Pure & Appl. Chem., Vol. 63, No. 1, pp. 81–88, 1991.
Printed in Great Britain.

206922209100008

Xanthophylls as precursors of retinoids

Takao Matsuno

Department of Natural Products Research, Kyoto Pharmaceutical University, Misasagi, Yamashina-ku, Kyoto 607, Japan

Abstract - Xanthophylls such as canthaxanthin, astaxanthin, zeaxanthin, lutein and tunaxanthin that are widely distributed in Nature have been found to be precursors of retinoids not only in freshwater fish and marine fish (yellowtail) but also in mammals (rat).

Reductive metabolic pathways from 3-dehydroretinol to retinol and from 3-dehydroretinal to retinal have been newly discovered by feeding experiments with rat and yellowtail, respectively.

INTRODUCTION

In many animals, the most important metabolic products of carotenoids are the retinoids, and the metabolic reactions of carotenoids in animals are essentially oxidative. However, pathways of reductive metabolism have recently been discovered, and this has opened up the possibility that xanthophylls could be precursors of retinoids (ref. 1).

A review on xanthophylls as retinoid precursors will be presented, based upon the original literature and on the results of our feeding experiments. The retinoids discussed so far in these studies are retinol (1), retinal (2), 3-dehydroretinol (3) and 3-dehydroretinal (4) (Fig. 1).

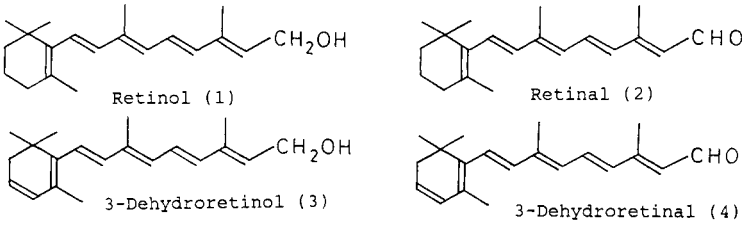


Fig. 1. Retinoids.

Only about 60 of the 600 known carotenoids (ref. 2) have been reported to be precursors of retinol, the main ones being α-carotene, γ-carotene, ß-cryptoxanthin, echinenone, ß-apo-12'-carotenal and, of course, ß-carotene (Fig. 2). From the structural point of view, retinol precursors must have at least one unsubstituted ß-ring attached to a conjugated polyene structure that is intact from C-7 to C-15 (Fig. 2).

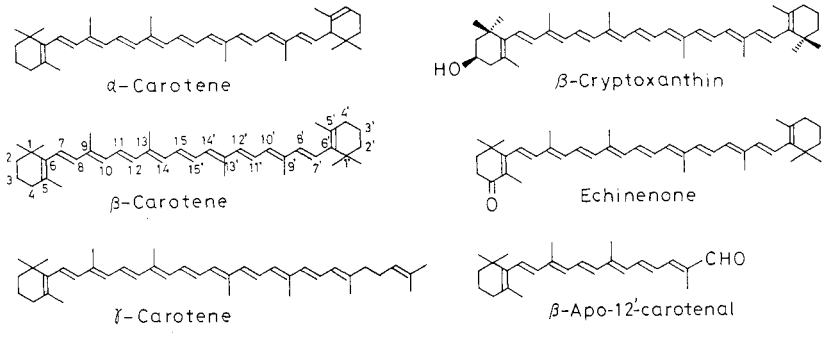


Fig. 2. Typical retinol precursors.

The familiar reactions of carotenoid metabolism in animals are essentially oxidative. Figure 3 shows the typical oxidative metabolic pathways that have been demonstrated in goldfish Carassius auratus (refs. 3 & 4) and fancy red carp Cyprinus carpio (ref. 5) and Figure 4 shows typical oxidative metabolic pathways in crustaceans (refs. 6 - 10).

