CXCI. THE RELATION BETWEEN LIPOCHROME PIGMENTS AND VITAMIN A IN THE NUTRITION OF YOUNG FISH.

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INTRODUCTION.

THE large stores of vitamin A found in the liver oils of many species of fish are of peculiar interest when it is borne in mind that frequently only traces of that substance can be detected in the food on which they normally subsist.

An explanation can be found by assuming that a fish such as the cod possesses the power to synthesise the vitamin, or, on the other hand, one can believe that the reserves merely represent an accumulation; it being remembered in the first place that although very little of the vitamin may be present in the individual organisms on which they live, most fish are voracious feeders, and, secondly, that substances such as vitamin A are readily retained by the liver.

An earlier investigation in this laboratory [Coward and Drummond, 1922] indicated that the vitamin A present in the eggs of fresh-water fish (trout) disappeared during the larval period of the development of the young fish. The evidence was, however, derived from rat-feeding tests, the technique of which at that time was much less satisfactory than it is to-day. The differentiation of vitamins A and D had not been established, and it is uncertain, therefore, in the light of recent knowledge whether the conclusions reached were justified.

It must be stated that there is growing support for the view that accumulation does occur. An investigation by MacPherson [1933] of the Dominion of Newfoundland Fishery Research Station has recently indicated that a correlation can be traced between the age of fish (length) and the reserves in the liver. Similar accumulation appears to occur also in man [Wolff, 1932; Moore, 1932].

From several points of view it appeared that the changes during the early stages of the development of young fish might be studied again with advantage, primarily because it was desired to apply the modern methods of spectroscopic assay of vitamin A, but also because we had in mind the extension of this method to the lipochrome pigments which might be biologically concerned.

The relation between carotene and vitamin A in the nutrition of mammals is now generally recognised, but there are reasons for thinking that equally important relationships in which other lipochrome pigments are concerned may be exhibited by other species.

Already it is suspected that xanthophyll, which was believed to be of far less physiological importance than carotene, may have a particular function in the growth processes of the domestic fowl and possibly also of certain herbivorous species such as the guinea-pig [Rydbom, 1933].

It appeared to be necessary, therefore, to ascertain whether any of the curious and little-studied lipochromes found so widely distributed amongst aquatic species are converted into vitamin A in the tissues of fish such as the cod. A related problem, which is not only of considerable interest but also of great practical importance, is to determine whether the red lipochromes present in such organisms as *Calanus* or *Euphausia*, which incidentally are known to differ in several respects from the recognised pigments of that class, are the origin of the large amount of vitamin A found in the livers of those species of whales which feed almost exclusively on zooplankton.

EXPERIMENTAL.

A convenient material for this investigation was available in the form of the fertilised eggs of trout. Both brown trout and rainbow trout were employed. The technique of hatching the eggs and of raising and feeding the young fish was that described in the communication already referred to [Coward and Drummond, 1922]. Periodically during the larval period a number of the young fish were removed, killed and the fatty materials extracted and examined. As soon as the fry were able to take solid food they were divided into three groups and fed on the following diets:

Group I. Pulped rat-liver.

Group II. Finely divided fresh cod-muscle.

Group III. Cod-muscle and fresh-water green algae.

The object of the third group was to ascertain whether the very young fish could convert the carotene or xanthophyll of the green algae into vitamin A, there being evidence that normally at this early period of their development they ingest a certain amount of "green" food. The alga employed was a motile form of *Chlamydomonas*, cultivated in tap-water¹. A culture of it was poured into the circulating water in the trout "ponds" each day at the same time as the finely divided cod-muscle was being given. Microscopic examination of fish revealed that green cells had been swallowed and digested but we had no means of judging how much was eaten. We suspect that it was quite a small amount by comparison with the other food consumed.

Again, at intervals specimens were taken for extraction and examination of the fats. In Fig. 1 there is plotted the absorption spectrum of the extract pre-

Fig. 1.

pared from the eggs of the brown trout. The absorption in the visible region does not entirely correspond with that of carotene. Saponification of the extract and application of the phase test revealed that the orange-red pigment is preferentially soluble in the light petroleum layer and, therefore, presumably it is a hydrocarbon or, at any rate, a lipochrome of low polarity.

