THE LIPIDS OF SEA-URCHIN SEMEN

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The constitution of the lipid material present in semen has received very scant attention. No reference appears in the literature relating to the component fatty acids of semen lipids, although the presence of neutral fat, free fatty acid, free and combined sterols, phosphatides, and sphingomyelin has been reported by Woodhouse (1928) in the sex organs of domestic animals and of humans; likewise, Sakaki (1913) reported the presence of phosphatides and sphingomyelin in the human placenta; Sano (1922) reported the presence of these phosphatides in the fresh 'sperm freed from membranes and connective tissue' of the cod, porgy and salmon. More recently, Lardy & Phillips (1941), in an investigation of the sources of energy for bull spermatozoa, showed that the phospholipid content of both semen and spermatozoa decreased when incubation was carried out in the absence of extracellular substrates but in the presence of oxygen.

Our knowledge of the physiology of sea-urchin spermatozoa is primarily due to Rothschild and co-workers who have shown (see Rothschild, 1951) that sea-urchin spermatozoa differ from those of mammals, in which the energy for motion is provided from outside the spermatozoa in the form of substrates in the seminal plasma. The energy necessary for the movement of sea-urchin spermatozoa does not arise to any great extent from enzymatic breakdown of substrates in their seminal plasma since they are capable of prolonged activity when suspended in pure sea water. Serious doubt has been cast on the view that the energy required is derived from breakdown of intracellular carbohydrate (Rothschild & Cleland, 1952). These authors have shown that the phospholipid content of the spermatozoa of *Echinus esculentus* amounts to about 5·5 % of the dry weight of the spermatozoon, while the seminal plasma contains much less. On aerobic incubation of the semen diluted with sea water the phospholipid content was shown to decrease, indicating that oxidative breakdown of the phospholipid material was the principal source of energy required.

It was considered that further information concerning the physiology of sea-urchin spermatozoa might be derived from a more detailed study of sea-urchin semen lipids.

METHODS AND RESULTS

1193 g. of the semen of *E. esculentus* kindly placed at our disposal by Lord Rothschild was extracted by refluxing with acetone. The solid tissue was pressed and re-extracted with acetone, this process being repeated three times, followed by a final extraction of the tissue with light petroleum (b.p. 40/60). The acetone extracts

were combined, acetone distilled off and the residue thoroughly extracted with light petroleum to which was added the final petroleum extract of the tissue. After removal of the petroleum, the lipid extract was heated at 100° C. under reduced pressure for a short time to remove the last traces of solvents and moisture, when 11·40 g. (0·95%) of lipids were obtained.

The phospholipids were separated from the total lipids by crystallization from acetone at 0° C. (4 ml./g.), the insoluble fraction then being freed from other entrained lipids by three further crystallizations from acetone at the same temperature and dilution. In this way the total lipids were resolved into 8·440 g. (74·0%) non-phosphorus-containing lipids and 2·960 g. (26·0%) phospholipids, the nitrogen (Kjeldahl) and phosphorus (molybdate) contents of the latter fraction being 2·0 and 3·2% respectively, giving an atomic ratio N : P of 1·4 : 1.

Table 1. *Sea-urchin semen lipids*

	Non-phospholipid 'glycerides'	Non-phospholipid 'glycerides' fatty acids excluding unsaponifiable	Phospholipid fatty acids
Saponification equivalent	415·1	288·1	286·1
Iodine value	199·5	246·6	189·8
Free fatty acids (as % oleic)	44·5	—	—
Unsaponifiable (wt. %) Sterols	12·4	—	—
Non-Sterols	24·7	—	—
Neutral fat	18·4	—	—

After determining the characteristics of the non-phosphorus-containing lipids the fraction was saponified and unsaponifiable material (2·960 g. iodine value 144·0) extracted from the soaps. Crystallization of the unsaponifiable material from light petroleum gave rosette form crystals. These were recrystallized from the same solvent, both mother liquors being combined and the petrol removed, the unsaponifiable material in this way being resolved into two fractions. The sterol content of each fraction was determined by the method described by Popjak (1943), the crystalline fraction containing 28%, that of the fraction recovered from the mother liquors containing 38%, the mean sterol content of the unsaponifiable material being 34%.