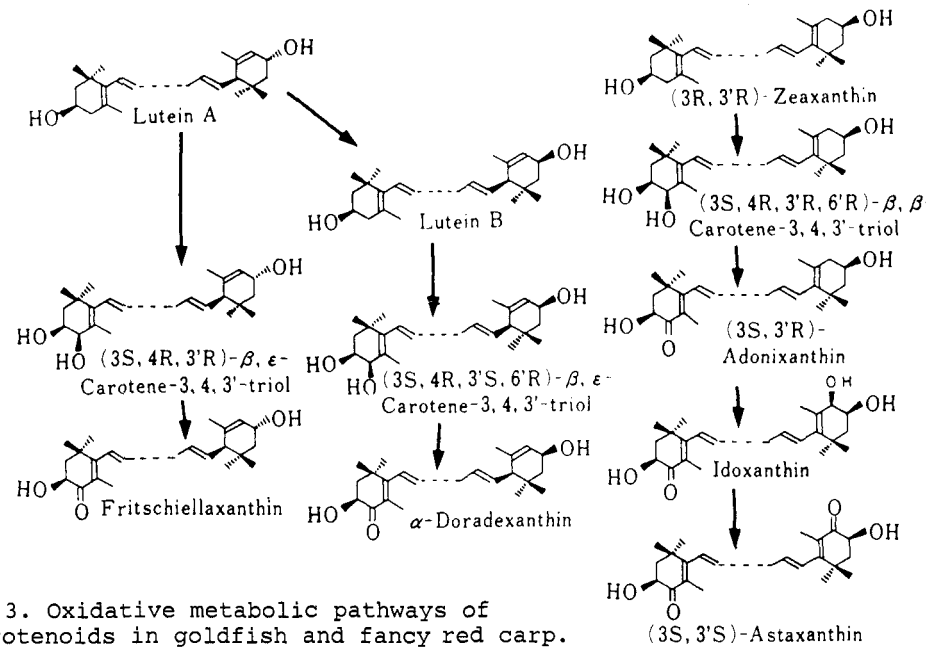


Fig. 3. Oxidative metabolic pathways of carotenoids in goldfish and fancy red carp.

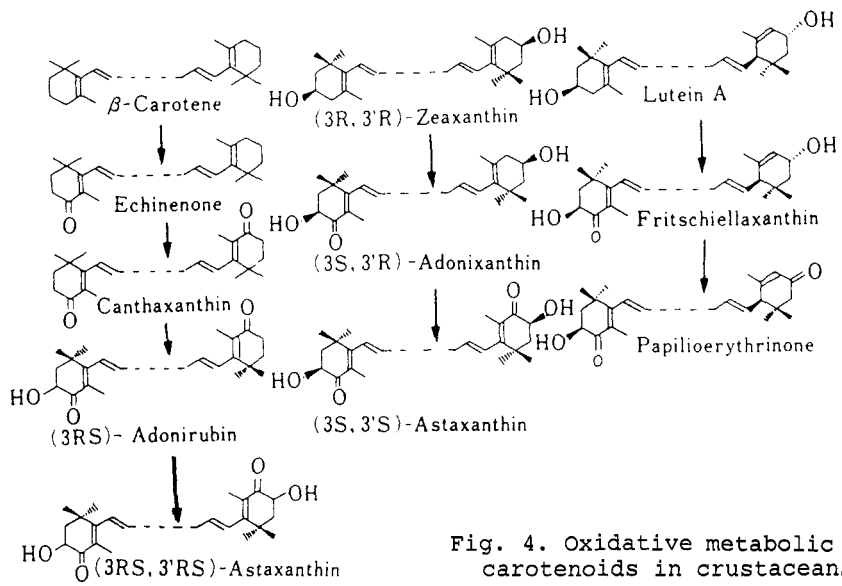


Fig. 4. Oxidative metabolic pathways of carotenoids in crustaceans.

However, pathways of reductive metabolism have recently been discovered. Figure 5 shows the reductive metabolic pathways of ketocarotenoids in rainbow trout Salmo gairdneri (refs. 11 - 12). These reductive metabolic reactions involve the stepwise removal of the keto-groups at C-4 and C-4'.

Figure 6 shows the reductive metabolic pathways of astaxanthin to tunaxanthin in yellowtail Seriola quinqueradiata (refs.13 & 14), flying fish Prognichthys agoo and dolphin Coryphaena hippurus (ref. 15), respectively. These metabolic pathways also involve the elimination of keto groups from C-4 and C-4' and also the reduction of keto-groups at C-3 and C-3' to secondary alcohols.

Grangaud et al. (ref. 16) first demonstrated that astaxanthin is converted into retinol in the liver of retinol-depleted freshwater fish in 1962. Gross and Budowski (ref. 17) in 1966 found evidence of the conversion of astaxanthin, canthaxanthin and isozeaxanthin, via ß-carotene, into retinol

