CXLV. PIGMENTATION OF THE LIVER OF THE MONK (OR ANGLER) FISH.

By JOHN ARNOLD LOVERN and RICHARD ALAN MORTON.

From the Torry Research Station, Aberdeen, and The University, Liverpool.

(*Received July 3rd, 1931.*)

In studying the livers of various species of fish, one of us (J. A. L.) noticed the frequent occurrence of a pronounced pink pigmentation in the interior portions of the liver of the monk fish (*Lophius piscatorius*). The livers of gadoid fish, such as codling, hake, *etc.*, are of uniform colour throughout, being in most cases almost white. Colouring is sometimes seen in the Elasmobranchii (*e.g. Squalus acanthias* and *Raia clavata*) but as a rule the livers are very pale. The exceptions are mottled in appearance, and the pigmentation is due to a grey-black substance, which is not extracted along with the oil.

The pigmentation of monk-livers is, therefore, a distinct and unusual phenomenon. The colouring matter is localised in irregularly shaped deposits found much more often in the interior than in the exterior portions of the liver. The intensity of the pigmentation varies between wide limits, not only from fish to fish, but also for the localised deposits in a particular liver. In an absolutely fresh liver the intensity of the pigment is much less than in the same liver after exposure to air, and oxidation thus appears to play a part in its formation.

Histological examination of the pigmented areas shows that not all the cells are pigmented, even in such areas. The distribution of pigment appears to be quite irregular. Under high power the pigment is seen to be present in the form of deep orange droplets (possibly dissolved in oil), of various sizes, but of the same order of magnitude as the liver cells. It is worth noting that the formaldehyde fixative used did not appreciably bleach the pigment. Bleaching experiments with a strong solution of ferric chloride in 50 % alcohol, in which pigmented histological sections were immersed, revealed a fairly rapid and complete bleaching. Two examples may be given.

Section from liver No. 1.

Pigmented section after 5 minutes in $FeCl_3$	No appreciable bleaching
Same section after 20 minutes in $FeCl_3$	Complete bleaching of pigmented areas

Section from liver No. 2.

Pigmented section after 5 minutes in $FeCl_3$	Some bleaching
Same section after 20 minutes in $FeCl_3$	Mainly bleached
Same section after 30 minutes in $FeCl_3$	Completely bleached

The pigment accompanies the oil when the livers are extracted by means of solvents. It is noteworthy that oil cannot be obtained from these livers by steaming, although the oil content is frequently as much as 50 %. In one experiment a small portion of the unsaponifiable matter was prepared and found to contain much of the pigment present in the original oil. This unsaponifiable matter (mainly cholesterol) was spread in a thin film on a glass slip and exposed to air and sunlight for some days. Bleaching was only gradual, over a week elapsing before the colour had all disappeared.

The nature of this pigment has not yet been fully elucidated, and the investigation is being continued in the hope of isolating a quantity sufficient for identification. Some interesting properties have, however, already emerged. Previously to commencing the study of monk-livers it had been found that oils prepared from the various parts of any particular fish-liver gave the same intensity of blue colour with antimony trichloride. In the case of the first three monk-livers examined, however, the following results were obtained (J. A. L.).

		Blue value (Carr-Price)
Liver (1)	Oil from pigmented interior	9·6
	Oil from non-pigmented exterior	3·6
Liver (2)	Oil from pigmented interior	4·4
	Oil from non-pigmented exterior	2·4
Liver (3)	Oil from pigmented interior	14·0
	Oil from non-pigmented exterior	3·2

In view of the well-known chromogenic properties of carotenoids it appeared worth while to investigate whether the pigment was carotenoid in type, especially since in the case of a monk-liver which apparently contained no pigment at all, the oils from the inside and outside layers gave the same intensity of blue colour. However, in later experiments it was found that this difference between the chromogenic power of the oils from the pigmented and non-pigmented parts is not always observed. Thus, in the case of a liver having a very intense localised pigmentation, the oils from the red and white parts of the liver respectively gave the same blue colour in the antimony trichloride test, namely 2·8 Carr-Price units. The intensities of the blue colours were thus not proportional to the redness of the oils.

It was decided to examine the oils spectroscopically in order to see whether additional evidence as to the nature of the coloured substance could be obtained. The following samples were studied.

Sample (A). The mixed oils from livers (1), (2) and (3). (These were unfortunately mixed for other purposes before experiments on the nature of the pigment were undertaken.)

Sample (A′). The unsaponifiable matter from A, dissolved in chloroform.

Sample (B). Oil from the inside of a monk-liver.

Sample (C). Oil from the outside of the same liver exhibiting little or no pigmentation.

Sample (D). Oil from non-pigmented parts of a liver with deeply pigmented deposits.

Sample (E). Oil from pigmented parts of the same liver.

Sample (F). Ether-extracted oil from several complete livers exhibiting a fairly uniform distribution of a rather low intensity of pigmentation.

All the oils showed the band at 328 $m\mu$ as a faint inflexion, superimposed on much more intense general absorption.

