A Noncarotene Provitamin A for Fishes

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In the course of other studies on the fat metabolism of marine organisms, a routine ether-acetone fat extraction was made of a mixed zooplankton haul consisting predominantly of the calanoid copepods *Temora turbinata* and *Centropages typicus*. The extract was 1.60% of the wet weight of the organisms. It was deep red in color and highly viscous. The oil was 11.6% nonsaponifiable. When assayed with the Evelyn photoelectric colorimeter with filter No. 440, the mixed carotenoid pigment content was shown to be 1.6 mg/g.

From its color and apparent carotenoid nature it was suspected that this material might prove to be effective as a provitamin A in fishes. It has already been shown by Morton and Creed (*5*) that carotene is an acceptable precursor for the formation of vitamin A in certain fishes.

TABLE 1

AVERAGE TOTAL LIVER VITAMIN A

Hours after feeding	Control	Plankton oil	Carotene
0	448		
12		308	950
24	208	2140	356
36	80	3825	...

Accordingly, a group of twenty small *Limanda ferruginea*, maintained in three live cars in the open bay, was divided into three experimental groups as follows: the first lot, containing six animals, was unfed except for occasional small organisms that gained access to the live car between its slats. The second group, six animals, was given a single forced feeding of one gelatin capsule containing 7.5 mg of a mixture of 95% beta- and 5% alpha-carotene dissolved in 0.500 g soya oil. The third set of eight animals each received a single capsule of the zooplankton oil. This contained 0.500 g oil containing a total of 0.800 mg carotene.

The fishes were killed in pairs at intervals of 12 hr after this single feeding. Assays for vitamin A and carotene were run in duplicate on the stomach, the pyloric cecae, the postcecal gut, and on the liver. Assays were conducted by the colorimetric method of the Association of Vitamin Chemists. Carotene was determined in the Evelyn colorimeter with filter No. 440 and a standardization curve.

Results, as they are reflected in the total liver storage of vitamin A, are presented in Table 1. There is a steady, significant increase in total vitamin A content of the livers of those fishes that had been fed plankton oil. The carotene-fed fish show an earlier response. The subsequent marked decrease in liver vitamin A in this group may merely reflect a toxicity which expressed itself more forcibly at 36 hr when the balance of the carotene-fed fish were found dead. Finally there is to be seen a steady progressive decline in the total reserves of vitamin A in the livers of the unfed control fish. This may possibly indicate the rate of utilization of vitamin A by these fish.

In this experiment, then, the administration of plankton oil is followed by a liver response at least 30 times as large as might have been predicted on the basis of the carotene content of the oil administered.

Numerous recent workers (*1, 2, 3, 4, 6, 7*) have adduced evidence to show that the locus of conversion of carotene to vitamin A in the mammal is the mucosa of the small intestine. To determine whether the fish might show similar reactions, homologous segments of the intestinal tract of *L. ferruginea* were tested. The cecal segment of the gut was removed from living fish, and homogenized with minimal quantities of buffered fish saline at pH 6.6. The liver of the same animals was similarly treated. The homogenates were then divided as follows: One lot of cecal brei was allowed to incubate with no added precursor; another lot was provided with plankton oil (0.417 mg/g) in stable emulsion. One lot of liver was incubated with no precursor, and to the other was added plankton oil emulsion in the amount of 0.442 mg/g. A third series was made up of liver and cecal breis mixed approximately equally. Half of this was incubated with no supplementation, and the other received plankton oil in the amount of 0.478 mg/g of mixture.

TABLE 2

RESULTS OF 24-HR INCUBATIONS

Wt of tissue	Additions	Vitamin A/g
2.310 g C.*	none	390
2.400 g C.	0.100 g P.O.†	640
2.150 g L.‡	none	1685
2.260 g L.	0.100 g P.O.	1560
1.122 g C.	none	1127
1.250 g L.	"	
1.220 g C.	0.100 g P.O.	1426
0.870 g L.	" " "	

* C.—ceca.
† P.O.—plankton oil.
‡ L.—liver.

Table 2 presents the results of such incubations. The cecal homogenate increased in potency by an average of 63.2% after the addition of the plankton oil. The liver homogenate showed no increase at all, and the increase in potency in the combined liver and ceca incubation was no greater than would have been expected due to the action of the cecal component alone. This evidence suggests that the locus of conversion of precursor to vitamin A in these forms is probably the small intestinal region which bears the pyloric cecae. Suggestive also is the fact that the conversion was effected *in vitro* in the same qualitative manner as *in vivo*.