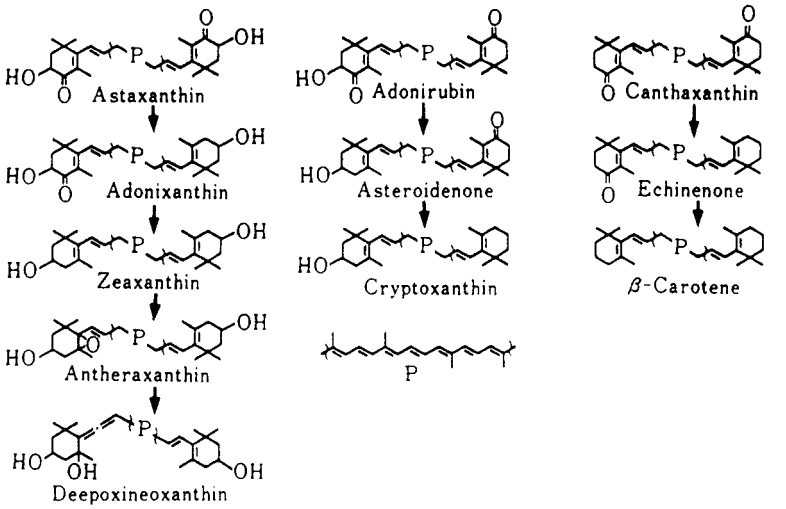


Fig. 5.
Reductive metabolic pathways of carotenoids in rainbow trout.

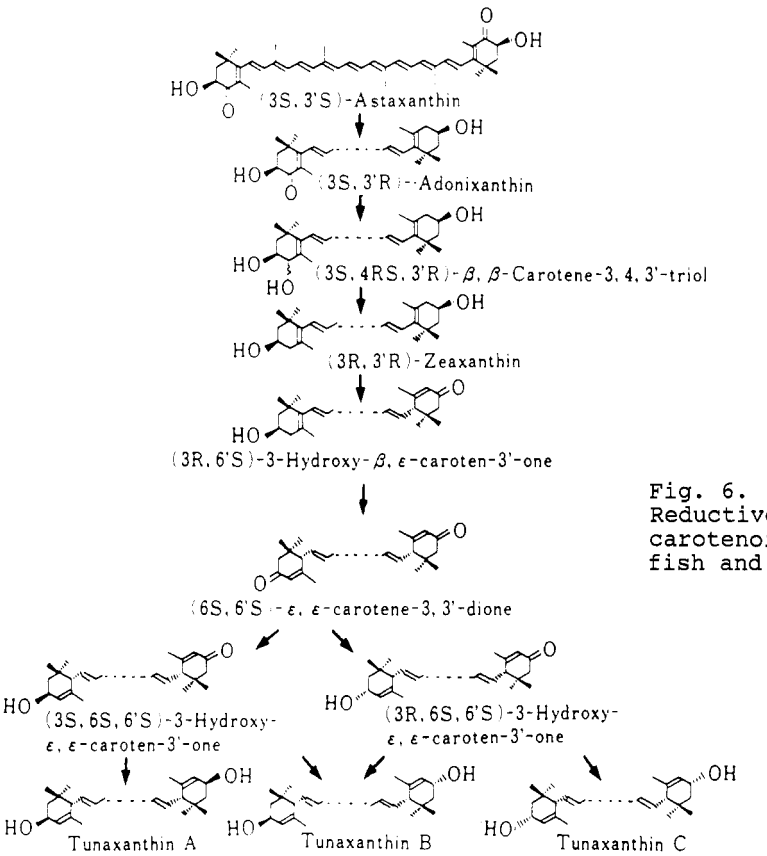


Fig. 6.
Reductive metabolic pathways of carotenoids in yellowtail, flying fish and dolphin.

and 3-dehydroretinol in freshwater fish, guppies and platies. Barua and Goswami (ref. 18) reported in 1977 that lutein is the precursor of 3-dehydroretinol in some freshwater fish. Recently, Schiedt et al. showed quite clearly that labelled retinol and 3-dehydroretinol are formed in retinol-depleted rainbow trout from any of a number of labelled carotenoids, particularly astaxanthin, zeaxanthin and canthaxanthin (refs. 11 & 19). B. W. Davies and B. H. Davies have reported the formation of retinol and 3-dehydroretinol from radio-labelled xanthophylls, i.e., canthaxanthin, zeaxanthin and lutein, in goldfish (ref. 20). All these investigations have been carried out with freshwater fish.

Our feeding trials were conducted to demonstrate the possibility of the bioconversion of some xanthophylls such as astaxanthin, zeaxanthin, cantha-xanthin, lutein and tunaxanthin into retinoids in retinoid-depleted freshwater fishes, marine fish and mammals.

TILAPIA

Figure 7 shows the contents of retinol and 3-dehydroretinol in liver of tilapia Tilapia nilotica after feeding trials with a retinoid-depleted diet supplemented with xanthophylls such as astaxanthin, canthaxanthin, zeaxanthin, lutein and tunaxanthin (ref. 21). The retinoid content increased in all these xanthophyll-fed groups, compared to that of the control group.

In the groups administered ß-carotene and canthaxanthin, retinol predominated over 3-dehydroretinol, whilst in the groups given xanthophylls, such as astaxanthin, zeaxanthin, lutein and tunaxanthin, 3-dehydroretinol predominated over retinol.

