Examination of some Components of Cephalopod and Sperm-Whale Liver Oils by the Chromatographic Method

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The whales commercially taken in Antarctic waters are of two main types, the whalebone whales (*Mystacoceti*), represented chiefly by the blue (*Balaenoptera musculus*), fin (*B. physalus*) and humpback (*Megaptera nodosa*), and the toothed whales (*Odontoceti*) represented only by the sperm (*Physeter catodon*).

The whalebone whales of Antarctic waters live almost entirely on krill (*Euphausia superba dana*), and the presence of preformed vitamin A in these creatures has been demonstrated by Batham, Fisher, Henry, Kon & Thompson (1951). The oils derived from all these whales are so similar as to justify their being mixed.

By contrast, however, the sperm whale feeds, in most if not all areas of the Antarctic, almost exclusively on cephalopods of the '10-armed' variety. The oil derived from the sperm whale is very different from that of the whalebone whales, being rich in hexadecyl alcohol and other waxy substances, and is carefully kept separate.

It was decided to examine the liver of the cephalopod to confirm the presence (long suspected in the whaling industry) of preformed vitamin A. At the same time the livers both of the cephalopod and the sperm whale were examined for carotenoids and derivatives of vitamin A. A sample of salps was also examined.

Most of this work was carried out on the Antarctic whaling grounds on board the floating factory ship *Balaena* during the 1951-2 whaling season. Detailed accounts of modern whaling operations are published by Cockrill (1951) and Sharp & Marsh (1952).

Materials and methods of collection

The only convenient source of cephalopods is the stomach of the sperm whale. Well-preserved specimens are rare, though examples up to about 3 ft. long are sometimes found practically intact and have previously been preserved and brought home for examination. Fragments of much larger specimens have been found, and by simple proportion it has been calculated that some of these would have body lengths of about 14 ft. (4.3 m.), implying

that the two long arms would be an additional 14 ft. (4.3 m.) long. Large quantities of the indigestible portions (beaks, eye lenses, gladii, etc.) are always found.

Dissection of an externally intact example often showed that the liver was in process of consumption by parasitic worms which occur in large quantities in the whale stomach. To secure a suitable liver it was, therefore, essential (i) that the whale should have eaten shortly before its death, (ii) that the interval between shooting and dissection on the deck of the factory ship should not exceed 3 hr., and (iii) that during the shooting and towing to the factory the whale should not disgorge its latest meal. Such a combination of circumstances was rare and only one suitable cephalopod was secured during the 1951-2 season. This creature was dissected by Mr S. Brown of the Discovery Committee and found to be a female of the species *Moroteuthis ingens* Smith with overall length of 36 in. (90 cm.). The only portion of the cephalopod so far examined is the liver, though other parts may contain vitamin A. The eyes are of interest since they are structurally not unlike the human eye and are very large.

On one occasion it was possible to examine the stomach content of a cephalopod, and this appeared to consist of fragments of krill. A readily accessible elementary account of the cephalopod has been published (Buchsbaum, 1951).

Sperm-whale liver. This was removed during dissection and portions weighing 0.5-1.0 kg. were brought to the laboratory, sampled, and worked up as quickly as possible. The liver portions are usually taken from the centre of the most readily accessible lobe, experiments having shown that the concentration both of oil and of vitamin A is subject to variation in different parts of the liver.

Salps. These were taken, among a variety of other creatures, by means of a small conical net towed occasionally from the stern of the factory ship. It was hoped to obtain fresh krill by this method, but in fact none was caught. The plankton occurred in large numbers in reddish patches on the sea surface. Such patches were previously considered to consist invariably of krill.

EXPERIMENTAL

Ultraviolet and visible absorption. A Beckman quartz spectrophotometer was used.

Solvents. Commercial cyclohexane, redistilled, was satisfactory for wavelengths above 260 $m\mu$. Light petroleum (b.p. 40–60°), *n*-hexane and absolute ethanol were used as supplied. CHCl_3 was washed repeatedly with water, dried over CaCl_2 and redistilled. Ether was redistilled from $\text{Fe}(\text{OH})_2$.

Liver from cephalopod. The material was brown and not very coherent. It weighed about 300 g. and was contained in a sac of tissue. It was removed piecemeal and then frozen. A portion was ground with Na_2SO_4 and the oil extracted with peroxide-free ether in a Soxhlet apparatus. The material was examined both in the visible and ultraviolet regions.