In Table 1 are summarized the data obtained from the separation and resolution of the lipids, together with the characteristics of the non-phosphorus-containing lipids and phospholipid fractions respectively.

From the above data it can be deduced that the semen lipids of *E. esculentus* consisted of 13·6% neutral fat, 32·9% free fatty acid, 26·0% phospholipids, 9·2% sterols, 18·3% other unsaponifiable material.

Component fatty acids of the non-phosphorus containing lipids. 4·95 g. mixed fatty acids (recovered from the soaps after extraction of the unsaponifiable material and comprising the free acids, together with those derived from the neutral glycerides) were resolved by crystallization from acetone (10 ml./g.) at −60° C. into two

TABLE 2. *Crystallization of sea-urchin mixed fatty acids*

Fraction	Conditions	Weight		Iodine value
		g.	% of total	
A	Insoluble in acetone at −60° C.	1·40	28·3	75·8
B	Soluble in acetone at −60° C.	3·55	71·7	296·6

Table 3. *Fractionation data for sea-urchin ester fractions*

Fraction	Weight (g.)	Saponification equivalent	Iodine value
	Methyl esters of acids A		
A1	0·55	283·1	37·5
A2	0·57	315·2	82·7
A3*	0·19	—	—
Total	1·31	—	—
	Methyl esters of acids B		
B1	0·60	288·1	216·6
B2	0·57	301·2	261·9
B3	0·58	310·2	284·1
B4	0·60	311·0	312·5
B5	0·59	317·4	321·6
B6	0·56	345·4†	294·0
Total	3·50	—	—

* A3 unsaponifiable matter (by weight) 0·05 g. Remaining esters calculated as sap. equiv. 315·2, iodine value 82·7 (A2).

† Equivalent of B6 esters freed from unsaponifiable matter 340·3. Unsaponifiable matter (by weight) 0·03 g.

Spectroscopic analysis of acids recovered from A1 and A2:

$E^{1\%}_{1cm.}$	234 mμ	268 mμ	301 mμ	315 mμ
A1 (unisomerized)	12·2	4·1	—	—
A1 (isomerized)	46·2	52·0	15·2	14·6
A2 (unisomerized)	19·4	6·0	—	—
A2 (isomerized)	90·4	113·4	32·1	31·9

Table 4. *Sea-urchin semen component fatty acids*

Acid	A (28·3 %)* (%)†	B (71·7 %)* (%)†	Total fatty acids excluding unsaponifiable	
			% (w/w)	% by mol.
Palmitic	35·0	—	10·1	11·5
Unsaturated C_{18}	—	2·8 (−3·0)	2·1 (−3·0)	2·4
Unsaturated C_{18}	19·0 (−2·5)	34·2 (−5·4)	30·4 (−4·9)	31·9
Unsaturated C_{20}	30·5 (−2·5)	49·8 (−8·1)	45·1 (−7·0)	43·4
Unsaturated C_{22}	11·5 (−2·5)	12·3 (−8·0)	12·3 (−6·5)	10·8
Unsaponifiable	4·0	0·9	—	—

* Groups as % (w/w) of total fat.

† Component acids as % w/w of group.

Figures in brackets denote mean unsaturation of each group of acids (Hilditch, 1947, p. 23).

fractions, consisting of mainly saturated and monoethenoid acids, and mainly polyethenoid acids respectively.

Each group of acids was methylated (Bjarnason & Meara, 1944), fractionated, and from the analytical data obtained the composition of each ester fraction was calculated (Hilditch, 1947, pp. 498–570), and therefrom the component acids in each group of acids, and ultimately the component fatty acids of the unsaponifiable free non-phosphorus-containing lipids.