From these results, we can conclude that these xanthophylls, astaxanthin, zeaxanthin, lutein and tunaxanthin, were probably directly bioconverted into 3-dehydroretinol without being first transformed into retinol (Fig. 8).

Recently, the bioconversion of astaxanthin into 3-dehydroretinol in mature rainbow trout has been reported by Guillou et al. (ref. 22).

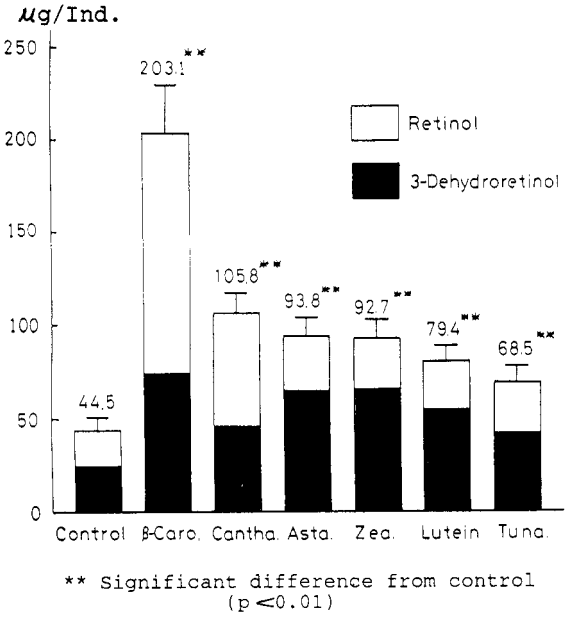


Fig. 7. Contents of retinoids in liver of tilapia after the feeding experiments.

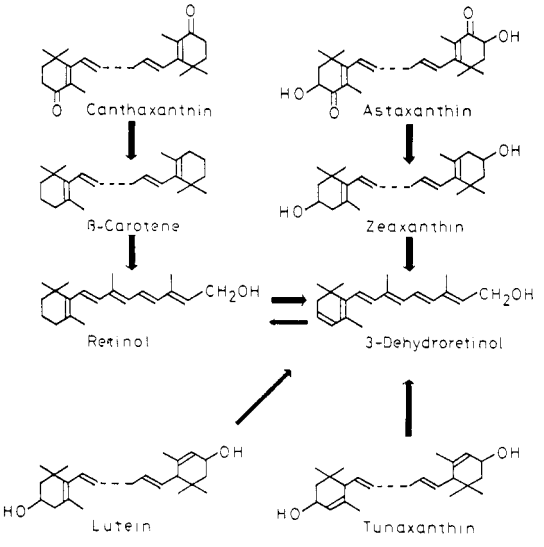


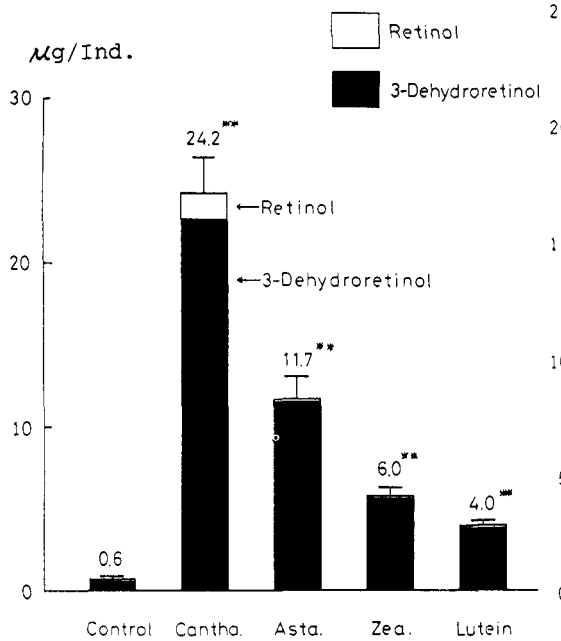
Fig. 8. Xanthophylls as retinoid precursors in tilapia.

BLACK BASS

Figure 9 shows the results of the feeding experiments with black bass Micropterus salmoides (ref. 23). In all the xanthophyll-fed groups, i.e. with canthaxanthin, astaxanthin, zeaxanthin and lutein, the retinoid concentration markedly increased, compared with that of the control group, and the retinoid fractions comprised a large amount of 3-dehydroretinol and only a trace of retinol except for the canthaxanthin group.
From these results, it is assumed that these xanthophylls were directly bioconverted into 3-dehydroretinol, whereas canthaxanthin was reductively bioconverted into retinol and the retinol thus formed was oxidised to 3-dehydroretinol.
With respect to its liver retinoids, the black bass is a typical 3-dehydroretinol-type freshwater fish.