Saponification of liver oil. (Gridgeman, Gibson & Savage, 1948). This method was found satisfactory for most of the materials examined. The oil (about 5 g.) was refluxed gently with conc. aqueous-ethanolic KOH for 25–40 min. and, after dilution with ethanol and water, so that there was a soap concentration of 2–4%, it was extracted with equal volumes of diethyl ether and light petroleum. Provided shaking was moderate, emulsions were avoided; four extracts were combined and the solvent removed, the last traces being blown off with N_2 .

Preparation of a solution containing astacene (Karrer & Jucker, 1950, pp. 21, 233). The cephalopod liver was extracted in the cold with acetone (gentle swirling was sufficient to cause the material to break up), and an equal volume of light petroleum added. On dilution with water, the pigment passed into the light petroleum layer. This was separated, washed first with water, then 90% methanol and then 2*N*-NaOH, together with sufficient ethanol to make the solution homogeneous. After standing in the dark for 5 hr., the solution was diluted with water and the upper layer separated. This was repeated and a further quantity of light petroleum separated. The ethanolic liquor was acidified with 20% (w/v) acetic acid, and a red oil separated. This was removed and, on washing with water, a quantity of white solid appeared, forming an orange emulsion. The white solid did not dissolve on making alkaline, and was removed by filtration. The filtrate was diluted and made slightly acid with 20% acetic acid. The colour readily passed into CHCl_3 and three extractions were made with this solvent.

The CHCl_3 extracts were combined, dried over Na_2SO_4 and the solvent removed, leaving a crystalline residue; 22.32 g. of liver gave 3.198 g. of this material.

Isolation of unsaponifiable matter from sperm liver

At first, direct saponification of raw liver was tried, but it proved difficult to secure complete reaction with the considerable quantities involved. It was found better to undertake batchwise Soxhlet extractions of the raw liver, usually for 4 hr., prior to saponification of the liver oil.

Chromatography of unsaponifiable matter (Gridgeman *et al.* 1948). The material is chromatographed on alumina from light petroleum solution, and developed with more light petroleum. If the column size and strength of alumina are correctly chosen, a yellow band consisting of oxidized material passes down the column and comes almost to rest about one-half to two-thirds of the way down, while weakly adsorbed substances like anhydrovitamin A are washed out of the column. Elution is effected with gradually increasing

amounts of diethyl ether in the light petroleum, and a fraction consisting of 'pure' vitamin A emerges just after the yellow band has left the column. With factory-produced liver oil this fraction is sufficiently pure (i.e. exhibiting the normal vitamin A absorption spectrum) to conform with the requirements of the World Health Organization Report (1950), the shape of the absorption curve being within 2% of that found by Morton & Stubbs (1948) between 310 and 350 $m\mu$. Following the vitamin A are the kitol and steroid fractions. The matter is more fully discussed below (p. 462).

Reaction with ethanolic hydrogen chloride. The solvent-free material is treated with 0.03*N* reagent at the rate of about 50 ml./g. and left to stand in the dark for 40 min. The material is extracted at least three times with diethyl ether and the combined extracts repeatedly washed with water to remove inorganic matter. The extract is dried over anhydrous Na_2SO_4 , the ether removed and chromatography carried out using the strongest adsorbent alumina available. The absorption spectrum of the first material to emerge is examined for the anhydrovitamin A maxima. Its elution can be seen from its orange fluorescence in ultraviolet light (Edisbury, Gillam, Heilbron & Morton, 1932; Shantz, Cawley & Embree, 1943).

Further chromatography of eluted materials. Further chromatography was usually carried out by the method of Karrer & Jucker (1950). A glass tube about 20 cm. long and 1 cm. int. diam. is plugged at the lower end by means of cotton wool and inserted into a bored rubber bung carrying a piece of glass tube of smaller diameter. This also carries another bung which can be inserted into a Büchner flask. The tube is filled with suitable alumina in small batches, using pressure from above and suction from beneath. The solution is poured on to the top of the column (the surface of which is protected by a thin layer of kieselguhr) and viewed if necessary in ultraviolet light. The chromatogram is developed with light petroleum or *n*-hexane, and when the bands are suitably separated the tube is removed and the contents extruded. The zones are separated by means of a spatula, and the various fractions are eluted in stoppered flasks with diethyl ether. After filtration the ether is removed under reduced pressure and the material examined with the Beckman spectrophotometer.

