XVII. THE STOMACH OIL OF THE FULMAR PETREL (*FULMARUS GLACIALIS*).

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In the course of an investigation on the suggested relationship between vitamin A and the arsenious chloride colour reaction of cod-liver oils [Rosenheim and Webster, 1926, 1], it seemed desirable to examine some other oils, differing essentially in their character and origin from cod-liver oil. Our attention was drawn by Sir Walter Fletcher to a communication by Malcolm [1926], in which the stomach oil of the Australasian mutton bird or white-headed Fulmar (*Oestrelata lessoni*) was stated to be a rich source of vitamin A. Purdy [1918] had previously suggested the body-fat of these birds as a substitute for cod-liver oil, and had found it effective in the treatment of bronchitic conditions and phthisis.

We were unable to obtain mutton bird oil in this country, as it appears to be produced commercially in small amounts only in certain islands near Tasmania and New Zealand. It seemed of interest therefore to examine the stomach oil of an arctic representative of the order Tubinares, the Fulmar petrel (*Fulmarus glacialis*), which corresponds most closely to the antarctic mutton bird. These birds breed in enormous numbers in St Kilda, and have extended their breeding colonies in recent years to an extraordinary extent in the Orkney and Shetland Islands [Coward, 1926]. They are purely oceanic wanderers, and seldom, if ever, come to land, except for the purpose of breeding. When disturbed on the nest, the bird ejects, as a defensive measure, with considerable force, some of the oil stored in the stomach.

We found that the stomach oil not only gives the arsenious chloride reaction to approximately the same degree as cod-liver oil, but that its content of growth-promoting vitamin A runs parallel to the colour value. The oil also contains the calcifying vitamin D.

A chemical examination showed that the oil is not a glyceride, but a "liquid wax," containing nearly 40 % of unsaponifiable matter. The latter appears to consist mainly of unsaturated higher alcohols of the same type as those found in sperm oil. The resemblance of the Fulmar stomach oil to that contained in the head cavity of the Spermaceti Whale (*Physeter macrocephalus*, L.) is so striking as to suggest a similarity in the origin and function of these oils which will be discussed later.

Experimental.

Through the kindness of Capt. S. R. Douglas we were able to examine a nearly full-grown bird, which was shot in August, at the end of the breeding season, in the island of Yell, Shetland. The bird weighed 800 g. and its wing measure was 290 mm. (adult birds = 318–330 mm.). The proventriculus was found to be completely filled with oil, which was collected by ligaturing the organ and making an incision. The oil, 50 cc. in amount, was perfectly clear, deep amber-coloured, and of a penetrating musty odour, resembling that of arctic plankton[1].

The natural untreated oil was used throughout this investigation, and was protected against light and air which rapidly bleach it.

The thick layer of blubber beneath the skin yielded 72 % of a nearly colourless fluid oil. This, as well as the fat of the liver and muscular tissue, was also examined for comparison with the stomach oil. In order to avoid destruction of the vitamin during the preparation of the fats, the tissues were immediately put into 5 % KOH solution. On standing at room temperature for 24 hours, the proteins dissolved and the fats were extracted with pure ether in a separating funnel. The ether extract was washed with water, dried with anhydrous Na_2SO_4 and concentrated in a current of nitrogen. The fats were finally dried *in vacuo*.

Colour tests.

Quantitative determinations of the blue colour given in Rosenheim and Drummond's $AsCl_3$ test [1925], were carried out by means of Lovibond's colorimeter. The results were identical with those obtained by the more convenient modified method, introduced by Carr and Price [1926], in which a chloroform solution of $SbCl_3$ is used instead of undiluted $AsCl_3$. We have, however, recently met with oils of low vitamin A content in which the modified method indicated the absence of vitamin A, whilst the results of the colorimetric "arsenic" test agreed with the biological growth test. This lack of sensitiveness in the "antimony" method is due to the dilution of both the oil and reagent and necessitates a control test with undiluted $AsCl_3$ in all apparently negative cases.

Table I.

	Arsenic and antimony reaction			Fearon reaction		
	Mg. oil in 1 cc. reagent	Units of blue		Mg. oil in 1 cc. reagent	Units of red	
		Measured	C.L.O. = 1*		Measured	C.L.O. = 1*
Cod-liver oil	20	10·0	1·0	20	20	1·0
Fulmar stomach oil	20	8·0	0·8	20	11·0	0·55
„ subcutaneous fat	100	2·0	0·04	100	12·0	0·12
„ muscle fat	20	2·2	0·22	20	1·5	0·07
„ liver fat	0·5	16·0	64·0	20	Nil	Nil

* C.L.O. = 1 signifies the numbers of blue or red units given by 20 mg. oil, taking cod-liver oil = 1.