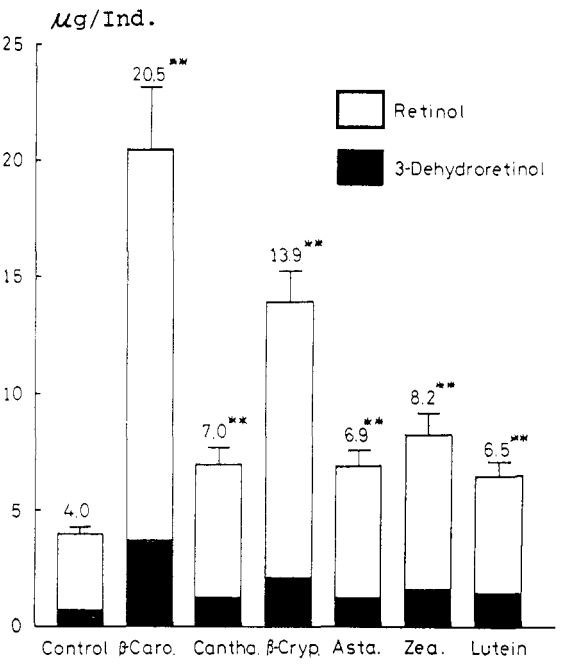
AYU

Figure 10 shows the results of feeding experiments with ayu Plecoglossus altivelis (ref. 24). In all test groups that were fed the xanthophylls and those that were fed ß-carotene and ß-cryptoxanthin which are known as retinol precursors, the concentration of retinoids increased compared with that of the control group.
With respect to its liver retinoids, the ayu is a retinol-type freshwater fish.



** Significant difference from control ($P<0.01$)

Fig. 9. Contents of retinoids in liver of black bass after the feeding experiments.



** Significant difference from control ($p<0.01$)

Fig. 10. Contents of retinoids in liver of ayu after the feeding experiments.

YELLOWTAIL

We have studied the bioconversion of administered astaxanthin in the eggs of a marine fish, namely yellowtail Seriola quinqueradiata during embryonic development (ref. 25). Figure 11 shows the composition of the feed used in this experiment and procedures for sampling eggs at different stages during embryonic development. The yellowtail was reared first on fresh mackerel, followed by moist pellets, and finally with moist pellets containing antarctic krill oil, and were then injected with gonadotropic hormone to promote ovulation. Eggs were removed after two days; both immature eggs (Sample No. 1) and ovulated eggs (Sample No. 2) were collected. After fertilization, fertilized eggs were collected (Sample No. 3), and then further eggs collected one day after fertilization (Sample No. 4) and two days after fertilization (the eyed stage) (Sample No. 5). Finally larval fish were collected (Sample No. 6). The six samples (Sample No. 1 - 6) were taken from the same one individual female yellowtail.

Figure 12 shows the dynamic aspects of the bioconversion of administered astaxanthin in yellowtail eggs during embryonic development. Adonixanthin, ß-carotene-3,4,3'-triol and zeaxanthin found in immature eggs of yellowtail are considered to be reductive metabolic products of astaxanthin derived from the administered antarctic krill oil. Both the carotenoid and retinoid contents of the eggs markedly increased with time and finally reached a maximum from the stage of immature eggs (Sample No. 1) to the stage of ovulated eggs (Sample No. 2). Both carotenoid and retinoid contents decreased progressively during the embryonic development. It was observed that the content of 3-dehydroretinal (4) which is derived from zeaxanthin, decreased, whilst the retinal (2) content increased from Sample No. 2 to No. 5. From the experimental results we have proposed a possible reductive pathway for the metabolism of 3-dehydroretinal (4) to retinal (2) (Fig. 13), because no transport of material occurs through the egg membrane in such a closed system as fish eggs during embryonic development. From the results, which show a dramatic decrease in both carotenoid and retinoid contents from ovulated eggs (Sample No. 2) to fertilized eggs (Sample No. 3), it may be concluded that both carotenoids and retinoids play an important role during fertilization.

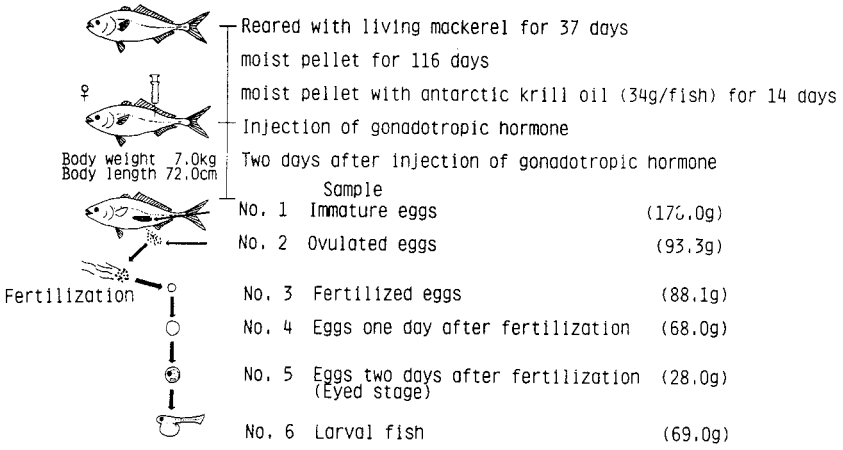


Fig. 11. Feed of yellowtail in the feeding experiment and procedures for sampling of eggs at different stages during embryonic development.