RESULTS

Liver of cephalopod

This was recovered from a 51 ft. male sperm whale on 21 December 1951, in position approximately 65° S., 165° E. It weighed about 300 g. and was found to resemble a fish liver in that the fat content was high (about 56%). The absorption spectrum of the oil was examined both in the visible and the ultraviolet regions.

The fat was reddish orange in colour and had a maximum in the visible region at 473 $m\mu$. with $E_{1\%}^{1\text{cm.}} = 0.11$. Karrer & Jucker (1950) quote 510 $m\mu$. as the maximum for astacene in carbon disulphide, but give no information for *n*-hexane. However, (on p. 57) they give a list showing maxima for various compounds in several solvents and say that, if carbon disulphide be replaced by hexane, a displacement of 30–40 $m\mu$. towards shorter wave-

lengths is to be expected. There is thus some evidence here for the presence of astacene, and this is supported by its behaviour on distribution between various solvents. Astacene is almost the only carotenoid which remains in the lower layer after shaking with light petroleum and alkali; this results from the formation of the sodium or potassium salt.

In the ultraviolet region (solvent, *n*-hexane), a series of small maxima was observed at 271.5, 283,

Fig. 1. Ultraviolet absorption spectrum of light petroleum-soluble matter from saponification of oil from cephalopod liver. (Solvent, chloroform.)

300.5 and 317.5 $m\mu$. The 283 $m\mu$ maximum was the greatest and had $E_{1\text{cm}}^{1\%} = 0.28$. A further quantity of liver was extracted and the oil saponified using the method of Gridgeman *et al.* (1948). The unsaponifiable matter was heterogeneous, consisting of reddish fluid and a white solid. The greater part of the red matter of the original oil, however, remained in the alkaline layer after saponification and extraction. The extract was taken up in chloroform and the absorption spectrum examined. This showed maxima at 276, 287, inflexions at 301–306 and the highest maximum at 326–331 $m\mu$, where $E_{1\text{cm}}^{1\%}$ was 2.78 (Fig. 1). The visible absorption spectrum showed faint inflexions only at 425, 450, 475 $m\mu$, and these may point to the presence of a carotenoid in very low concentration.

A portion of liver was treated by the method of Karrer & Jucker (1950), and the resulting material gave (in chloroform) a maximum at 488 $m\mu$ with $E_{1\text{cm}}^{1\%} = 0.18$. The maximum is not sharp. There is as yet no evidence for the presence of astaxanthin in the original material.

Confirmation of presence of vitamin A

A belief that the liver of this species contains vitamin A has been held for many years. This belief is based on the Carr-Price test and on the ultraviolet-absorption spectrum of ethereal extracts. To con-

firm the presence of vitamin A, some of the unsaponifiable fraction was treated with anhydrous ethanolic hydrogen chloride and the absorption spectrum of the product examined for evidence of the presence of anhydrovitamin A. Absorption curves showing the effect of 15 min. treatment with 0.03 *N*-ethanolic hydrogen chloride are reproduced (Fig. 2). For further confirmation, some more material was allowed to stand with this reagent for 40 min. and the product in *n*-hexane was then chromatographed on weakened alumina. Development was with *n*-hexane, followed by *n*-hexane containing gradually increasing amounts of diethyl ether, then pure ether.

Fig. 2. Effect of reaction of ethanolic hydrogen chloride on a solution supposed to contain vitamin A. Time of contact 15 min. (Solvent, *n*-hexane.) $\circ$ — $\circ$, Without ethanolic HCl; $\times$ — $\times$, with ethanolic HCl.

The following fractions were collected in 5 ml. tubes:

Tube nos.	Carr-Price spot test	Fluorescence in ultraviolet light
1–5	Pale blue	Orange
6	Nil	Nil
7–10	Pale blue	Intense yellow
11–14	Pale purple	Nil
15–17	Nil	Nil
18–20	Pale blue	Intense yellow
21–30	Nil	Nil

Some pink material remained uneluted at the top of the column. The absorption curve of tubes 1–5 closely resembled that of impure anhydrovitamin A. It had the following characteristics (solvent, *n*-hexane; Fig. 3; *E* values are referred to weight of reaction product):

Wavelength of maxima ($m\mu$)	$E_{1\text{cm}}^{1\%}$	$E_{\lambda_{\text{max}}}/E_{371}$
353.5 (λ_a)*	1.24	0.743
371 (λ_b)	1.68	1
392 (λ_c)	1.34	0.798
Slope change at approx. 332.5–340 $m\mu$. (λ_1).		
* See p. 464.		

