XIII. NOTE ON THE UNSAPONIFIABLE MATTER FROM THE STOMACH OIL OF *SCYMNORHINUS LICHIA*.

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In a previous communication [Heilbron, Kamm and Owens, 1926] it was reported that oil had been obtained from the stomach of the elasmobranch fish *Scymnorhinus lichia*, and that this oil contained the hydrocarbon squalene.

An investigation of the oil has now been made in some detail.

This fish, known to the fishermen as "Darkie Alf," is commonly spoken of by them as having an oil bag in the stomach, and the oil is at times used by them as fuel for their lamps.

The specimens here to be described were in excellent condition, and averaged 1 m. in length and 5 to 6 kg. in weight. Before dissection, the pylorus was ligatured, and the stomach was then removed. This was in all cases distended, sometimes very largely so, and was full either of clear oil, or, if the fish had been feeding just before capture, of an oily mass of undigested food. The content of oil was usually about 250 cc. As will be seen below, this oil is almost identical with the liver oil, and no entirely satisfactory explanation of its function is yet forthcoming.

While it has only been possible to obtain specimens during three months of the year (March to May), it is to be noted that stomach oil was present in every member of the species examined, while members of other closely related species of shark or dog-fish contained no readily recognisable quantities of oil in their stomachs.

It is tentatively suggested that some of the liver oil may have come into, and been temporarily stored in, the gall bladder, whence it may have been forced through the bile duct into the small intestine and hence into the stomach. Whether this process is a normal one of the living animal or takes place *post mortem* on the release of pressure consequent on the sudden ascent from its normal habitat of more than 100 fathoms to sea level, must still be a matter for conjecture.

EXAMINATION OF THE OIL.

Extraction and examination of the unsaponifiable matter.

The oil (2065 g.) from the stomachs of 9 fish was subjected to distillation at a pressure of 2 mm., whereby 1000 g. of a liquid boiling at 228–230° were obtained. This had n_D^{20} 1.4967, d_{20}^{20} 0.8536, and on treatment in ether solution with gaseous hydrogen chloride, gave a quantitative yield of squalene hexahydrochloride (m.p. 112–120°) [cf. Heilbron, Kamm and Owens, 1926].

The residues were then saponified by boiling with 5000 cc. of 10 % alcoholic NaOH for 8 hours. The alcohol having been distilled off, the soap solution was mixed with an equal weight of fine silver sand, and was taken to dryness on the steam-bath. The dried soaps were exhaustively extracted with acetone, the acetone extract concentrated by distillation, and the concentrate dissolved in ether, washed with water until neutral, and dried.

After removal of the solvent, the residue was now again distilled *in vacuo*, a fraction B.P. 210–233°/3–4 mm. being removed. This was shown, by the hydrochloride test, to be almost pure squalene.

The residue (50 g.) was resaponified with an alcoholic solution of sodium ethoxide, the neutral portion (15 g.) being again extracted with ether. Distillation of this yielded an oil (B.P. 190–225°/0.55 mm.) which was not further separable by fractional distillation. A small sample taken at this point showed a negative digitonide test for cholesterol.

After slight dilution with pentane, the whole fraction was allowed to stand undisturbed in the ice-chest for several weeks, when a white crystalline solid started to separate, and was filtered off from time to time. This was recrystallised four times from absolute alcohol, 3 g. of pure white crystals being obtained. These were alcoholic in nature, and were saturated to bromine solution. They melted at 67°, and when mixed with pure batyl alcohol at 67.5°; while pure batyl alcohol melted at 69°. C = 72.4, 72.3, H = 12.6, 12.6 % ($C_{20}H_{42}O_3$ requires C = 72.7, H = 12.7 %) [cf. Tsujimoto and Toyama, 1922; Toyama, 1924; Weidemann, 1926].

A small portion of the residue, which could not be induced to crystallise further, was now again tested for squalene, and was found to contain only 9 % of this compound.

The remainder (9 g.) was therefore dissolved in 100 cc. of ethyl acetate and was hydrogenated, a rapid stream of hydrogen being passed through it at 18° for 7 hours in the presence of a palladium catalyst supported on Kahlbaum's charcoal. After removal of the catalyst by filtration and of most of the solvent by distillation *in vacuo*, a further quantity of batyl alcohol separated, showing that in all probability selachyl alcohol ($C_{20}H_{40}O_3$) had also been present. This result was to be expected since Tsujimoto, Toyama and Weidemann have shown that this alcohol usually occurs associated with batyl alcohol.

The constitution of the unsaponifiable matter may therefore be summarised as follows:

<i>Unsaponifiable matter</i>	
Squalene (by distillation)	1000 g.
Residue (by saponification)	45 g.
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	1045 g. = 50.6 % of original oil
This consists of	
Squalene (distillation)	1000 g.
(distillation of unsap.)	23 g.
(estimated as present in residue)	1 g.
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	1024 g. = 98 %
Batyl alcohol	3 g. = 0.28 %
Residue (chiefly selachyl alcohol)	8 g. = 0.96 %

In conclusion I desire to thank Profs. I. M. Heilbron and J. Johnstone for advice and help, Dr W. M. Owens for a pure specimen of batyl alcohol, and the Food Investigation Board of the Department of Scientific and Industrial Research for a grant which enabled the research to be undertaken.

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