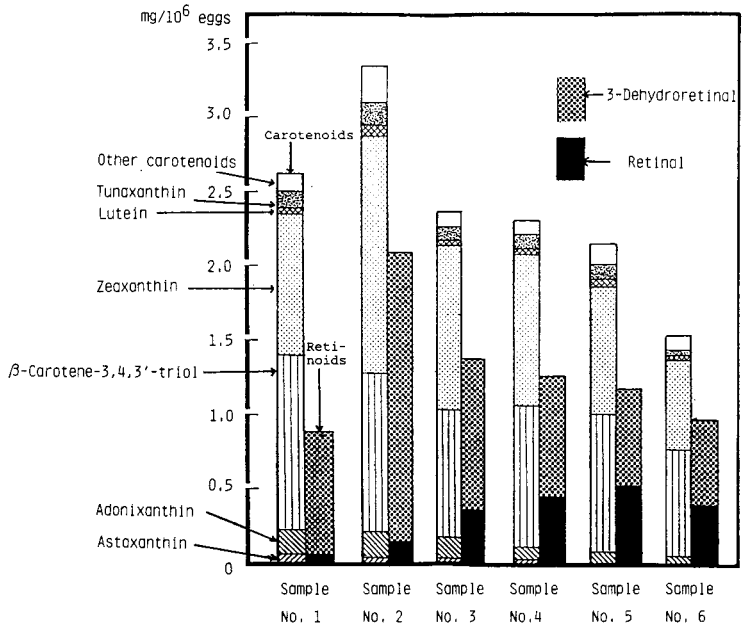


Fig. 12. Dynamic aspects of the bioconversion of administered astaxanthin in yellowtail eggs during embryonic development.

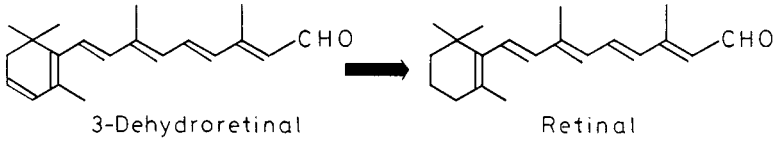
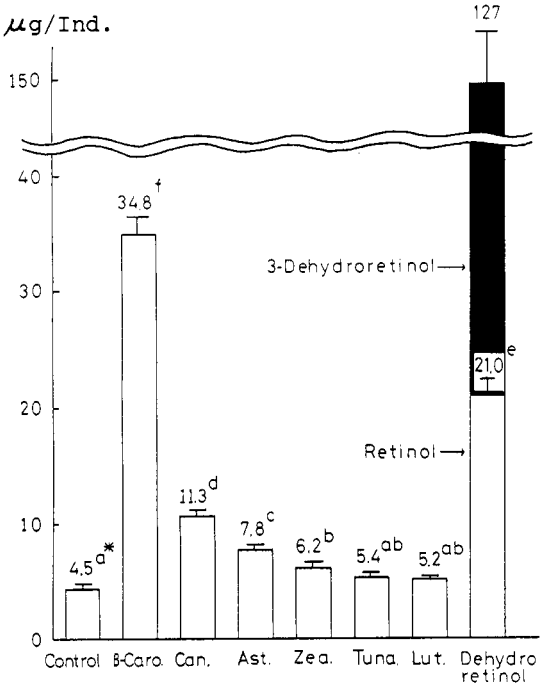


Fig. 13. Reduction of 3-dehydroretinal to retinal in yellowtail during embryonic development.

RAT

Figure 14 shows the retinol content in the liver of weanling male rats after feeding trials (ref. 26). There is a significant difference between most xanthophyll-fed groups and the control group, except that there is little significant difference between the tunaxanthin and lutein groups and the control group. A large amount of unchanged 3-dehydroretinol and a comparatively small amount of retinol were found in the liver of the rats that had been administered 3-dehydroretinol. This indicated that some of the orally administered 3-dehydroretinol was reductively metabolized to retinol.



* Values with different superscripts are significantly different ($p < 0.05$).