Shantz *et al.* (1943) give the following figures for anhydrovitamin A in ethanol: λ_{max} 351, 371, 391 (relative *E* values 0.685, 1.0, 0.871). The change of slope at about 335 $m\mu$ is usual in preparations

believed to contain some *iso*anhydrovitamin A. There may, however, be less of that material present than usual since E_{392} is greater than $E_{383.5}$ approximating more closely to the figures for anhydrovitamin A.

Fig. 3. Ultraviolet absorption spectrum of anhydrovitamin A from chromatogram of product of reaction between ethanolic hydrogen chloride and material from cephalopod believed to contain vitamin A. Time of contact 40 min. The slope change at approx. 330–340 $m\mu$. can be clearly seen. (Solvent, *n*-hexane.)

The remaining tubes also showed features of interest (solvent, *n*-hexane):

Tube nos.	$\lambda_{\max.}$ ($m\mu$.)	$E_{1\text{ cm.}}^{1\%}$ at $\lambda_{\max.}$	Inflexions ($m\mu$.) (approx.)
7–10	327.5	0.85	360
11–14	298	0.44	315, 330
19	330.5	5.8	347.5, 362.5

Carr-Price colour, shape of curve and lack of fluorescence give little help in identifying the materials contained in tubes 11–14. In the case of tubes 7–10 and 19, however, it is possible that there is some correspondence with the work of Karrer (1947). Thus, in the reaction of chloroform and acid with vitamin A epoxide, he obtained two substances: (1) had maxima at 333.5, 350 and 367.5 $m\mu$., and agreed with $C_{20}H_{30}O_2$ (solvent, ethanol); (2) had the same spectrum as vitamin A but was biologically inactive.

(1) would seem to correspond with tube 19, and (2) with tubes 7–10.

Evidence for the presence of provitamin D

The solvent was removed from the contents of tubes 21–30 and the residue taken up in ethanol. In the ultraviolet region, this solution gave the greatest $E_{1\text{ cm.}}^{1\%}$ value (1.03) at 282 $m\mu$.

Wavelengths ($m\mu$.) of maxima in ethanol

Provitamins D (Rosenberg, 1945)	260	270	281	293.5
This material	Obscured	272	282	292

This is the only evidence so far forthcoming for the presence of a provitamin D in Antarctic cephalopods. Its presence was expected, however, since similar and better defined absorption curves were obtained on chromatography of the unsaponifiable matter from two cephalopods (*Loligo forbesii* and *Sepia officinalis*) from the Plymouth area. In this case, however, there were greater concentrations of vitamin D and provitamin D while vitamin A was not found.

The cephalopod liver appeared to contain no kitol.

Liver of sperm whale

The chromatographic method of Gridgeman *et al.* (1948) is primarily intended for the isolation of vitamin A alcohol from the unsaponifiable matter of factory-produced whale-liver oil, that is, after drying and extraction of batches of minced liver on the large scale. Measurements made in this country with the Unicam quartz spectrophotometer on chromatographed fractions showed that numbers of samples of factory-produced liver oil gave figures for the intensity of absorption expressed as fractions of that at $\lambda_{\max.}$ closely corresponding to the Morton & Stubbs ratios (1948) between the wavelengths 310 and 350 $m\mu$., so that the 1900 conversion factor could be applied directly to the $E_{1\text{ cm.}}^{1\%}$ value at 326 $m\mu$. without correction (World Health Organization Report, 1950). (Solvent, *cyclohexane*.)

