LIPIDS OF PROTOZOA.
II. LIPIDS OF *Paramecium aurelia* AND *Paramecium caudatum*

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The phospholipid composition of two species of infusorians (Ciliophora) has been investigated. It has been shown that the alkyl form is present in the phosphatidylethanolamines and their phosphonic analogs, and in the phosphatidylcholine, phosphatidylserine, and phosphatidylinositol. The main fatty acid in the total lipid extracts was arachidonic. The main alkyl ethers of glycerol were batyl and selachyl alcohols. It has been shown that free-living infusorians may form promising materials for an all-sided study of the metabolism of alkyl-containing glycerophospholipids and sources for their preparative isolation.

We have continued the study of the phospholipid (PL) and fatty acid (FA) compositions of protozoa [1].

The PL and FA compositions have been studied for only two laboratory species of the genus *Paramecium* — *P. aurelia* and *P. tetraurelia* [2]. Infusorians and some other representatives of the protozoa are of interest by virtue of the fact that they contain a large amount of phosphonic alkyl- and alkenylglycerolipids [3-5]. The physiological functions of the phosphonic and alkyl-containing lipids in biological membranes have not yet been adequately elucidated [6].

We have investigated the PL composition of two species of protozoa, *P. aurelia* and *P. caudatum* from natural water bodies, by using a combination of the methods of qualitatively determining the plasmalogen, alkyl-acyl, and diacyl varieties of glycerophospholipids [7] and also the technique of the TLC separation of the phospho and phosphono analogs [8, 9].

As the results of an investigation of the PL composition of the infusorians showed (Table 1), the main representatives were amino-PLs, their sums being approximately the same for the two species: 62.0 and 61%. Alkyl forms were found in the phosphatidylethanolamines and their phosphonic analogs, and also in the phosphatidylserines, phosphatidylcholines, and phosphatidylinositols. The proportions of the alkyl forms in the phosphatidylserine were 23.4 and 44.6%, in the phosphatidylcholine 39.5 and 37.4%, and in the phosphatidylinositol 13.6 and 29.1%.

An investigation of the FA composition of a total lipid extract of whole cells is of definite interest in connection with the fact that infusorians contain a high percentage of polyunsaturated FAs, especially arachidonic acid. We have previously shown that the infusorian *Colpoda steinii* contains a high percentage of this FA [10]. Investigations of the FA composition of the infusorians (Table 2) showed that the predominating FAs were the 16:0, 18:1, 18:2, and 20:4 varieties.

Glycerol alkyl ethers were isolated from the total lipid extracts. Saturated and monoenic alkyl esters were identified: the main alcohols were the 16:0 (72.3 and 57.9%) and 18:1 (25.7 and 41.3%) species. The 16:1 and 18:0 species were identified as minor components (Table 3).

Thus, in infusorians (*Ciliophora*) of the *Paramecium* genus, there are large amounts of alkyl-containing phospholipids, a high percentage of arachidonic acid, and predominantly two representatives of the alkyl ethers. Free-living infusorians are apparently the most promising model objects among the living world for the investigation of the metabolism of alkyl-acyl glycero-PLs.

EXPERIMENTAL

The free-living infusorians *P. aurelia* and *P. caudatum* were isolated from natural water bodies in the region of Tol'yatti. The necessary biomass was obtained by cultivation under artificial conditions. Lipids were extracted by a known method [1]. The amounts of alkyl-acyl- and diacylglycero-PLs were determined by published methods [1, 7-9]. Fatty acid methyl ethers and isopropylidene derivatives of alkyl ethers of glycerol were analyzed by GLC and were identified by Dembitskii's method [11].

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TABLE 1. Phospholipid Composition of Infusorians of the Genus *Paramecium*

Class of phospholipids	P. aurelia	P. caudatum
1-O-Alkyl-2-O-acyl-sn-glycero-3-phosphoethanolamine	4,5 (8,9)*	8,7 (17,9)
1,2-Di-O-acyl-sn-glycero-3-phosphoethanolamine	18,9 (37,6)	17,9 (36,8)
O-Alkyl-2-O-acyl-3-sn-glyceryl (2-aminoethyl)phosphonate	23,2 (46,1)	19,8 (40,7)
1,2-Di-O-acyl-3-sn-glyceryl(2-aminoethyl)phosphonate	3,7 (7,4)	2,2 (4,6)
Lysophosphatidylethanolamine	1,6	1,4
Ceramide aminoethylphosphonate	5,4	6,2
1-O-Alkyl-2-O-acyl-sn-glycero-3-phosphoserine	1,1 (23,4)**	2,5 (44,6)
1,2-Di-O-acyl-sn-glycero-3-phosphoserine	3,6 (76,6)	3,1 (55,4)
Sum of the amino-PLs	62,9	61,8
1-O-Alkyl-2-O-acyl-PCs	9,0 (39,5)***	8,6 (37,4)
1,2-Di-O-acyl-PCs	13,8 (60,5)	14,4 (62,6)
Lyso-PCs	1,8	2,6
Sum of the choline-containing PLs	24,6	25,6
1-O-Alkyl-2-O-acyl-PIs	0,8 (13,6)****	1,6 (29,1)
1,2-Di-O-acyl-PIs	5,1 (86,4)	3,9 (70,9)
Cardiolipin	3,4	2,6
Phosphatidic acid	0,9	—
Sphingo-PLs	3,2	4,5
Percentage of PLs in the total lipids	53,6	56,2