Fig. 14. Contents of retinoids in liver of rat after the feeding experiments.

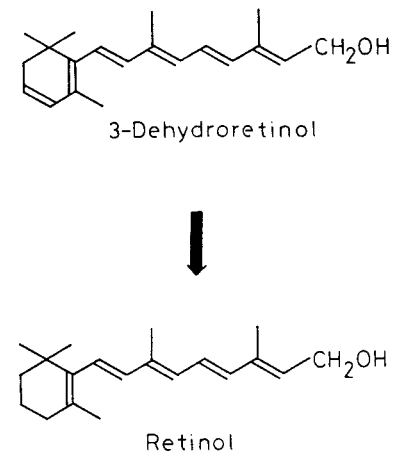


Fig. 15. Reduction of 3-dehydroretinol in rat liver.

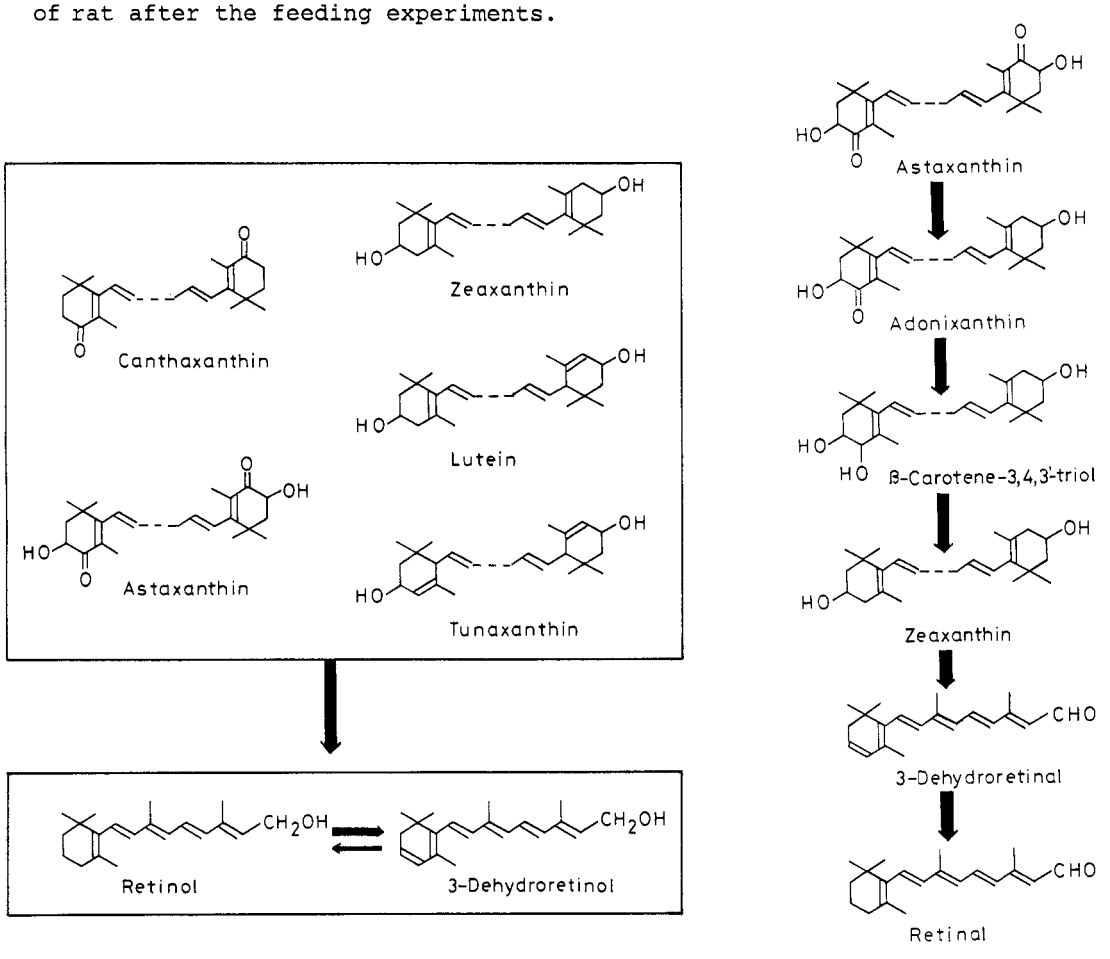


Fig. 16. Xanthophylls as retinoid precursors.

