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LIPIDS FROM THE SAND DOLLAR *Scaphechinus mirabilis*

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The composition of lipids and their fatty acids in tissues of scalp, gonads, and internal organs of the sand dollar Scaphechinus mirabilis *was determined using one- and two-dimensional TLC and GC—MS. Lipids of* S. mirabilis *scalp consisting of several neutral and phospholipids were characterized for the first time. A significant quantity of eicosapentaenoic acid (20:5) was found in scalp lipids.*

Key words: *Scaphechinus mirabilis*, sand dollar, neutral and phospholipids, fatty acids, GC—MS.

Sea urchins are rather well studied because some of them are commercial sources of caviar used in food and some are used as models for genetic and biochemical experiments [1, 2]. Research on the lipid composition is performed most often on sea urchin gonads, sperm, eggs, and embryos [2-7].

Only one report on lipids of *S. mirabilis* has appeared among those on sea urchin species [8]. This species is common in the Sea of Japan and the Bering Sea and differs externally from other species (in the true sea urchin classification). It has an unusual flat shell, almost disk-shaped, that is covered so much by fine chocolate-colored spines that its surface seems velvety rather than spiny [9]. It has been shown [8] that lipids from internal organs of *S. mirabilis* contain up to 7% α-glyceryl ethers (GE), compounds that are derivatives of glycerine and hydrocarbons bound through an ether bond to one or several positions of the glycerine. These compounds are remarkable because one α-GE (batyl alcohol, C_{18} radical) is listed in the Russian pharmacopeia and possesses radioprotective properties [10].

The goal of our research was to characterize and compare the lipid composition of skin with spines (scalp), gonads, and soft internal tissues of *S. mirabilis*. It is known that gonads are responsible for reproduction; internal organs, for vital functions. There is no information on the function of the scalp. It can only be assumed that one of its functions is protection from external environmental factors.

Lipids were extracted from samples by the method of Bligh and Dyer [11]. The content of total lipids (TL) in scalp was 0.80%; in female gonads, 1.45; in male gonads, 4.20; in internal organs, 1.36.

Literature data on total lipids from sand dollars *Echinarachnius parma* and *S. griseus* vary from 0.5 to 2.4%, respectively. The same variation in TL is observed for ordinary (not flat) sea urchin species, from 0.8 to 3.1% [12]. The TL content in male gonads is greater than 4% and is comparable with TL from gonads of other sea urchin species *S. purpuratus*, *Hemicentrotus pulcherimus*, and *Anthocidaris crassispina* [13] but differs from the literature data [14] for *Strogylocentrotus intermedius*, for which the TL content in female gonads was almost two times greater than in male gonads, possibly due to the season in which they were caught. Data were presented for animals caught in September whereas ours were caught in July. In our work the TL content in male gonads was three times greater than in female gonads. According to the literature [14], the TL content in *S. intermedius* shell was 0.5%, which is less than in the scalp in our work.

Neutral lipids (NL) were analyzed by one-dimensional TLC. Phospholipids (PL) remain at the origin in the selected solvent system; NL rise higher. NL from samples of scalp and internal organs are identical and contain the following compounds with R_f values (from bottom up): 0, origin, PL; 0.05, glycerine 1-*O*-monoalkylethers (GE); 0.10, 1,2-diacylglycerides (DG); 0.19, sterols; 0.24, 1,3-DG; 0.31, glycerine dialkylethers; 0.55, triglycerides (TG); 0.85, glycerine trialkylethers. NL from female and male gonads do not contain glycerine di- and trialkylethers and TG. Eggs (female gonads) contain significant amounts of 1,3-DG (according to the spot intensity) in contrast with sperm (male gonads) and scalp.