Samples (A), (E) and (F) gave bands with maxima at 480–485 $m\mu$, 450–457 $m\mu$ and 422–430 $m\mu$, in the same region and of the same type as those given by carotenoid pigments such as carotene or xanthophyll. At the same time the maxima do not agree exactly with those of carotene dissolved in an oily medium (*e.g.* arachis oil). Identification of definite members of the carotenoid group is a difficult matter, especially in the presence of vitamin A,

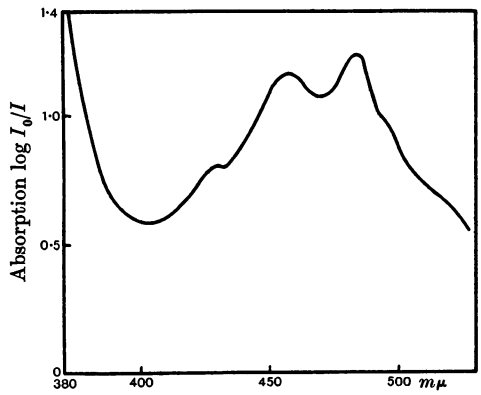

Fig. 1. Monk-liver oil F_1. $\frac{1}{2}$ mm. layer.

so that although the evidence suggests a carotenoid, it is by no means conclusive. It may be significant that materials containing the pigment (both in oils and extracts of the unsaponifiable matter dissolved in ether or chloroform) respond to many of the colour tests for carotenoids, *e.g.* with strong sulphuric acid there is a gradual development of an intense green colour, which, although gradually fading, persists for 24 hours. There is some doubt as to the product of the interaction of the pigment and antimony trichloride. The blue solution obtained with carotene exhibits a maximum near 590 $m\mu$, and this could not be detected, whilst the blue colour with monk-liver oil exhibited the two bands with maxima at 572 $m\mu$ and 606 $m\mu$ respectively associated with vitamin A. The presence of these maxima may mask absorption in the region of the 592 $m\mu$ band and render detection difficult.

It would thus appear that whilst some of the evidence suggests a carotenoid nature for the pigment, there is no satisfactory evidence as to any particular carotenoid—as stated above it is hoped to isolate the pure pigment in sufficient quantity for identification. If it should prove to be carotenoid in nature, it

may well provide additional evidence in the carotene-vitamin A problem. It is interesting to note that the vitamin A content of the livers of *Lophius piscatorius* is generally low compared with other species.

Experiments on monk-liver oils.

Oil	$E^{1\%}_{1\,cm.}$ 328 $m\mu$ (gross)	Bands in the visible spectrum	$E^{1\%}_{1\,cm.}$ 572 $m\mu$	$E^{1\%}_{1\,cm.}$ 606 $m\mu$
A	1·4	480, 450, 422 $m\mu$ (½ mm. oil itself)	1·0	0·55
Unsaponifiable matter	—	482, 452, 420, 400 $m\mu$	—	—
B	0·26	Ill-defined	0·24	0·2
C	—	Ill-defined	0·2	0·2
D	0·7	Inflexion only	0·42	0·12
E	0·85	485, 455, 422 $m\mu$ (½ mm. oil itself)	0·44	0·24
F	0·82	485, 455, 430 $m\mu$ (½ mm. oil itself)	0·825	0·25

Discussion.

The spectroscopic data on the monk-liver oils indicate that the vitamin A potency varies between 0·2 and 0·8 of that associated with an average cod-liver oil (1·0 spectroscopic unit or about 12–13 Carr-Price units). The particular samples studied all showed a clear maximum at 572 $m\mu$ in the antimony trichloride colour test, but the capacity to give the more usual 606 $m\mu$ maximum was latent [*cf.* Heilbron, Gillam and Morton, 1931]. The ultra-violet absorption band at 328 $m\mu$, characteristic of vitamin A appears to be present in the intensity to be expected from the colour test in all the oils, but owing to superimposed, more general absorption, the band only appears as a marked inflexion.

There appears to be no direct connection between the pigment and vitamin A, as the non-pigmented oils cannot be differentiated from the pigmented oils by any property other than colour.

The pigment resembles carotene in its absorption in the visible spectrum. Since 0·5 mm. layers of different oils exhibit the three bands at 480, 450 and 422 $m\mu$, the points of maximum absorption corresponding with

$$E = \log I_0/I = 0{\cdot}8 - 1{\cdot}2,$$

we have

$$E^{1\%}_{1\,cm.}\ 450\,m\mu = 0{\cdot}16 - 0{\cdot}24.$$

Now for carotene itself $E^{1\%}_{1\,cm.}$ 450–460 $m\mu$ = 1400, whilst the intensity of the blue solution obtained with carotene and antimony trichloride is of the order $E^{1\%}_{1\,cm.}$ 590 $m\mu$ = 300–400. Accepting the higher figure, we can calculate approximately the intensity of a blue colour due to the quantity of pigment present in monk-liver oil on the assumption that it is carotene. The value for $E^{1\%}_{1\,cm.}$ 590 $m\mu$ is 0·04 to 0·07. Now in the present series of monk-liver oils the values for $E^{1\%}_{1\,cm.}$ 572 and $E^{1\%}_{1\,cm.}$ 606 $m\mu$ are never less than 0·20. Hence we have

the rather important conclusion that, deeply pigmented as the monk-oils are, the quantity of vitamin A present even in oils having only one-fifth of the potency of cod-liver oil, is sufficient to give a blue colour from 3 to 6 times as intense as that to be expected from the pigment on the basis that it is a carotenoid. It is thus not very surprising that the pigment did not give rise to a distinct detectable band in the colour test.

There is now considerable evidence that carotene is heterogeneous, containing two or more closely related substances. From the present point of view this fact increases the difficulties in identifying the pigment of monk-livers. We are endeavouring to secure pigmented monk-liver oils which are either very poor in vitamin A or relatively very rich in pigment, but, as the conditions governing seasonal and other variations in potency and pigmentation are unknown, the test may well prove difficult.

SUMMARY.

Monk-livers often contain irregular deposits of a red fat-soluble pigment, hitherto not found in fish-livers. The pigment appears to act as a chromogen towards antimony trichloride, but quite small quantities of vitamin A are so intensely chromogenic as to obscure the rôle of the pigment. Such evidence as is available points to the pigment being carotenoid in type.

We are indebted to the Department for Scientific and Industrial Research for support granted to this investigation.

REFERENCE.

Heilbron, Gillam and Morton (1931). *Biochem. J.* **25**, 1352.