In an attempt still further to identify the fraction of the plankton oil which was responsible for this provitamin A activity, chromatographic fractionation was employed using a mixture of 3 parts $CaCO_3$ and 1 part celite as ad-

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sorbent, and petroleum ether as solvent. Both the nonsaponifiable fraction and the whole oil were fractionated. In both instances a significant proportion of the material originally put on the column was not retained but appeared in the filtrate. On removal of the solvent this was a yellow oil which darkened and solidified on refrigeration. This fraction constituted 83.25% of the whole oil chromatogram. The same pale yellow filtrate appeared when the nonsaponifiable fraction was chromatographed. In this instance it constituted 76.4% of the total recovery.

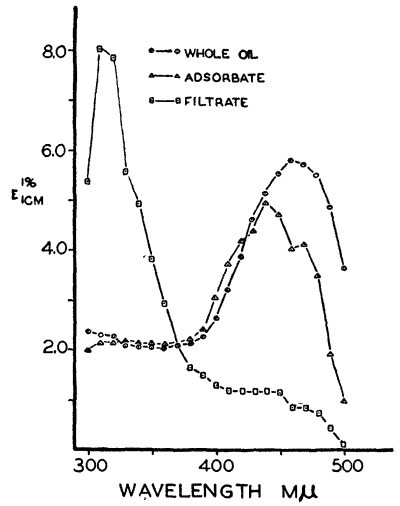

FIG. 1. Absorption spectra of plankton oil and of chromatographic fractions.

Spectrophotometric characteristics of this filtrate, of the combined eluate of the adsorbed pigments, and of the original whole oil are shown in Fig. 1. The adsorbed pigments show typical carotenoid absorption peaks. The primary ingredient of the filtrate material absorbs maximally in the neighborhood of 310 mμ in petroleum ether. There is no evidence of the 325–328 peak characteristic of vitamin A, nor are maxima found in the common carotenoid range.

TABLE 3
EFFECTS OF INCUBATION OF CECAE WITH PLANKTON OIL FRACTIONS

Time in hr	Filtrate	Adsorbate	Control
0	70 vitamin A/g	78 vitamin A/g	68 vitamin A/g
2	91	60	33
4	121	70	24
5	97	34	22

The biological activity of these two chromatographic fractions was determined by incubating them with homogenates of the pyloric cecae. When added to the cecal brei in amounts proportional to their concentration in whole oil the resulting increase in vitamin A potency is represented in Table 3. It will be seen that all the provitamin A activity resides in the noncarotenoid filtrate fraction of the plankton oil.

Thus it appears that some fishes can utilize zooplankton pigments other than the common carotenoids as raw materials for the elaboration of vitamin A.

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Pantothenic Acid in Copper Deficiency in Rats[1]

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According to the work of Free (*1*), the graying effect in rats could be due either to a lack of vitamins or to a deficiency of copper, and of several other elements. Henderson and his co-workers (*3*) reported that supplementation of the diet with 100 μg of calcium pantothenate per day had no effect on preventing the graying of piebald rats on a copper-deficient diet composed of whole milk supplemented with iron and manganese, whereas additions of 50 μg of copper sulfate corrected the condition. Other workers have studied the relationship between panthothenic acid and achromotrichia. Unna and Sampson (*4*) stated that doses of 5, 10, or 20 μg of calcium pantothenate were insufficient to prevent graying, whereas 40 μg gave inconsistent results. György and Poling (*2*) found that 75–100 μg of pantothenic acid daily caused definite restoration of pigmentation in 5–7 weeks when administered to rats deficient in pantothenic acid.

In our experiment, two groups of piebald and black rats, 22 days old, were placed on simplified diets designed primarily to study a comparison of the weight gains of the animals. In each group there were 15 animals with an average initial weight of 36 g. Group 1 was placed on a basal ration composed of whole dried milk (KLIM) 50.0%, sucrose 49.5%, NaCl 0.49%, manganous sulfate 0.0008%, ferrous sulfate 0.002%, and thiamine hydrochloride 0.00034%. Group 2 was fed the basal ration augmented with sufficient copper sulfate to give an analytical value of 20 ppm copper for the ration. The analytical value of copper obtained for the ration of Group 1 was less than 1 ppm. At the end of a 60-day trial, the two groups showed approximately the same rate of weight gain. The animals of Group 1 at the end of 7 weeks showed a consistent peculiar type of graying identical to

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