¹ We are indebted to Prof. Salisbury for identifying the organism we employed.

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A comparison of this curve with that recorded by Lovern and Morton [1931] for a pigment found in monk fish-liver oils shows a striking similarity.

The exhaustive researches of Lönnberg and Hellström [1932] on the pigments of fish suggest that this fat-soluble colouring matter may be widely distributed.

The absorption spectrum of the extract from the eggs of the rainbow trout is similar to that of the brown trout eggs, but much less pigment is present.

In Table I the absorption at 4800 Å. near the maximum of the carotenoid band and the absorption at 3280 Å. have been evaluated for the brown trout. The figures express the extinction coefficient for a concentration where 1 cc. of extract (CHCl₃) is equivalent to 1 g. of fish.

Table I.

1	2	3	4	5	6	7	8	9
Group	Days from commencement	Absorption at 4800 Å.	Absorption at 3280 Å.	Corrected value at 3280 Å.	Weight	Weight × absorption at 4800 Å.	Weight × corrected absorption at 3280 Å.	Column 7 + column 8
Eggs	0	2.6	2.0	0	0.083	0.215	0	0.215
Larval trout	6	2.2	2.08	0.39	0.091	0.200	0.0355	0.236
with egg sacs	15	2.2	2.10	0.41	0.096	0.211	0.0394	0.250
	23	1.8	1.80	0.41	0.108	0.204	0.0442	0.248
	30	1.1	1.4	0.55	0.136	0.150	0.0748	0.225
Feeding begun:								
Group I	37	0.98	1.96	1.21	0.126	0.124	0.1525	0.277
	51	1.12	2.08	1.22	0.104	0.117	0.1269	0.244
	62	0.70	2.10	1.57	0.140	0.098	0.2198	0.318
	76	0.325	5.20	4.95	0.165	0.054	0.8168	0.871
	92	0.82	10.70	10.07	0.162	0.133	1.6313	1.734
- A for 16 days	92	0.72	8.4	7.85	0.143	0.103	1.1221	1.225
Group II	37	0.82	1.3	0.67	0.152	0.125	0.1018	0.227
	51	1.06	1.9	1.08	0.104	0.110	0.1123	0.222
	62	0.45	1.92	1.57	0.120	0.054	0.1884	0.242
	76	0.35	1.62	1.35	0.138	0.048	0.1863	0.234
Group III	37	0.98	1.20	0.55	0.128	0.125	0.0704	0.195
	51	1.06	1.85	1.03	0.104	0.110	0.1071	0.217
	62	1.15	2.22	1.33	0.095	0.109	0.1264	0.235
	76	0.40	2.01	1.70	0.120	0.048	0.204	0.252

Mean for eggs and Groups II and III: 0.231

While the absorption at 4800 Å. is a measure of the concentration of pigment, the figure obtained at 3280 Å. must be regarded as probably compounded of three components—the absorption of any vitamin A present, the absorption due to the pigment and that due to other irrelevant chloroform-soluble materials. If the absorption of the pigment in this region is subtracted from the gross absorption, the resulting values represent changes in the content of vitamin A provided that the irrelevant substances are present in constant concentration. This latter requirement has been assumed in the results to be described, but the possibility of variations in concentration of substances other than vitamin A absorbing at 3280 Å. cannot be disregarded.

It was necessary, therefore, to allow for the absorption at 3280 Å. of the pigment, but since there was always the possibility of the presence of some vitamin A in the extracts of the eggs, an upper limit only could be set for this absorption, i.e. the extinction coefficient where the ratio of absorption at

3280 Å. to absorption at 4800 Å. is at a minimum. This condition was fulfilled in the earliest experiment on the eggs.

It will be seen (Table I) that the ratio is 0.77, so that we have assumed that at this stage the vitamin A absorption is zero, and all subsequent values at 3280 Å. have been "corrected" in a similar manner by subtracting from them 0.77 of the absorption at 4800 Å. It cannot be claimed that the "corrected" values express true absorption due to vitamin A alone, but we have eliminated by this means any variations brought about by the pigment, so that those which do occur probably arise from varying concentration of vitamin A. By multiplying this value by the mean weight of the fish, figures are obtained which represent the amounts of carotenoid (column 7) and of vitamin A (column 8) present in each fish.