[1] We had occasion to notice the smell of plankton also in the stomach contents of a whale (*Balaenoptera rostrata*), which had been alive for 3 days after having been stranded on the East Coast.

In Table I the results obtained with an active Newfoundland cod-liver oil are included for comparison, together with those of Fearon's [1925] colour test.

It will be seen that the Fulmar stomach oil possesses four-fifths of the colorimetric value of the standard cod-liver oil, whilst that of the subcutaneous and muscle fat is much less. On the other hand, the liver fat is 64 times as strong as cod-liver oil. The amount of this liver fat available was not sufficient for a growth test, but we found liver fats of other animals of equally high colour value, the growth-promoting power of which we are at present investigating.

Fearon's test has no relation to vitamin A, as we have previously shown, and the results are interesting only in confirming our statement that the reaction is negative in the case of liver fats of birds [Rosenheim and Webster, 1926, 1].

Feeding tests.

(1) *Vitamin A.* The technique described by Drummond, Coward and Handy [1925] was employed, in which a constant supply of vitamin D (irradiated cholesterol) is given throughout the experiment. In the course of this and similar investigations we found it convenient to correlate the dose of the oil to be tested with its colorimetric value by taking as the standard the number of blue units (Lovibond's scale) given by 10 mg. and 20 mg. of cod-liver oil in the colour test. Under the conditions adopted 20 mg. of Newfoundland oil equal 10 units of blue, and administered daily restore normal growth, *i.e.* induce a weekly increase in body-weight of 8–10 g. In the present work the Fulmar oil was administered in daily doses of 10 mg. and 20 mg., representing 4 and 8 units of blue respectively. In our experience the animal test, as at present developed, is not sensitive enough to differentiate variations of ± 2 units of blue, *i.e.* deviations of ± 4 mg. from the average dose of 20 mg. The results are represented in Fig. 1.

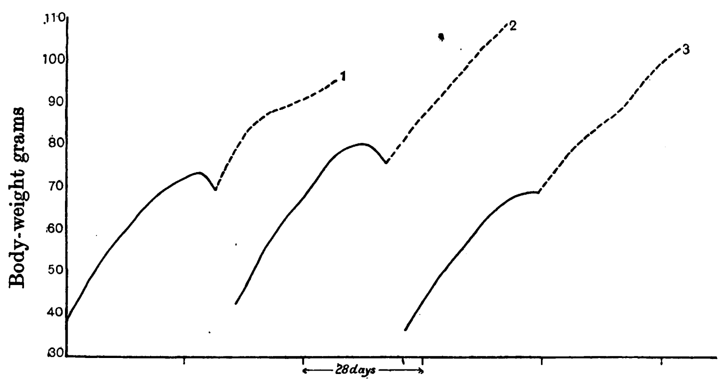

Fig. 1.

- - - - 1 = 10 mg. Fulmar oil = 4 Blue units.
- - - - 2 = 20 mg. ,, ,, = 8 Blue units.
- - - - 3 = 20 mg. cod-liver oil = 10 Blue units.

It will be seen that 20 mg. (= 8 blue units) of the Fulmar stomach oil produced growth of the same order as the same quantity of the standard cod-liver oil (= 10 blue units). Xerophthalmia had developed in all cases during the preparatory period, which cleared up rapidly on administration of the oil.

The growth tests made with mutton bird oil by Malcolm [1926] are open to criticism, since a constant supply of vitamin D had not been given. The statement "that mutton bird oil is one of the richest known natural sources of vitamin A" requires modification, as a good Newfoundland cod-liver oil induces similar growth increments in the doses given by Malcolm.

(2) *Vitamin D.* The technique previously described [Rosenheim and Webster, 1926, 2] was used. A daily dose of 20 mg. gave partial protection, whilst 40 mg. completely protected the animals against rickets. The presence of vitamin D in mutton bird oil has up till now not been demonstrated.

Chemical examination.

The untreated stomach oil of the Fulmar is a clear, deep orange-red, non-drying oil which begins to solidify when cooled to 8–9°, becomes waxy at 5° and sets to a translucent solid at 0°. Table II contains the constants of the oil, determined by the standard methods, together with those of mutton bird oil[1] [Carter, 1921], a genuine sperm oil [Dunlop, 1908], and an average cod-liver oil.

