Composition and Distribution of Lipids in Tissues of Bogue *(Boops boops)*

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Total lipids from liver, head, skin and muscle of Bogue were separately isolated and their composition was investigated by a combination of analytical determinations, and column and thin layer chromatography.

The major components of the neutral lipid fractions from all tissues studied were triglycerides, followed by cholesterol. The triglyceride fraction of skin and head contains significant amounts of glyceryl ether analogs. Low contents of free fatty alcohols were also identified, decreasing in the order: head, muscle, skin and liver.

The major components of all phospholipid fractions was phosphatidylcholine (*viz.* 36–59% of total phospholipids) followed by phosphatidylethanolamine (*viz.* 23–34% of total phospholipids). Low amounts of sphingomyelin and phosphatidylserine were also identified in all cases. All the tissues studied were found to contain plasmalogens, as well as glyceryl ether analogs in both, the depot fats and the phospholipid fractions.

Introduction

Bogue a Teleostean costal Sparid, is a characteristic species of the Aegean and Adriatic sea [1].

Lipids of this Teleostean have not been studied yet, except the fatty acids of Bogue [2] and the lipids in the growing oocytes of Bogue [3]. A great deal of information has been accumulated concerning the amount and the composition of triglyceride fat in those fishes, which use the skeletal musculature as a fat depot, and less for other lipids, use as phospholipids.

In the overwhelming majority of species the depot lipids consist of triglycerides.

On the contrary, in most species of Elasmobranchs triglycerides are either accompanied or entirely replaced by alckoxyglycerides.

This paper is a report of the composition and distribution of neutral lipids, glycolipids, sulfolipids and phospholipids in the main tissues of Bogue namely in flesh, liver, skin and head.

Materials and Methods

Preparation of lipid extracts

Bogues from Aegean sea were caught during January, February and early March, with body lengths between 24–30 cm (5 fishes), 26–32 cm (3 fishes) and 22–33 cm (9 fishes) respectively.

The experimental animals were used right after being caught. Their skin, liver, muscle tissue and head were dissected and immediately homogenized in a Omni-mixer (Ivan Sorvall, Inc.) for 2–10 min at 7000–10000 rpm, while kept in an ice bath. Total lipids of each tissue homogenate were then extracted according to the method of Bligh and Dyer [4].

Silicic acid column chromatography

Column chromatography on silicic acid was performed according to Thompson and Hanahan [5].

The organic solvents used for elution were purified by the methods of Vogel [6].

The total lipid extracts obtained from each tissue, as mentioned above, were submitted to silicic acid column chromatographic fractionation in two stages: First, they were fractioned into 4 lipid classes (*viz.* by eluting neutral lipids with chloroform plus chloroform:methanol (19:1 v/v), glycolipids with chloroform:acetone (1:1 v/v), sulfolipids with acetone and phospholipids with methanol. Second, phospholipid fraction was further resolved into 6 fractions by rechromatography on silicic acid columns. They were eluted with chloroform:methanol mixtures of increasing polarity (*viz.* 100:0, 19:1, 4:1, 3:2, 1:1, 1:9 by volume).

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Thin layer chromatography

Thin layer chromatography of lipids was performed on silica gel G layers using the following systems (by volume).

I) Petroleum ether:diethyl ether:acetic acid (70:30:1) for neutral lipids, II) Chloroform:methanol:water (95:35:4) for phospholipids.

Spots were detected by exposure to iodine vapors, by charring at 100 °C after spraying with 50% sulfuric acid, and by using the molybdenum blue and ninhydrin spray reagents for detection of phospholipids respectively.

Analytical methods

Lipid phosphorus was determined by the method of Bartlett [7] as modified by Marinetti [8].

For phosphorus assays after TLC separation, the phosphomolybdenum blue colour formed by the above procedure, was extracted with 5 ml of ethyl acetate [9] and its optical density measured at 780 nm.