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TABLE 1. Composition and Content of Fatty Acids in Scalp, Gonads, and Internal Organs of the Sand Dollar *S. mirabilis*, % of Fatty-Acid Mass

FA	Scalp	Female gonads	Male gonads	Internal organs	FA	Scalp	Female gonads	Male gonads	Internal organs
13:0	0.9				18:4n-3	1.1			2.3
14:0	5.8	6.5	5.2	4.8	19:0		2.0	2.1	
14:0i		4.2		4.4	19:1n-9	0.8			1.7
15:0	6.3		5.9		19:2				1.8
16:0	9.5	21.4	18.4	10.5	20:0	0.7	1.5	1.8	
16:0i				3.4	20:1n-11	8.9	4.5	4.7	5.9
16:1n-7	10.7	11.8	7.4	16.0	20:1n-9	2.4	11.5	8.7	3.0
16:3n-3	0.9			2.5	20:2n-9	4.4	3.2		
16:4n-3	1.1			3.5	20:2n-6	3.9	4.8	1.8	1.2
17:0		4.3	5.0		20:4n-3	5.6	2.0	2.9	2.3
18:0	3.7	11.6	13.5	3.1	20:5n-3	**23.6**		3.5	**25.8**
18:0i				2.2	21:1n-11	1.2			
18:1n-11			5.1		22:1n-11	1.3			
18:1n-9	1.2	6.8	4.5	Tr.	22:2n-9	1.1			Tr.
18:1n-7	2.2	3.9	6.9	2.8	22:6n-3	2.4			2.8

The compounds were identified by comparison with the literature [15]. The observation in *S. mirabilis* of α-GE and other glycerine ethers, in particular, di- and triethers, is consistent with unpublished data of S. V. Isay, according to which the *S. mirabilis* GE (in addition to α-GE) fraction isolated preparatively was 20.6%; in *S. griseus*, 85.1%. In general, the NL in sperm and eggs differ little from those in other sea urchin species [13, 14]. TLC showed that male gonads contained traces of sterols whereas the female gonads contained more.

Two-dimensional micro-TLC of PL used known systems (see Experimental) with variable amounts of water because of the high humidity. PL were analyzed for scalp. Despite the fact that material of animal origin was investigated, we used systems for tissue of both animal and plant origin. Results were clearer for systems used for tissues of plant origin. Skin with spines contained the following polar lipids: phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS), phosphatidylinosite (PI), monogalactosyldiacylglycerine (MGDG), pigment, and an unidentified compound migrating closer to the solvent front in the center of the plate. There are no reports of the PL composition from sea urchin skin. It can be stated that the main set of classical PL reported in the literature for the most studied sea urchin tissues, e.g., gonads [16, 17], was also observed in spines.

Fatty acids (FA) from the studied tissues were analyzed by GC and GC—MS. Table 1 lists the results. As it turned out, scalp contained the same variety of FA as the internal organs. The scalp and internal organs of sand dollar were dominated by 20:5 n-3, the content of which was 24-26%. Certain rare FA (16:3, 16:4, 18:4) were found only in scalp and internal organs. The FA compositions of female and male gonads differed little from each other. Palmitic acid (16:0) dominated them. Certain odd FA (17:0 and 19:0) were found only in gonads. Female and male gonads also had polyunsaturated FA (PUFA). However, their fraction of the total FA was less than reported in the literature for gonads of certain species of common sea urchins. Thus, female gonads of *S. intermedius* contained 20:5 PUFA in the range 15.1-32.5% depending on the habitat; male gonads, 21.1-36.5% [18]. The content of 20:5 PUFA in gonads of *S. droebachiensis* during spawning season (December-June) varied in the range 22.5-19.1% [19]; shell lipids, up to 30% 20:5 PUFA [19]. It is thought that the composition of gonads varies during the year [19]. Significant amounts of arachidonic (20:4) and 20:5 PUFA have been found in spematozoa of *H. pulcherrimus* and *A. crassispina* [17]. Literature data [20] for the effect of diet on the FA composition of gonads of the aquarium sea urchin *Psammechinus miliaris* are comparable with our data for saturated FA 14:0, 16:0, and 20:0 in gonads. The exception was 18:0 FA. In our specimen, its content was markedly higher. Data for monoenoic FA were similar. The difference was in 18:4 FA. We did not observe it in *S. mirabilis* gonads whereas its content was high for certain diets in *P. miliaris* gonads. The 16:0 FA dominated in internal soft tissues, including gonads, of *Paracentrotus lividus* [21], in contrast with soft tissues of *S. mirabilis*. Lipids from soft tissues of *P. lividus* contained a significant amount of 20:5 PUFA (12.2% of total FA). The predominant component in soft tissues from *S. mirabilis* (Table 1, internal organs) was 20:5 PUFA. It was up to 26% of the total FA.