Table II.

	Fulmar oil	Mutton bird oil	Sperm oil	Cod-liver oil
Specific gravity	0·884	0·884	0·880	0·925
Saponification value	122	120	122	185
Iodine value (Wijs)	118	130	70	168
Higher alcohols	37·7 %	38·4 %	41·2 %	0·5–1·5 %
M.P. of ditto	31·5°–32·5°	31°–31·5°	31·5°–32·5°	?
Free fatty acid	1·6	2·2	1·4	0·3–1·5
Cold test	8°–9°	6°	7°–9·5°	Variable

These figures justify the conclusion that the composition of the stomach oil of these representatives of the arctic and antarctic petrels is practically identical. Both oils differ fundamentally from cod-liver oil in their low specific gravity and their high percentage of unsaponifiable substances (alcohols) which characterise them as "liquid waxes." On the other hand, they both resemble sperm oil, their higher iodine value being due to the nature of the unsaturated fatty acids they contain.

The fatty acids were fluid at room temperature. In the elaidin test they solidified only partially, thus indicating the presence of a certain amount of oleic acid. Their mean molecular weight was 292 and the iodine value 156. The high iodine value points to the presence of highly unsaturated fatty acids and the bromination test was therefore carried out.

[1] The earlier analyses of mutton bird oil [Smith, 1911; Evers and Foster, 1920] are in general agreement with that by Carter [1921].

1·134 g. of the fatty acids gave 0·238 g. = 20·9 % of ether-insoluble bromides. The white substance did not melt when heated in a capillary to 200° and began to darken at 240°, turning black without fusing at 250°. The product, being insoluble in organic solvents, was purified by extraction at the boiling-point with ether and benzene, in which it was practically insoluble. The behaviour on heating remained unchanged, thus excluding the presence of the hexabromide of linolenic acid (M.P. 175°).

Analysis: 0·0620 g. gave 0·1038 g. AgBr. Found 71·3 % Br.
Calculated for $C_{22}H_{34}O_2Br_{10}$: 70·9 %
,, ,, $C_{20}H_{32}O_2Br_8$: 67·8 %.

The substance is therefore the decabromide of clupanodonic acid, which has been found by Tsujimoto [1922] as a characteristic constituent of sardine oil and other fish oils. The amount of ether-insoluble bromides in sperm oil is only 1·1–2·3 % [Dunlop, 1908], and this fact explains the difference in the iodine value of Fulmar and sperm oil.

The fatty acids give Fearon's test, which is ascribed by Rosenheim and Webster [1926, 3] to aldehydic oxidation products of clupanodonic acid.

The alcohols. The unsaponifiable fraction of the oil is a deep ruby-red wax, which rapidly bleaches on exposure to light and air. The $AsCl_3$ reaction is given in great intensity by freshly prepared specimens. Liebermann's cholesterol reaction is positive and Fearon's reaction negative, as in the unsaponifiable fraction of cod-liver oil. The alcohols were recrystallised from light petroleum, filtered at a low temperature and were thus obtained as a pale yellow wax, melting at 32°, easily soluble in organic solvents. Their iodine value is 54, and on bromination a small amount of a white bromide (cholesterol bromide?) is obtained. A quantitative estimation, by means of digitonin, gave 2·4 % cholesterol, showing that the bulk of the alcohols is unsaturated. On acetylation with acetic anhydride the acetates were obtained as oils, which solidified at 0° and melted at 5°. The acetates formed a clear solution in acetic anhydride, indicating the absence of any considerable amount of hydrocarbons. We were unable to obtain any direct evidence for the presence of cetyl alcohol, which is assumed to constitute the bulk of the alcohols of mutton bird oil [Carter, 1921]. In their general character the alcohols of the Fulmar oil resemble the unsaturated alcohols of unknown constitution, which have been obtained from sperm oil by Lewkowitsch [1892]. Recently oleic alcohol has been isolated from the unsaponifiable fraction of arctic sperm oil by Tsujimoto [1925].

The similarity in composition of the two oils is further exemplified by the fact that we were able to isolate only 1·4 % of glycerol from Fulmar oil, and that sperm oil also yields not more than 1·3–2·5 % glycerol [Dunlop, 1908].