The method was applied to the unsaponifiable matter from a sample of raw sperm-whale liver, and the results compared with the ratios for vitamin A derived from a fish-liver oil (Morton & Stubbs, 1948) and for synthetic *all-trans*-vitamin A (Cama, Collins & Morton, 1951) (Tables 1 and 2). It would seem that the figures correspond to a different proportion of *all-trans*- and neovitamin A from that present in factory-produced whale-liver oil, so that some isomerization takes place during drying and extraction by factory methods. By analogy with other properties of oils from raw livers, such as the shape of absorption curve, it is possible that, while the percentage of neovitamin A is variable in raw materials, it becomes more constant in the factory-produced liver oil. It also would appear that some substance with its maximum at a higher wavelength than that of vitamin A is a contaminant of the vitamin A fraction of the oil from raw liver. This is thought to be derived from the 'oxidized material' in which there is at least one substance not yet found in factory-produced liver oil. Several mixtures of alumina, supplied by Unilever Ltd. and of Grade 'O' alumina (Peter Spence Ltd.) were tried in the chromatographic method, but it did not prove possible to reduce the ratios on the portion of the curve to the long wavelength side of the maximum. An examination of the material leaving the column before the vitamin A was therefore made.

Table 1. *Vitamin A fraction isolated chromatographically from raw sperm-whale liver compared with standard vitamin A from a natural source (Morton & Stubbs, 1948) and with synthetic all-trans-vitamin A (Cama, Collins & Morton, 1951)*

(Solvent, cyclohexane. The figures in the third column show the ratio of the extinction at the given wavelength to the extinction at 326 m μ . In the fourth and sixth columns are the corresponding figures for Morton's vitamin A. As most of the differences in the fifth and seventh columns exceed 0.020, it is necessary to apply the Morton correction.)

Wavelength (m μ .)	Extinction (<i>E</i>)	Sperm liver ratios	1948	Difference	1951	Difference
260	0.061	0.117	0.123	-0.006	0.107	+0.010
265	0.059	0.113	0.127	-0.014	0.108	+0.005
270	0.066	0.127	0.150	-0.023	0.130	-0.003
275	0.083	0.160	0.187	-0.027	0.161	-0.001
280	0.107	0.206	0.236	-0.030	0.212	-0.006
285	0.141	0.271	0.300	-0.029	0.274	-0.003
290	0.180	0.346	0.393	-0.047	0.346	0
295	0.233	0.448	0.515	-0.067	0.448	0
300	0.290	0.557	0.611	-0.054	0.553	+0.004
305	0.319 (?)	0.614 (?)	0.718	-0.104 (?)	0.669	-0.055 (?)
310	0.412	0.793	0.841	-0.048	0.811	-0.018
313	0.448	0.862	0.888	-0.026	0.869	-0.007
315	0.463	0.890	0.906	-0.016	0.893	-0.003
317.5	0.477	0.918	0.923	-0.005	0.913	+0.005
320	0.490	0.942	0.950	-0.008	0.936	+0.006
322.5	0.501	0.964	0.978	-0.014	0.968*	-0.004
325	0.517	0.995	0.998	-0.003	0.987	-0.008
326	0.520	1.000	1.000	0	1.000	0
327	0.522	1.002	0.998	+0.004	1.000	+0.002
328	0.522	1.002	0.994	+0.008	0.995	+0.007
329	0.521	1.001	—	—	0.987	+0.014
330	0.519	0.998	0.972	+0.026	0.982	+0.016
332.5	0.501	0.964	—	—	0.936*	+0.028
335	0.478	0.919	0.880	+0.039	0.888	+0.031
336	0.467	0.898	0.858	+0.040	0.869	+0.029
340	0.422	0.812	0.776	+0.036	0.790	+0.022
345	0.357	0.686	0.646	+0.040	0.667	+0.019
350	0.284	0.546	0.513	+0.033	0.517	+0.029

* Interpolated.

Table 2. *Summarized results for the liver samples*

(Sperm whale 51 ft. male, taken 7 December 1951. About 12 hr. post mortem 258 g. liver gave 4.41 g. oil and 0.938 g. unsaponifiable material. The quantity 'c.f.' or 'conversion factor' in the fifth column is given by

$$1900 \times E_{326 \text{ m}\mu} (\text{corr.}) / E_{326 \text{ m}\mu} (\text{gross}).$$

It represents the proportion of the gross $E_{1 \text{ cm}}^{1\%}$ value at 326 m μ , which is due to vitamin A. The vitamin A figures are obtained by multiplying the value for $E_{1 \text{ cm}}^{1\%}$ 326 m μ , (corr.) by 1900.)

