giving cryptoxanthin for 10 d. It may be mentioned that *H. fossilis*, *Channa striatus* (Barua *et al.* 1973; Barua & Goswami, 1977; Goswami & Barua, 1981*b*), *Clarias batrachus* and *Ompok pabo* (Goswami & Bhattacharjee, 1982), *Mystus tengara* and *Mystus vittatus* (Goswami, 1984) can convert lutein into 3-hydroxyretinol and 3-dehydroretinol. *H. fossilis* can easily convert 3-hydroxyretinol into 3-dehydroretinol via

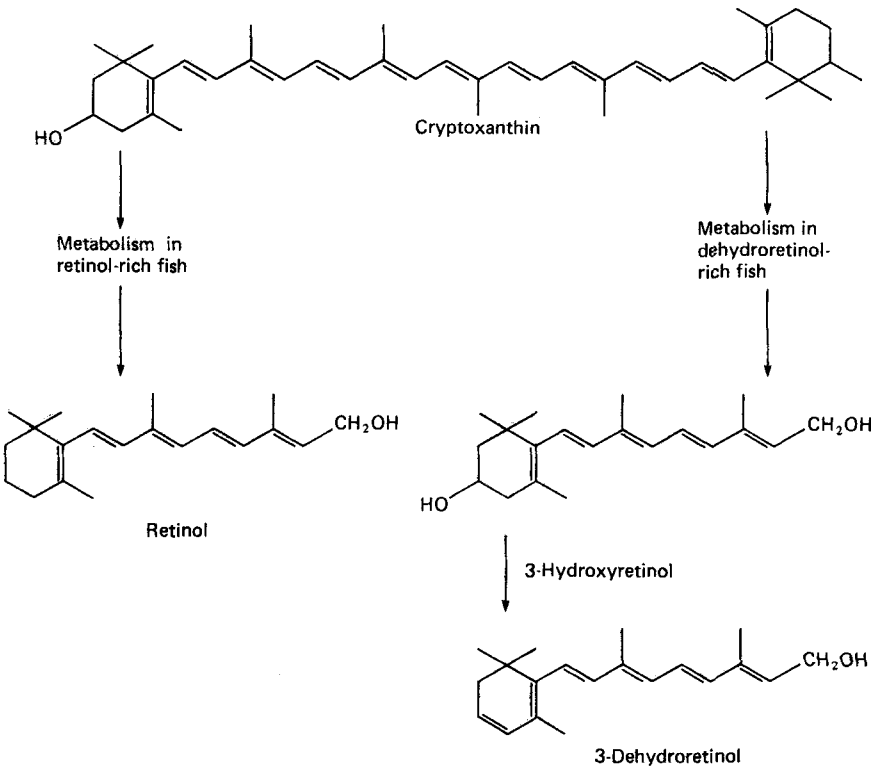

Fig. 1. Metabolism of cryptoxanthin in freshwater fish.

3-hydroxyanhydroretinol (Barua *et al.* 1979). Therefore it can be concluded that cryptoxanthin, which is $\beta\beta$-caroten-3-ol, is split to give 3-hydroxyretinol, which is further converted into 3-dehydroretinol (Fig. 1). In the present experiment we were unable to establish the presence in intestine and liver-lipid extracts obtained from fish given cryptoxanthin of 3-hydroxyanhydroretinol, which might be completely converted into 3-dehydroretinol (Barua *et al.* 1979); there might have been formation of a very small amount which escaped detection.

The present experiments suggest that the metabolism of cryptoxanthin in retinol-rich fish is similar to that in mammalian species but different in dehydroretinol-rich freshwater fish where, as with lutein, it is converted into 3-hydroxyretinol and 3-dehydroretinol.

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