The lipochromes. The solution of the pigments in light petroleum, obtained on recrystallisation of the alcohols (see above) was subjected to the Kraus phase-test [Willstätter and Stoll, 1913]. The pigment was found to be entirely epiphasic, thus showing it to be related to, or identical with, carotene, and proving the absence of xanthophyll. This was confirmed by the chromatographic analysis according to Tswett, in which the light petroleum solution passed completely through the chalk column without showing any xanthophyll layers. Spectroscopic examination also indicated the presence of carotene, although the second and third absorption bands were partially obscured by general absorption.

The pigment is easily absorbed by charcoal (norit) from an ethereal solution of the oil. The colourless filtrate, after removal of the ether, gave quantitatively the same amount of blue colour with $AsCl_3$ as the original oil. The presence of the lipochrome therefore does not interfere with the colour test.

Possible function of the stomach oil.

We were unable to find any previous reference in the literature throwing light on the presence of the large amounts of oil in the stomach of the Fulmar petrel, except that it appears to be peculiar to all the members of the order Tubinares [Challenger Reports, 1882]. Since the oil, like other waxes, is resistent to hydrolysis by lipoclastic enzymes, it might be assumed to represent the indigestible residue of fatty foodstuffs. Such an assumption has indeed been made by Carter and Malcolm [1926], who were unable, however, to find any evidence for the presence of similar waxes or their alcohols in the fats of fishes, or any form of marine life, which might serve as food for these birds.

The resemblance of this fluid wax in its chemical character to that secreted by the "preen" glands of terrestrial birds suggests a different explanation. The rump gland wax of geese and ducks contains, like the stomach oil of petrels, about 40 % of alcohols, in which the presence of octadecyl alcohol has been definitely established by Röhmann [1904]. This wax has been shown by Röhmann to be a true secretory product and its obvious use to the bird in preening is to protect the feathers against the action of water, for which it is better suited than the easily saponifiable fats.

We would suggest that the stomach oil of the petrels has a similar origin and fulfils a similar function in these marine birds, which owing to their environmental conditions require a larger supply of preening material than terrestrial birds. Such a suggestion is strengthened by the presence of a distinctive structural feature in the order Tubinares, *i.e.* "their prominent tubular nostrils and their bills which consist of several horny pieces, separated by deep grooves" [Godman, 1910]. Through these structures the oil may be distributed over the beak, thus facilitating the preening operation when the bird is resting on the sea[1]. Another peculiarity of the oil, shared by sperm oil, is its low viscosity, which according to our determination is 0·216 at body temperature (36°). This characteristic feature distinguishes it from animal

[1] It might be mentioned that the petrels are said to eject the oil "through their nostrils" when disturbed. Certain statements in the literature lend support to the suggestion that the secretory gland may have to be searched for near the nasal cavity, the proventriculus serving merely as a store for the secretion.

fats and renders easy the transference of the oil from the proventriculus to the beak and aids in its distribution over the feathers. The presence of unsaturated fatty acids and alcohols in the wax helps to keep it fluid at relatively low temperatures, a property especially useful to a bird whose life is spent in the arctic regions.

The above view is rendered still more likely when we consider the analogous composition and function of similar waxes in certain aquatic mammals, the cetaceans. The liquid wax, sperm oil, present in the head cavity of many whales is practically of the same composition as the Fulmar stomach oil (see above), whilst that of dolphins and porpoises appears to form an intermediate link between liquid waxes and blubber oils [Lewkowitsch, 1921]. In the whale a canal running along the whole length of the body to the tail communicates with the head cavity and, probably in a way at present not properly understood, enables the secretion to reach and to spread over the surface of the skin, thus protecting the animal against the action of the sea water [Röhmann, 1908].

This suggested explanation for the function of the stomach oil does not account for the presence in it of vitamins. It may be pointed out, however, that the oil which we examined came from a juvenile bird which had not yet left the nest. The food received from the parents would probably supply more than the necessary amount of fat-soluble vitamins and thus explain their presence in the stomach oil. The examination of the stomach contents of a non-breeding adult bird would be of interest in this respect, and we intend to examine this and other questions during the next breeding season.

Summary.

1. The stomach oil of the Fulmar petrel (*Fulmarus glacialis*) has been examined chemically and biologically. It gives the arsenious chloride reaction approximately to the same degree as cod-liver oil, and its content of vitamin A runs parallel to the colour value. The oil also contains vitamin D.

2. The oil is not a glyceride, but a liquid wax of similar composition to sperm oil.

3. An explanation for the origin and the function of the oil is suggested.

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