Glyceryl alkyl ethers were isolated by submitting the lipid samples (0.1–0.2 μmol of lipid phosphorus) to acetolysis and saponification [10].

Plasmalogens were determined by the method of Gottfried *et al.* [11].

Cholesterol was determined by the method of Huang *et al.* [12]. Since a number of aldehydic compounds and sugars yield an interfering colour in the cholesterol assay, the presence of cholesterol in several fractions was confirmed – apart from analytical determinations by spraying the thin-layer chromatograms with 50% H_2SO_4. By heating at 180 °C, spots corresponding to cholesterol and cholesteryl esters appear very soon, with a characteristic violet colour.

Acyl esters were determined by the method of Snyder *et al.* [13].

Hexose was determined by the method of Dubois *et al.* [14] as modified by Galanos *et al.* [15].

Results and Discussion

As shown in Table I, the average values of total phospholipid contents per gram of wet liver and head show a slight gradual increase during growth (from January to March), whereas the respective values of skin and muscle remain almost unchanged. However, qualitative and semiquantitative examination of the neutral and polar lipid fractions by TLC indicated on gross differences in their overall patterns.

Table I. Preliminary data on the total phospholipid content of the Bogue.

Fish sample	γP/g wet tissue			
	Liver	Skin	Head	Muscle
1. (5 fishes)	814	313	358	214
2. (3 fishes)	870	322	370	212
3. (9 fishes)	1143	336	479	214

The total phospholipid content of the studied tissues of Bogue was found as expected, to be, quite different from one another. Thus, the total phospholipid values in mg/g of wet tissues 28.2 for liver, 12.0 for head, 8.0 for skin and 5.3 for the muscle.

Comparison data on muscle total phospholipids of Bogue and of a series of Teleostean and Elasmobranch fishes reveal a general similarity among them [16–19].

The TLC patterns of neutral and polar lipids of the above total lipid extracts are shown in Figs. 1 and 2 respectively. Quantitative data of the lipid classes of each tissue, obtained by column chromatography (see Methods) are depicted in Table II.

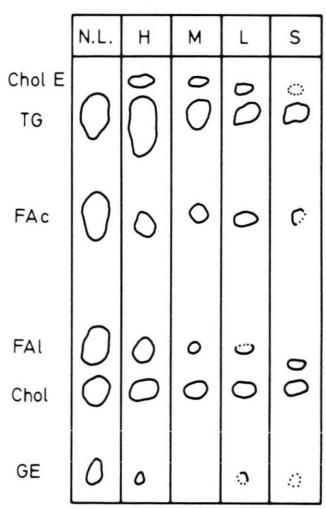

Fig. 1. Thin-layer chromatographic separation of total lipid extracts from head (H), skin (S), liver (L) and muscle (M) tissue of Bogue. N.L. = Neutral lipid standard, composed by triolein, oleic acid, cetyl alcohol, cholesterol and selachyl alcohol (from up to down).

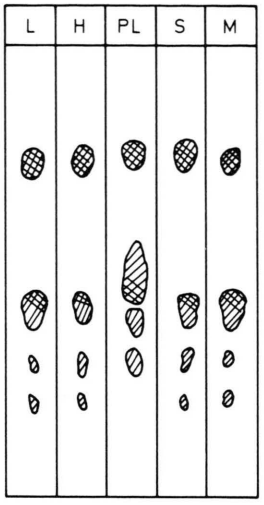

Fig. 2. Thin-layer chromatographic separation of total phospholipids from liver (L), head (H), skin (S) and muscle (M) tissue of Bogue. P.L. = phospholipid standard, composed by phosphatidylethanolamine, phosphatidylserine, phosphatidylinositol, phosphatidylcholine and sphingomyelin (from up to down).

= Positive to ninhydrin spray

= Positive to molybdenum blue

Table II. Analytical data of lipid classes.