DISCUSSION

If it can be assumed that the seminal plasma of sea-urchin semen is as deficient in non-phosphorus-containing lipids as it is in phospholipids, then the present investigation gives some indication of the nature of the lipids of sea-urchin spermatozoa.

The component fatty acids of the non-phospholipid fraction indicate that this portion of the total lipids conforms to the general character of marine oils by virtue of the low saturated acid content and high content of highly unsaturated acids of the C_{18}, C_{20} and C_{22} series. This is seen by comparing sea-urchin semen fatty acids with those of the lipids from two other invertebrate marine animals, the mussel (*Mytilus edulis*) (Lovern, 1938), and the prawn *Leander serratis* (Klem, 1935) (Table 5).

The content of unsaturated C_{16} acids in the sea-urchin semen mixed fatty acids is somewhat lower, and the unsaturated C_{20} acids content correspondingly higher than those of the other two invertebrate fats recorded, this relationship holding for the depot fats and liver lipids of most marine animals. Although for obvious reasons no rigid comparison can be made between sea-urchin semen fatty acids and those present in the fats from different organs of other marine organisms, nevertheless, there is sufficient resemblance between these fats to indicate that the composition of the fatty acids derived from the sea-urchin semen lipids is typically marine in type, containing a characteristically low amount of saturated acids and a high proportion of highly unsaturated acids of the C_{18}, C_{20} and C_{22} series. The possibility that these unsaturated fatty acids may play some role in the fertilization reaction has been discussed by Rothschild (1952).

No explanation can as yet be given to account for the high free acidity of the semen fat. It is probable that this is due to lipolytic hydrolysis of part of the original neutral fat and possibly of sterol esters having proceeded to a considerable extent. On the other hand, it has often been observed in these laboratories that lipids extracted from fresh livers, where fat is being actively metabolized, have a relatively high free acidity, and it is possible that a somewhat similar process is operating in sea-urchin spermatozoa. A further observation is that high free acidity appears to be concomitant with a relatively high unsaponifiable (and therefore sterol) content, this tendency also being observed in the liver lipids of the ox, sheep and pig (Hilditch & Shorland, 1937) and of the elephant (Cama, 1952). In the present investigation it was not found possible to determine whether the sterols occurred free or combined, but if the sterols of sea-urchin spermatozoa resemble

those found in other marine organisms it is probable that the bulk occurs as free sterols.

The high content of phospholipids (26%) in sea-urchin semen lipids is of the same order as that found (40%) in the lipids of the common mussel. This phospholipid content, being of the order of 2·3% calculated on a dry weight basis appears to be in keeping with the value of 5·5 obtained by Rothschild & Cleland (1952), indicating that about 60% of the phospholipid content has been metabolized in the interval between collecting the semen and its extraction. It is not possible to say, however, whether any of the non-phosphorus-containing lipids have also been metabolized.

Table 5. *Component fatty acids of aquatic invertebrate animal lipids* (%, *w*/*w*)

	Sea-urchin semen non-phospholipid 'glycerides'	Common mussel non-phospholipid 'glycerides'	Prawn total lipids
Myristic	10·1	1·9	1·5
Palmitic		16·7	9·5
Stearic		1·7	2·0
Unsaturated C_{16}	2·1 (−3·0)	10·9 (−2·5)	13·5 (−2·0)
Unsaturated C_{18}	30·4 (−4·9)	21·5 (−4·1)	32·0 (−3·3)
Unsaturated C_{20}	45·1 (−7·0)	29·9 (−7·3)	34·0 (−6·0)
Unsaturated C_{22}	12·3 (−6·5)	13·9 (−9·3)	7·0 (−10·0)
Unsaturated C_{24}	—	3·5 (−?)	—

The N : P ratio of the crude phospholipids gives some indication as to the nature of their constituents. This ratio is of the order of 1 : 1 for vegetable phospholipids but of the order of 1·5 : 1 for phospholipids of animal origin. The ratio in the case of the sea-urchin semen phosphatides, being 1·4 : 1, is in agreement with this value and indicates the presence of monoaminophosphatides, together with a certain amount of diaminophosphatides, either sphingomyelin or related substances (which are known to occur in sex organs, the placenta and fish sperm), contaminated possibly with smaller amounts of non-phosphorus-containing lipoproteins.

