least seven components which may be divided into Jacob's groups I, II and III.

3. Whereas groups II and III of codling bear similarities to the corresponding groups of rabbit, group I is quite different.

4. Group II appears to be particularly significant in relation to the denaturation of these protein mixtures.

5. The mobilities of codling muscle proteins are appreciably greater than those of rabbit.

6. The influence of ionic strength on the mobilities of these proteins has been examined.

7. On dialysis of a 0·05*I* extract against distilled water, component 2 precipitates completely, and all the components, except perhaps 5, suffer a partial loss of solubility. The precipitate of globulin X is only partially re-soluble; the soluble portion shows three main components which on the basis of mobility can be related to components in the original extract. However, estimates of composition reveal that the relationship is complex.

The work described in this paper was carried out as part of the programme of the Food Investigation Organization of the Department of Scientific and Industrial Research.

REFERENCES

Bailey, K. (1948). *Biochem. J.* **43**, 271.
Bate-Smith, E. C. (1937). *Proc. Roy. Soc.* B, **124**, 136.
Bosch, M. W. (1951). *Biochim. biophys. Acta*, **7**, 61.
Davis, B. D. & Cohn, E. J. (1939). *J. Amer. chem. Soc.* **61**, 2092.
Dole, V. P. (1945). *J. Amer. chem. Soc.* **67**, 1119.
Dyer, W. J., French, H. V. & Snow, J. M. (1950). *J. Fish. Res. Bd Can.* **7**, 585.
Hamoir, G. (1949). *Bull. Soc. Chim. biol., Paris*, **31**, 118.
Hamoir, G. (1951*a*). *Biochem. J.* **48**, 146.
Hamoir, G. (1951*b*). *Biochem. Soc. Symp.* no. 6, p. 8.
Hamoir, G. (1951*c*). *Biochem. J.* **50**, 140.
Jacob, J. J. C. (1945). *Bull. Soc. Sci. Liege*, no. **4**, 231.
Jacob, J. J. C. (1947). *Biochem. J.* **41**, 83.
Jacob, J. J. C. (1948). *Biochem. J.* **42**, 71.
Kekwick, R. A., Lyttleton, J. W., Brewer, E. & Dreblow, E. S. (1951). *Biochem. J.* **49**, 253.
Longsworth, L. G. & MacInnes, D. M. (1940). *J. Amer. chem. Soc.* **62**, 705.
Raffel, S., Pait, C. F. & Terry, M. C. (1940). *J. Immunol.* **39**, 317.
Reay, G. A. (1935). *Rep. Fd Invest. Bd, Lond.*, no. 65.
Reay, G. A. & Kuchel, C. C. (1936). *Rep. Fd Invest. Bd, Lond.*, no. 93.
Roth, E. (1947). *Biochem. Z.* **318**, 74.
Subba Rao, G. N. (1948). Physico-chemical investigations on the muscle proteins of some commercial species of fish caught in N.E. Scottish waters. Ph.D. Thesis, University of Aberdeen.
Svedberg, T. & Pedersen, K. O. (1940). *The Ultracentrifuge*, p. 296. London: Oxford University Press.
Svensson, H. (1941). *J. biol. Chem.* **139**, 805.
Svensson, H. (1946). *Ark. Kemi Min. Geol.* **22** A, no. 10, p. 1.
Tiselius, A. & Kabat, E. K. (1939). *J. exp. Med.* **69**, 119.
Tiselius, A. & Svensson, H. (1940). *Trans. Faraday Soc.* **36**, 16.
Weber, H. H. & Meyer, K. (1933). *Biochem. Z.* **266**, 137.
Wiedemann, E. (1947). *Helv. chim. Acta.* **30**, 892.

The Lipids of Fish

1. CONTENT AND CONDITION OF LIPIDS IN THE FLESH OF THE HADDOCK (*GADUS AEGLEFINUS*)

By J. A. LOVERN
Department of Scientific and Industrial Research, Torry Research Station, Aberdeen

(*Received* 1 *September* 1952)

The series of papers which it is hoped to present under the general title 'The Lipids of Fish' will describe studies preparatory to investigations of the lipoprotein constituents of fish tissues. From the outset, therefore, considerable emphasis has been placed on the relative ease with which any particular lipid may be extracted from the tissue, since much of the evidence for the existence of lipid-protein complexes is based on the occurrence of so-called 'bound' lipids, i.e. lipids which cannot be extracted from the fresh tissue by solvents in which they are normally readily soluble. At the same time, segregation of constituents according to ease of extraction may well assist in the separation of the extremely complex mixture of lipids present in the tissue.

The flesh of the haddock has been chosen as the most suitable raw material for the present series of studies, since it is quite free from adipose tissue, it can be obtained absolutely fresh in virtually unlimited quantity from this laboratory's own fishing vessel, and, the haddock being a small fish, it offers the advantage that lipid samples are usually representative of a very large number of individuals.