Assay	Results in μmol/g wet tissue			
	Liver	Head	Skin	Muscle
Neutral lipids				
Cholesterol	25.00	50.75	115.00	7.90
Acyl esters	120.30	284.20	372.00	28.60
Glyceryl ethers	0.93	36.66	58.38	0.25
Glycolipids				
Hexose	0.37	0.32	0.16	0.09
Sulfolipids				
Hexose	0.19	0.10	0.08	0.07
Phospholipids				
Lipid P	36.38	15.07	10.50	6.80
Acyl esters	69.60	23.57	17.60	12.60
Glyceryl ethers	1.12	0.65	0.44	0.37
Plasmalogen	0.71	1.70	0.69	0.37

The data of Table II revealed a rather non-uniform distribution of lipids in the tissues of Bogue studied.

This is especially evident with respect to the neutral lipid content of the total lipid extracts, as indicated by the corresponding percentages of acyl esters given in Table II. On a weight basis these acyl esters contents correspond to 62.6%, 91.2%, 95.1% and 68.7% of the acyl esters of the total lipid extracts of liver, head, skin and muscle respectively. The cholesterol data of the 4 tissues studied is given in Table II.

The data on liver cholesterol of Bogue (Table II), are very similar with those on the liver cholesterol of pike as given by Wilber (cited by Lovern, ref. [16]).

Examination of the results of Table II and Fig. 1, leads to the conclusion that the depot lipids of Bogue consist essentially of triglycerides which in head and skin are accompanied by significant amounts of glyceryl ether analogs, molar ratios of glyceryl ethers to acyl esters 0.129 and 0.157 respectively.

This feature is in common with some species of Teleostean fishes, whereas the overwhelming majority of Teleostean utilize triglycerides alone as depot lipids (cited by Lovern) [16].

Minor amounts of free fatty alcohols occur in all tissues, decreasing in the order: head, flesh, skin, liver (Fig. 1).

The total glycolipid and sulfolipid contents of all the tissues studied were very low, as indicated by the respective hexose values depicted in Table II.

The phospholipids fractions were investigated in more detail (see methods) and the data is given in Table II.

By combining all these data, as well as the results of P assays after TLC separation of each column fraction, an overall composition for each of the phospholipid fractions of the 4 tissues of Bogue investigated is summarized in Table III.

As shown in Table II, the phospholipids of all the tissues studied contain glyceryl ether and plasmalogen analogs. However, the plasmalogen assays on the individual phospholipid fraction indicated exten-

Table III. The overall composition of the phospholipid fractions of the tissues of Bogue.

Phospholipid	Composition (mol % of total phospholipid)			
	Liver	Head	Skin	Muscle
Cardiolipin	–	0.4	0.15	0.6
Phosphatidylethanolamine	23.0	31.9	33.7	23.7
Phosphatidylserine	3.3	4.4	9.0	5.7
Phosphatidylcholine	56.5	41.1	36.2	59.0
Lysophosphatidylcholine	10.7	22.2	3.2	4.0
Sphingomyelin	6.5		17.75	7.0

sive losses of plasmalogenic constituents. This, combined with the rather significant amounts of lyso-phospholipids found by the TLC analysis (Table III) suggests that an extensive hydrolysis of plasmalogens to lyso-phospholipids occured during the column chromatographic fractionation of phospholipids.

This effect was more pronounced in the liver phospholipids (see Table III).

The main phospholipids of all tissues of Bogue studied are phosphatidylcholine and phosphatidylethanolamine (Table III).

The data on flesh phosphatidylcholine, phosphatidylethanolamine and phosphatidylserine as given by Lovern [16, 19] and Bligh [20] for other Teleostean, are very similar to the data for Bogue described in the present work (Table III). On the contrary phosphatidylinositol was not determined in muscle of Bogue, although it is present in the flesh of other fishes [18, 19].

The data on liver total phospholipids of Bogue are in agreement with the average of total phospholipids of sardine liver (*Clupanodon melanostica,* 2.2%) but not with those of hake (*Merlucius merlucius,* 15%), which is richer in phospholipids.

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