Material	$E_{326 \text{ m}\mu}$ gross	$E_{1 \text{ cm}}^{1\%}$ 326 m μ corr.	'c.f.'	Vitamin A (i.u./g.)	λ_{max} (m μ .)
Unsaponifiable	506.4	373.1	1400	709 000	315-325 (flat)
Oil via unsaponifiable	107.5	79.3	1400	151 000	—
Vitamin A eluates on unsaponifiable	346.6	322	1760	611 700	327.5
Vitamin A eluates on oil	73.6	68.5	1760	130 000	—
Tube 24 (5 ml.). Following vitamin A (on unsaponifiable material)	0.73	0.34	890	650	—
Tubes 16-17 (10 ml.) preceding vitamin A (on unsaponifiable material)	0.54	—	—	—	337.5-347.5

The eluate was divided as follows (groups of 5 ml. tubes):

Tube nos.	Colour in Carr-Price spot test	Wavelengths of maxima (m μ .)	$E_{1\text{ cm.}}^{1\%}$ at max. referred to wt. of unsaponifiable matter
1-5	Pale blue	334, 352, 371, 392.5	1.68 at 371 m μ .
6-8	—	358-370 (flat)	0.32
9-13	Pale purple	352.5	1.53
15-17	Pale steel blue	337.5-347.5 (flat)	0.59

The anhydrovitamin A fraction (tubes 1-5). The two specimens of this crude fraction from raw liver had, in addition to the usual anhydrovitamin A

Fig. 4. Visible absorption spectrum of carotene fraction from chromatogram of unsaponifiable matter from raw sperm-whale liver. (Solvent, *n*-hexane.)

maxima at approx. 350, 370 and 392 m μ ., an additional small maximum close to 334 m μ . In factory-produced liver oils we have invariably seen a slope change at 330-335 m μ . in this fraction, and on chromatography this material gave, besides purer anhydrovitamin A, a crude fraction with inflexion at 334, maxima at 348 and 365.5 m μ . (solvent, *cyclohexane*), believed to correspond with the *iso*-anhydrovitamin A of Embree & Shantz (1943). As the positions of the maxima have been found to vary somewhat in crude fractions, the 335 position has been termed λ_1 , the 350 position λ_2 , the 370 position λ_3 , and the 390 position λ_4 . Two typical anhydrovitamin A fractions are here compared with anhydrovitamin A.

Material	Solvent	λ_1	λ_2	λ_3	λ_4	$E_{\lambda_2}/E_{\lambda_3}$	$E_{\lambda_4}/E_{\lambda_3}$
Raw liver	<i>cyclo</i> Hexane	334	352	371	392.5	0.84	0.76
Factory-produced liver oil	<i>cyclo</i> Hexane	Slope change	352	369.5	392	0.76	0.78
Anhydrovitamin A Shantz <i>et al.</i> (1943)	Ethanol	—	351	371	392	0.685	0.87

While these substances may be of natural occurrence in the liver, it seems more likely that they are to some extent due to post-mortem changes, since the liver becomes acid in the sometimes con-

siderable interval between killing of the whale and the time when the liver becomes available. However, the possibility of the additional maximum being due to the presence of anhydrosurvitamin A is not excluded (Embree & Shantz, 1943).

The carotenoid fraction. Closely following the anhydrovitamin A fraction was a very small amount of a substance absorbing in the visible region. This has not yet been found in factory-produced liver oil. After further chromatography this was taken up in the minimum of solvent (4 ml.) and examined on the Beckman spectrophotometer. The following figures were recorded (solvent, *n*-hexane):

$E_{1\text{ cm.}}^{1\%}$	This material (Fig. 4) Maxima (m μ .)	α -Carotene Maxima (m μ .)	β -Carotene Maxima (m μ .)
—	395	395	—
0.55	424 (Infl.)	420	425
0.74	446	445	450
0.64	474	475	477

The figures for α - and β -carotene are taken from Karrer & Jucker (1950). The belief that a carotenoid, probably carotene, occurs here is not proved, though the ease of elution certainly points to the presence of a hydrocarbon.