The present paper reports the results of successive extractions of haddock flesh with a range of solvents.

EXPERIMENTAL

A lipid is generally considered to be 'free' when it can be extracted from the tissue with a solvent which does not denature protein, such as, for example, light petroleum and ethyl ether. Extraction after treatment with such a solvent as ethanol is considered to yield much of the lipid originally linked to protein.

Finely minced haddock flesh exhaustively extracted with light petroleum, either by grinding with sand or by use of a Waring Blendor, yielded about 0·1% of lipid. (All percentages are w/w.) If the extracted tissue was then dried under vacuum and re-extracted with light petroleum, a further trace (about 0·02%) of lipid could be recovered. Subsequent extraction with ethyl ether yielded about 0·05% of additional lipid. Thus the total 'free lipid' amounted to about 0·17%. There were, however, considerable variations from fish to fish and the range encountered in a considerable number of experiments was from 0·06 to 0·13%, 0·02 to 0·03% and 0·03 to 0·11% for the three extracts respectively. The I_2 values of these extracts varied even more widely; from 82 to 188, 13 to 90 and 28 to 132 respectively. Such great individual variations emphasize the desirability of obtaining lipid samples representative of a large number of individuals.

If the exhausted tissue was next extracted with cold ethanol, a further yield of about 0·1% (range 0·06–0·15%) of the original fresh tissue weight was obtained. Hot ethanol, on the other hand, extracted about 0·5% of lipid together with an equal quantity of non-lipid substances.

Tissue exhausted with cold ethanol yielded a further 0·15% or so of lipid to cold $CHCl_3$, but tissue exhausted by hot ethanol yielded only about 0·05%, even to hot $CHCl_3$. The ethanol extracts had I_2 values of about 40 and the $CHCl_3$ extracts about 60, although again there were considerable variations from fish to fish.

The final exhausted tissue still contained lipid, as could be shown by acid hydrolysis of the protein followed by recovery of the freed fatty material. If only cold solvents had been used, a further 0·4–0·6% of lipid could be recovered after acid treatment, but if hot ethanol and $CHCl_3$ had been used only about 0·1% of additional lipid could be obtained after hydrolysis. This lipid liberated by hydrolysis was presumably largely in the form of free fatty acids and its I_2 value ranged from 90 to 164.

The total lipid content of haddock flesh thus extracted ranged from 0·75 to 1·1%. In order to prepare large enough samples of lipid for detailed investigation a different series of solvents was used after preliminary studies of their relative effectiveness.

A total of 42 kg. of minced haddock flesh was extracted in batches of 7 kg. with large volumes of acetone at room temperature (approx. 15°), through a total of nine successive extractions for each batch. The first acetone extract of each batch consisted mainly of water-soluble, light petroleum-insoluble, non-lipid matter (about 2% of the tissue) and had a marked smell of NH_3, even when obtained from material extracted at sea from fish immediately after death. Nevertheless, these first extracts did contain some true lipid matter. The ninth acetone extract in all cases contained so little lipid that it was clear that almost all lipid available to this solvent had been removed. The combined extracts 2–9 were evaporated, the residue taken up in light petroleum to remove traces of non-lipid matter, the solution combined with the petroleum extract of acetone-extract 1, and the whole evaporated to yield 241 g. of lipid (=0·57%). It may be noted that acetone has been found to have an effect comparable to that of ethanol in freeing 'bound' lipid (Delage, 1935). The exhausted tissue was next extracted at room temperature with large volumes of ethanol/ether (3:1, v/v), again through a total of nine extractions, the last extract containing negligible amounts of lipid. The total lipid obtained in this fraction was 94 g. =0·22%. The residual tissue was then extracted nine times with a boiling benzene/ethanol (1:2, v/v) mixture allowing 30 min. boiling at each extraction, to give a total of 79 g. (0·19%) of lipid; subsequent similar extraction with a boiling $CHCl_3$/methanol (1:1, v/v) mixture gave 103 g. (0·24%) and, lastly, extraction with pyridine at 100° gave 65 g. (0·15%) of lipid. The total lipid obtained was thus 1·37% of the original tissue, compared with the range 0·75–1·1% obtained in the preliminary experiments. Acid hydrolysis of the final exhausted tissue showed that it contained only negligible traces of residual lipid.