According to the literature, *S. griseus* contained 18.8% 20:5 whereas its content was 3.5% in another sand dollar species *Echinorachnius parma*. The false sea urchin *Echinocardium cordatum* contained 47% 20:5 PUFA [12]. Significant amounts of 20:4 and 20:5 PUFA were found in true sea urchin species *S. intermedius* and *S. nudus* [12]. These acids are direct precursors of prostaglandins (PG), the presence of which was demonstrated for certain sea urchin species. Thus, Lomova et al. detected PG in *S. intermedius* eggs during early embryogenesis [22]. Both fertile and infertile sea urchin eggs can accumulate PGA_1 [23]. Korotchenko et al. found that internal organs of *S. intermedius* and *S. nudus* have PG activity [24].

Thus, the lipid composition of *S. mirabilis* scalp was characterized for the first time. Scalp contains neutral PL and glycolipid (monogalactosyldiacylglycerine). The FA composition of scalp consists of a variable set of FA. The predominant one is 20:5 PUFA. Its content in the total FA approaches 24%.

EXPERIMENTAL

Specimens. Animals were collected in July in Troitsa Bay of Peter the Great Gulf in the Sea of Japan. Lipid composition was studied in scalp from 12 freshly collected animals that were washed with distilled water and dried under a descending stream of air. Scalp (fragments of the collagen covering of the carbonate backbone with spines) was scraped off to give a sample as a powder after grinding (36.5 g). Female (1.38 g) and male (1.42 g) gonads were extracted from frozen animals to produce highly homogeneous tissues of these organs. Internal organs of *S. mirabilis* were extracted from freshly collected animals (64.1 g).

Lipid Extraction. Scalp was extracted as usual [11] with the exception that the ratio of volumes (sample:solvent) was 1:3 and the extraction intensified by ultrasound was carried out twice. A small amount of water was added to the combined extracts to layer the phases and remove nonlipid impurities. The lower organic layer resulting from centrifugation (15 min at 3000 rpm) was transferred by glass pipette into a flask and reduced in volume by evaporation in vacuo. The concentrated extract was transferred to a 2-mm vial. Residual solvent was evaporated by a stream of inert gas. The resulting analytical sample was stored at -20°C until analysis.

Soft tissues (gonads, internal organs) were extracted the same way without treatment of the samples with ultrasound. All extracts were combined for each sample. Each was treated with an amount of distilled water equal to the amount of methanol used for the initial extraction. The mixture was stirred and left in a refrigerator until fully layered. Water can be removed best from the organic phase by adding about one tenth the volume of benzene to the mixture after addition of the water. Solvents were removed in a rotary evaporator in a water bath at less than 40°C. If necessary, traces of water in the extracts were removed by adding benzene:ethanol (9:1) to form an azeotrope.

TLC of Lipid Extracts. Neutral and phospholipids were separated using plates on an aluminum support (silica gel STKh-1A, 110 μm thick, silicasol binder, 10 × 10 cm, Sorbfil, Krasnodar, Russia). NL were developed using hexane:Et_2O:CH_3CO_2H (85:15:1) [15]. NL were identified by R_f values taken from the literature [15].

Two-dimensional micro-TLC of PL was performed on plates (Sorbfil, 6 × 6 cm) using the systems (first direction) $CHCl_3$:acetone:MeOH:HCOOH (5:2:1:1) and (second direction) acetone:C_6H_6:HCOOH:H_2O (200:30:3:1). Spots were observed by heating after immersing plates in a beaker with H_2SO_4 (10%) in EtOH.

GC of FA. Methyl esters of FA were prepared as before [25]. We used an Agilent Technology 6850 chromatograph (Germany), HP-5-MS-5% capillary column (phenylmethylsiloxane, 30 m × 250 μm × 0.25 μm), Ar carrier gas, t = 220°C, and isothermal conditions.

GC—MS of FA was performed in an HP GC/MSD 5393, HP-5-MS capillary column, temperature programmed at 3°/min for 140-280°C using US NBS (mass spectra database).

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