In Table I it will be seen that in the young fish before feeding began the content of pigment decreased, but that this was accompanied by a corresponding rise in vitamin A content. This state of affairs continued throughout the tests on groups II and III, the two types of food showing little difference. In column 9 the sums of the values contained in columns 7 and 8 are given and these show remarkable constancy, indicating that as absorption at 3280 Å. increases, an approximately equal decrease at 4800 Å. occurs. One explanation of this would be that the pigment is being converted into vitamin A. Now $E_{1\text{ cm.}}^{1\%}$ for carotene is about 2500 at 4800 Å. [McNicholas, 1931], while for the purest vitamin A preparation $E_{1\text{ cm.}}^{1\%}$ is 1600 [Carr and Jewell, 1933]; thus we should expect for efficient conversion of carotene into vitamin A a rather greater decrease in absorption at 4800 Å. than the increase at 3280 Å. Since it is reasonable to expect that the pigment of the trout is roughly of the same absorbing power as carotene, the conversion appears to be an efficient one.

Group I fed with rat-liver showed a substantial increase both in weight and in vitamin A content as compared with the fish fed on cod-muscle. When the former were deprived of liver and were given only cod-muscle for 16 days they decreased considerably in weight and showed a reduced amount of substance absorbing at 3280 Å.

The results with the rainbow trout are expressed in the form of graphs. In Fig. 2 the superior growth on the liver diet is well marked and there is

Fig. 2.

●—● Cod-muscle diet. — Rat-liver diet. —●—● Cod-muscle diet + algae.

evidence that supplementing the "white fish" diet with green algae was beneficial. From the curves in Fig. 3, there is also an indication of storage of carotene

Fig. 3. (a) Maintained on liver diet, (b) transferred to cod-muscle diet.

•—• Cod-muscle diet. —•—• Cod-muscle diet + algae. •—• Rat-liver diet.

Fig. 4. (a) Maintained on liver diet, (b) transferred to cod-muscle diet.

•—• Cod-muscle diet. —•—• Cod-muscle diet + algae. •—• Rat-liver diet.

or a similar pigment by the fish in group III. In Fig. 4 the vitamin A content in the group fed with rat-liver is shown to rise very steeply, and to fall as sharply when deprived of vitamin A for 16 days by feeding exclusively on the cod flesh.

diet. The concentration attained would appear therefore to be abnormally high. Corresponding with the rapid decline in the amount of vitamin A in the case of fish deprived of liver is a sharp decrease in weight (Fig. 2).

It will be noticed that in groups II and III the reciprocal effects of decreasing pigmentation and increasing nett absorption at 3280 Å. indicated in Table I, are also apparent (Figs. 3 and 4). It would seem, however, that the rainbow trout eggs originally possess less pigment but more vitamin A. The mean value for the constant corresponding to that in column 8 of Table I is considerably lower (0.181). For group I this figure rapidly increased until it was greater than for the brown trout. The relative vitalities of the two kinds varied in like manner. In the initial stages the rainbow trout had a high mortality but in later stages they were more hardy than the brown variety, a fact which would indicate that the "constant" referred to may be a measure of vitamin A reserves.

It is apparent that our conclusions are at variance with those derived from the previous investigation in this laboratory [Coward and Drummond, 1922] but as we have already remarked, the feeding tests which formed the basis of those experiments are open to criticism in the light of present day knowledge.

SUMMARY.

1. The ova of brown and rainbow trout contain a pigment similar to, but not identical with, carotene. It is probably a hydrocarbon¹.
2. In the development of the young fish the amount of this pigment decreases, and there is a corresponding increase of absorption at 3280 Å., suggesting a conversion into vitamin A.
3. Indications were obtained that the lipochrome content of young fish may be increased when they are fed on a diet containing a typical green alga (*Chlamydomonas*). It is uncertain whether the pigment stored is unchanged carotene or a substance showing similar absorption.

¹ [Note added in proof.] Kuhn and Lederer [*Ber. deutsch. chem. Ges.* 1933, **66**, 488] have recently described a pigment "astacin" isolated from the lobster. Its formula is given as $C_{27}H_{32}O_3$ and a lactone structure is indicated by the fact that its distribution in the phase test can be reversed by changing the reaction. It is possible that this pigment is related to that we have described.

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