The 'oxidized material' fraction. This consisted of two and sometimes three main fractions, the first (tubes 9-13 above) having $\lambda_{\text{max.}}$ at about 352 m μ ., and the second having a long flat maximum from about 337-347 m μ . and giving distinctive colours in the Carr-Price spot test. One sample of liver (only three were examined) gave a third fraction with $\lambda_{\text{max.}}$ 280-285 m μ . The solvents for these crude fractions are not quoted, since the solutions were prepared by diluting the ether-light petroleum mixture from the chromatogram with *cyclohexane* for the spectrophotometer. Other less well characterized fractions were found. When subjected to further chromatography the second fraction was readily enriched, giving at first a maximum at 356 m μ . (solvent, *n*-hexane) and $E_{1\text{ cm.}}^{1\%} = 5.04$.

Further chromatography of the combined material from five initial chromatograms gave 0.031 g. of material with maximum absorption at 355 m μ . and $E_{1\text{ cm.}}^{1\%} = 105$ (solvent, *n*-hexane). This substance, which was easily freed from selectively

absorbing contaminants, is of unknown structure. Changes in the ratios $E\lambda/E\lambda_{\text{max.}}$ on chromatography are shown (Table 3). Above this on the chromatogram there was often a brown layer which

on elution gave a solution with maximum at 314–325 m μ . and $E_{1\text{cm.}}^{1\%} = 21.4$, with the maximum less well defined.

Table 3. *Material obtained from middle band of chromatogram of oxidized material from sperm liver oil*

(Solvent, *n*-hexane. The two columns show a comparison of the ratio $E_{\lambda}/E_{\lambda\text{max.}}$ for the materials from the second and third chromatographic separations for wavelengths between 320 and 400 m μ .)

Wavelength (m μ .)	Second	Third
320	0.699	0.701
5	0.764	0.772
30	0.824	0.833
5	0.879	0.895
40	0.925	0.934
2.5	0.938	0.950
5	0.954	0.965
7.5	0.969	0.978
50	0.984	0.987
2.5	1.000	0.998
5	1.000	1.000
7.5	1.000	0.997
60	0.995	0.986
2.5	0.984	0.978
5	0.969	0.963
70	0.941	0.930
5	0.903	0.884
80	0.830	0.823
5	0.728	0.733
90	0.641	0.631
5	0.527	0.530
400	0.434	0.438

By treatment of vitamin A with acid potassium permanganate, Meunier & Jouanneteau (1948) obtained, in addition to vitamin A aldehyde, a substance which gave a maximum at 345 m μ . in chloroform which they considered to be an epoxide of vitamin A aldehyde, and it is possible that these materials are related.

DISCUSSION

It is not yet possible to say that the cephalopod liver contains astaxanthin rather than astacene, although this is expected to be the case. In the salps, however (see Appendix), there is a slight indication of the presence of astaxanthin. In the case of Antarctic whalebone whales, Fisher, Kon & Thompson (1952) have shown that pigments are not absorbed in the gut and that they become steadily more concentrated in their passage along the alimentary canal. The brown colour of sperm whale faeces is, however, in contrast to the red colour of those of whalebone whales, and further work is needed. Occasionally, too, the whalebone

whales seem to absorb pigments, which are laid down either in the liver, giving rise to red-tinged oils seen sometimes during factory production, or in the blubber. 'Red whale oil' was examined by Schmidt-Nielsen, Sorensen & Trumpy (1932) and by Burkhardt, Heilbron, Jackson, Parry & Lovren (1934), and astacene was detected. The red oils occasionally extracted from livers on F.F.S. *Balaena* have up to the present shown no astacene or astaxanthin but exhibit a general absorption from 370 to 405 m μ . dropping sharply in the blue or violet regions. Further examination of these materials will be needed as and when they can be found. Their vitamin A potency appeared to be unusually high.

It is unfortunate that there is insufficient information to compare the amounts of vitamin A ingested and deposited in the sperm liver. Because of the great variation in size of the cephalopods, the problem is very different from the corresponding one in the case of the whalebone whales feeding on krill. Very little is known at present about the rate of consumption of the food of the sperm whale, or of the total amount of vitamin A contained therein.

SUMMARY

1. The presence of vitamin A in the liver of an Antarctic cephalopod was confirmed, and the presence of provitamin D, astacene (or astaxanthin) was detected.
2. Vitamin A isolated chromatographically from raw sperm-whale liver was compared with the Morton standards of 1948 and 1951.
3. The anhydrovitamin A fraction of raw sperm liver was found to contain a carotenoid, probably carotene.
4. A substance of unknown nature present in the 'oxidized material' fraction was detected and partially purified.

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