*Percentage of the alkyl and acyl forms on the sum of the forms in the PEs.

**In the PSs.

***In the PCs.

****In the PIs.

TABLE 2. Fatty Acid Compositions of the Total Lipids of Extracts of Infusorians

Fatty acids	Amount, wt. % (GLC)		Fatty acids	Amount, wt. % (GLC)	
	P. aurelia	P. caudatum		P. aurelia	P. caudatum
14:0	0,5	0,5	20:2 ω 9 (+ ω 6)	0,8	1,1
15:0	0,6	0,3	20:3 ω 6 (+ ω 9)	3,6	3,2
16:0	12,4	14,6	20:4 ω 6	28,0	22,3
16:1 ω 9 (+ω 7)	0,9	1,1	20:5 ω 3	2,9	2,4
17:0	—	0,2	22:4 ω 6	—	0,3
17:1 ω 9 (+ ω 8)	—	0,5	22:6 ω 3	—	0,7
18:0	3,1	3,8	Monoenic	13,7	20,6
18:1 ω 9 (+ ω 7)	11,9	16,9	Dienic	20,1	20,0
18:2 ω 6	19,3	18,9	Polyenic	47,7	39,7
18:3 ω 6	13,2	10,8	Saturated	18,5	19,7
20:0	1,9	0,3			
20:1 ω 9	0,9	2,1			

TABLE 3. Composition of the Alkyl Ethers of Glycerol from the Lipids of Infusorian Extracts

Alcohol	Amount, wt. % (GLC)	
	P. aurelia	P. caudatum
1-O-Hexadecyl-sn-glycerol	72,3	57,9
1-O-Hexadecenyl-sn-glycerol	0,7	—
1-O-Octadecyl-sn-glycerol	1,3	0,8
1-O-Octadec-11-enyl-sn-glycerol (paramecyl alcohol)	25,7	41,3

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LIPIDS OF ELAEAGNUS FRUIT

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The composition of the classes of lipids of the fruit of three morphological forms of the elaeagnus Elaeagnus angustifolia *L. have been studied. Their compositions were identical. The amounts of the main lipid classes of extracts of the seeds and pericarps, the fatty acid compositions of the acyl-containing classes of lipids, and the compositions of the carbohydrates and sterols have been determined. The fatty oil of the seeds contained linoleic acid, while the main fatty acids of the pericarp extracts were the 16:0, 18:1, and 18:2 acids. The 16:1 acid that is characteristic for sea buckthorn oil was detected in the elaeagnus fruit in insignificant amounts. The class of sterols, both in the free and in the esterified states, was represented by β-sitosterol. The main hydrocarbon of the pericarps and seeds was nonacosane.*

The family Elaeagnaceae includes three species of plants: *Hippophäe* L., *Elaeagnus* L., and *Sheferdia* L. Widespread on the territory of the Soviet Union are sea buckthorn (*Hippophäe rhamnoides* L.) and two species of elaeagnus — Russian olive (*Elaeagnus angustifolia* L.) and eastern elaeagnus (*Elaeagnus orientalis* L.) [1-3].

In folk medicine, plants of this family have long been known as medicinal. Elaeagnus fruit is used as an astringent, a tincture of the ripe fruit is used in homeopathy, and the leaves (in the form of a lotion) for rheumatism, while the essential oil stimulates the action of the heart [2]. The fatty oil of the sea buckthorn, which is a unique natural concentrate of vitamins and other biologically active compounds, is widely used for various types of tissue disorders. Industry is incapable of satisfying the ever-increasing demands for medicine in the form of sea buckthorn oil and, therefore, the problem of the search for a natural or synthetic analog of it is an urgent one.

It is known that the closer to one another individual plants are in the system, the larger are the amounts of similar chemical ingredients in their compositions [4]. Many studies have been devoted to the composition of the lipids of the sea buckthorn [5-8], but elaeagnus lipids have not previously been studied. The absence of information on the lipid composition and also the fact that elaeagnus belongs to the same family as the sea buckthorn has served as a reason for the study of this plant. Furthermore, this investigation is of interest also from the point of view of elucidating chemotaxonomic characteristics within the family.

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