From the characteristics of the mixed fatty acids recovered from the phospholipids, sapon. equiv. 286·1, iodine value 189·9, it is seen that these acids also can be considered to be normal for those derived from the phospholipids of marine species.

It appears, therefore, that there are adequate reserves of lipid material, both glyceridic and possibly in the form of free fatty acids, in addition to phospholipids, to provide a considerable amount of energy for the motility of sea-urchin spermatozoa when suspended in sea water.

SUMMARY

1. The specimen of sea-urchin semen examined contained 0·95% lipid material consisting of 13·6% neutral fat, 32·9% free fatty acids, 26·0% phospholipids, 9·2% sterols and 18·3% other unsaponifiable material.

2. The component fatty acids of the non-phosphorus-containing lipids have been computed to be: palmitic 10·1, unsaturated C_{16} 2·1 (−3·0), C_{18} 30·4 (−4·9), C_{20} 45·1 (−7·0), C_{22} 12·3 (−6·5)%, w/w, this being a composition which can be regarded as typical of a marine animal fat.

3. The N : P ratio of the phospholipids indicated the presence of mono- and diaminophosphatides and possibly the presence of non-phosphorus-containing lipoproteins.

4. Adequate reserves of non-phosphorus-containing lipids are present in the spermatozoa, in addition to phospholipids to serve as a source of energy required for movement.

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REFERENCES

BJARNASON, O. B. & MEARA, M. L. (1944). The mixed unsaturated glycerides of liquid fats. *J. Soc. chem. Ind., Lond.*, **63**, 61.

CAMA, J. S. (1952). Elephant lipids. Ph.D. Thesis. University of Liverpool.

HILDITCH, T. P. (1947). *The Chemical Constitution of Natural Fats*, 2nd ed. London; Chapman and Hall.

HILDITCH, T. P. & SHORLAND, F. B. (1937). The composition of the liver fats of some New Zealand farm animals. *Biochem. J.* **31**, 1499.

KLEM, A. (1935). Marine crustacea fats. *Hvalrdd Skr.* **11**, 49.

LARDY, H. A. & PHILLIPS, P. H. (1941). The interrelation of oxidative and glycollytic processes as sources of energy for bull spermatozoa. *Amer. J. Physiol.* **133**, 602.

LOVERN, J. A. (1938). Fat metabolism in fishes. *Biochem. J.* **32**, 1214.

POPJAK, G. (1943). Colorimetric determinations of total, free, and ester cholesterol in tissue extracts. *Biochem. J.* **37**, 468.

ROTHSCHILD, LORD (1951). Sea-urchin spermatozoa. *Biol. Rev.* **26**, 1.

ROTHSCHILD, LORD (1952). Spermatozoa. *Sci. Progr.* **40**, 1.

ROTHSCHILD, LORD & CLELAND, K. W. (1952). The physiology of sea-urchin spermatozoa. The nature and location of the endogenous substrate. *J. Exp. Biol.* **29**, 66.

SAKAKI, C. (1913). Some phosphatides of human placenta. *Biochem. Z.* **49**, 317.

SANO, M. (1922). Phosphatides of fish sperm. *J. Biochem., Tokyo*, **1**, no. 1, 17.

WOODHOUSE, D. L. (1928). The fat, lipin and cholesterol constituents of adrenals and gonads in cases of mental disease. *Biochem. J.* **22**, 1087.