To our knowledge, this is the first finding of the reductive bioconversion of 3-dehydroretinol into retinol (Fig. 15).
A large amount of retinol was found in the group fed ß-carotene, compared with that in any xanthophyll-fed group. Each of the xanthophylls was less efficiently bioconverted into retinol than was ß-carotene. After feeding rats with canthaxanthin, we have observed the presence of echinenone in the rat liver extract, and have concluded that canthaxanthin was reductively metabolized via echinenone to ß-carotene and then finally to retinol.
In the case of the astaxanthin-fed group, a reasonable explanation for the formation of retinol is that astaxanthin is bioconverted reductively into zeaxanthin, and the zeaxanthin thus formed is metabolized to retinol via 3-dehydroretinol by a reductive metabolic reaction. A similar explanation can be proposed for the formation of retinol in the groups fed tunaxanthin and lutein.

In conclusion, xanthophylls such as canthaxanthin, astaxanthin, zeaxanthin, lutein and tunaxanthin, which are widely distributed in Nature have been found to be precursors of retinoids not only in freshwater fish and marine fish (yellowtail) but also in a mammal (rat) as shown in Fig. 16.

Acknowledgements I am most grateful to the coworkers responsible for most of the experimental work outlined in this article:- Dr. M. Katsuyama, Miss M. Tsushima and Mr. E. Yamashita, of the Kyoto Pharmaceutical University and Dr. S. Arai, of the National Research Institute of Aquaculture, for the feeding experiments with tilapia, yellowtail, ayu, black bass and rat, and Dr. T. Maoka, of the Kyoto Pharmaceutical University, for numerous HPLC analyses of carotenoids and retinoids.

REFERENCES

1. T. W. Goodwin, Ann. Rev. Nutr. 6, 273-297 (1986).
2. H. Pfander, Key to Carotenoids 2nd ed, Birkhäuser, Basel (1987).
3. M. Hata and M. Hata, Nippon Suisan Gakkaishi 38, 331-338 (1972).
4. T. Matsuno, H. Matsutaka and S. Nagata, Nippon Suisan Gakkaishi 47, 605-611 (1981).
5. T. Matsuno, S. Nagata, M. Iwahashi, T. Koike and M. Okada, Nippon Suisan Gakkaishi 45, 627-632 (1979).
6. B. H. Davies, W-J. Hsu and C. O. Chichester, Comp. Biochem. Physiol. 33, 601-615 (1970).
7. T. Katayama, M. Shimaya, M. Sameshima and C. O. Chichester, Int. J. Biochemistry 4, 223-226 (1973).
8. T. Matsuno, T. Kusumoto, T. Watanabe and Y. Ishihara, Nippon Suisan Gakkaishi 39, 43-50 (1973).
9. T. Matsuno and M. Ookubo, Nippon Suisan Gakkaishi 48, 653-659 (1982).
10. T. Matsuno and T. Maoka, Nippon Suisan Gakkaishi 54, 1437-1442 (1988).
11. K. Schiedt, F. J. Leuenberger, M. Vecchi and E. Glinz, Pure Appl. Chem. 57, 685-692 (1985).
12. S. Ando and M. Hatano, Comp. Biochem. Physiol. 87B, 411-416 (1987).
13. T. Fujita, M. Satake, S. Hikichi, M. Takeda, S. Shimeno, H. Kuwabara, W. Miki, K. Yamaguchi and S. Konosu, Nippon Suisan Gakkaishi 49, 1595-1600 (1983).
14. W. Miki, K. Yamaguchi, S. Konosu, T. Takane, M. Satake, T. Fujita, H. Kuwabara, S. Shimeno and M. Takeda, Comp. Biochem. Physiol. 80B, 195-201 (1985).
15. T. Matsuno, M. Katsuyama, T. Maoka, T. Hirono and T. Komori, Comp. Biochem. Physiol. 80B, 779-789 (1985).
16. R. Grangaud, R. Massonet, T. Conquy and J. Ridolfo, Compt. Rend. Acad. Sci. Paris. 254, 579-581 (1962).
17. J. Gross and P. Budowski, Biochem. J. 101, 747-754 (1966).
18. A. B. Barua and U. C. Goswami, Biochem. J. 166, 133-136 (1977).
19. K. Schiedt, M. Vecchi and E. Glinz, Comp. Biochem. Physiol. 83B, 9-12 (1986).
20. B. W. Davies and B. H. Davies, Biochem. Soc. Trans. 14, 952 (1986).
21. M. Katsuyama and T. Matsuno, Comp. Biochem. Physiol. 90B, 131-139 (1988).
22. A. Guillou, G. Choubert, T. Storebakken, J. De La Noue and S. Kaushik, Comp. Biochem. Physiol. 94B, 481-485 (1989).
23. T. Matsuno, E. Yamashita and S. Arai, unpublished data.
24. T. Matsuno, M. Katsuyama and S. Arai, unpublished data.
25. T. Matsuno, M. Katsuyama and S. Arai, unpublished data.
26. T. Matsuno, M. Tsushima and M. Katsuyama, unpublished data.