DISCUSSION

Little appears to be known concerning the lipids of haddock flesh, or indeed of fish muscle tissue in general, work on the lipids of fish having been primarily concerned with adipose tissue whether mixed with the skeletal musculature or not. Bähr & Wille (1931) reported on the 'lecithin' content of fish flesh, including haddock flesh, but their method of extraction of the air-dried flesh with an ethanol/benzene mixture followed by determination of phosphorus in the extract and calculation of all phosphorus found as lecithin phosphorus is open to criticism. They showed, however, that fish flesh varied widely in 'lecithin' so determined, according to species, age, season of year, and stage of maturity. Thus haddock flesh in June contained 0·17% lecithin, whereas comparable material in October contained 0·48%. Kaucher, Galbraith, Button & Williams (1943) reported that codfish muscle contained a total of 9·47% lipid on a dry-weight basis, which would be about 2·5% on the wet tissue. This was extracted from freeze-dried tissue by hot ethanol followed by ethyl ether. Cod and haddock belong to the same genus, and it is rather surprising that the former should contain nearly twice as much lipid as the latter. The fractionation of these lipids by Kaucher *et al.* is discussed in the following paper.

The only large fat depot in the haddock is the liver. It would appear from the present results of extraction with light petroleum and ether that the major part of the lipids of haddock skeletal muscle are in 'bound' form. There seem to be several types of linkage holding this bound lipid, of varying ease of rupture, e.g. some lipid is freed by alcohol, some still bound and liberated after acid hydrolysis. The results of the large-scale extraction, although giving

a qualitatively and quantitatively different picture, also suggest a variety of linkages between the lipids and the protein of the flesh.

The various large-scale extracts revealed considerable differences in appearance, consistency, etc., and it seems probable that they have been separated not only according to the relative strength of their linkages to protein, but also according to their solubility properties. A considerable overlap in their composition would, therefore, be expected. The following paper records the results of investigation of two sub-fractions of the first (acetone) extract.

SUMMARY

1. Haddock flesh contains a total of about 1% lipid, but only about 0·1–0·2% of 'free' lipid, the rest being probably 'bound' to protein.

2. Extraction with a series of solvents suggests the presence of more than one type of linkage between lipid and protein although the picture is complicated by probable differences in solubility of the various lipid constituents.

This work has been carried out as part of the programme of the Food Investigation Organization of the Department of Scientific and Industrial Research.

REFERENCES

Bähr, O. & Wille, O. (1931). *Fischwirtschaft*, **7**, 129.
Delage, B. (1935). *Bull. Soc. Chim. biol., Paris*, **17**, 923.
Kaucher, M., Galbraith, H., Button, V. & Williams, H. H. (1943). *Arch. Biochem.* **3**, 203.

The Lipids of Fish

2. THE ACETONE-SOLUBLE LIPIDS OF THE FLESH OF THE HADDOCK

By J. A. LOVERN and JUNE OLLEY
Department of Scientific and Industrial Research, Torry Research Station, Aberdeen

(*Received* 1 *September* 1952)

In the previous paper (Lovern, 1953) the preparation of a series of lipid extracts from the flesh of the haddock (*Gadus aeglefinus*) was described. The first of these consisted of material extracted from the tissue by acetone and soluble in light petroleum. It is well known that in the presence of such soluble lipids as triglycerides, sterols and sterol esters, acetone will dissolve other lipids, e.g. phospholipins, which alone are insoluble in acetone. This particular extract contained 2·6% phosphorus and 1·6% nitrogen. There has been controversy, particularly in earlier work, about the occurrence of phospholipins genuinely soluble in acetone, i.e. soluble in the absence of such lipids as fats and sterols. MacLean (1914) showed that such phospholipins do occur but can be completely precipitated from acetone in the presence of traces of electrolytes. Removal of impurities, presumably electrolytes, can render an originally acetone-insoluble phospholipin soluble in acetone (MacLean, 1909*a*). In the purification of phospholipin preparations by repeated precipitation from ether or light petroleum solution by a large excess of acetone, many workers add an electrolyte, commonly magnesium chloride. There are, however, objections that such a procedure gives a variable yield according to the amount of electrolyte added, and that the nature of the phospholipin is altered in the process, e.g. the proportion of ether-insoluble phospholipin increases with increasing amount of electrolyte (Sinclair & Dolan, 1942). Cahn, Houget & Agid (1949) discussed in detail the relative merits of precipitation with acetone in the presence or absence of added electrolytes and showed that electrolytes also precipitate large proportions of non-lipid contaminants.

We considered it desirable to examine separately the phospholipin fraction genuinely soluble in acetone in the absence of added electrolytes and that present in the total acetone extract but precipitable when accompanying non-phosphatidic lipids had been removed. The technique of counter-current distribution, used in our earlier studies on lipid separation (Lovern, 1952; Olley, 1953), was employed to examine the fractions obtained by acetone precipitation. The non-phosphatidic lipids, together with the acetone-soluble phospholipins, form the subject of the present paper.

Part of this work was presented at the Second International Congress of Biochemistry, Paris, 1952.