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Phosphatidylcholine Is an Endogenous Substrate for Energy Metabolism in Spermatozoa of Sea Urchins of the Order Echinoidea

MASATOSHI MITA[1] and MASARU NAKAMURA[2]

[1]*Department of Biochemistry, Teikyo University School of Medicine, Itabashi-ku, Tokyo 173, Japan and* [2]*Department of Biology, Faculty of Medicine, Teikyo University, Hachioji, Tokyo 192-03, Japan*

ABSTRACT—A study was conducted to examine an endogenous substrate for energy metabolism in spermatozoa of six species of sea urchin, *Anthocidaris crassispina, Echinometra mathaei, Pseudocentrotus depressus, Strongylocentrotus intermedius, Strongylocentrotus nudus* and *Temnopleurus hardwickii,* which belong to the order Echinoidea. These spermatozoa contained cholesterol and several kinds of phospholipids, including phosphatidylcholine (PC), phosphatidylserine, phosphatidylethanolamine and cardiolipin. After dilution of dry sperm from these species in seawater, a rapid decrease in the level of PC was observed, but other phospholipids remained at constant levels. The preferential hydrolysis of PC was related to the properties of phospholipase A_2. Ultrastructural study showed that lipid bodies were present within mitochondria of the sperm midpieces of *A. crassispina, E. mathaei* and *P. depressus.* After incubation in seawater, the lipid bodies became small or disappeared. Thus it is concluded that spermatozoa of sea urchins of the order Echinoidea commonly use PC located in the lipid bodies within mitochondria as a substrate for energy metabolism.

INTRODUCTION

It is known that spermatozoa are stored for months as immotile cells in male sea urchins [7, 24]. After being spawned in seawater, spermatozoa begin movement, and respiration is activated. The energy for swimming is produced by mitochondrial respiration, and ATP is utilized almost exclusively by the dynein ATPase of the flagellar axoneme [4–6]. The transportation of high-energy phosphate from the midpiece to the tail is associated with a creatine-phosphate shuttle [27, 28]. However, it seems unlikely that sea urchin spermatozoa are capable of using an exogenous substrate for energy metabolism, since hardly any nutrients are present in seawater. Previous studies have shown that sea urchin spermatozoa obtain energy for swimming through oxidation of endogenous phospholipids [20–22, 25]. Sperm respiration also supports the phospholipid metabolism [20–22, 25]. Furthermore, it has been shown that following incubation in seawater of spermatozoa from *Hemicentrotus pulcherrimus,* a sea urchin of the order Echinoidea, the content of endogenous phosphatidylcholine (PC) decreases significantly, with no change in the levels of other phospholipids [16, 18, 19]. Thus, *H. pulcherrimus* spermatozoa possibly use PC as a substrate for energy metabolism. It is important to determine whether PC is a common preferred substrate for energy metabolism in sea urchin spermatozoa. For further clarification of energy metabolism using PC, the present study examined the substrate for energy metabolism in spermatozoa of six species of sea urchin of the order Echinoidea; *Anthocidaris crassispina, Echinometra mathaei, Pseudocentrotus depressus, Strongylocentrotus intermedius, Strongylocentrotus nudus* and *Temnopleurus hardwickii.*

Recently, PC has been shown to be abundant in *H. pulcherrimus* sperm midpieces [13]. Following the initiation of motility, the PC content of sperm midpieces decreases significantly, while that in sperm heads and tails does not change [13]. Fur-

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thermore, the sperm midpiece of *H. pulcherrimus* has been shown to contain lipid bodies in the intramembrane space of the mitochondrion [14]. Interestingly, the lipid bodies become small gradually, coincident with a decrease in the level of PC [14]. Therefore, an ultrastructural examination of the sperm midpieces of *A. crassispina, E. mathaei* and *P. depressus* was also carried out.

MATERIALS AND METHODS

Sea urchins

Six species of sea urchin belonging to the order Echinoidea: *A. crassispina, E. mathaei, P. depressus, S. intermedius, S. nudus* and *T. hardwickii,* were collected at Asamushi (Aomori, Japan), Misaki (Kanagawa, Japan), Tateyama (Chiba, Japan) and Kagoshima (Kagoshima, Japan). *E. mathaei* consists of four different types (A, B, C, and D types) which are distinguishable by color pattern of spines, shape of spicules and so on [29, 30]. A type of *E. mathaei* was used in this study. Spermatozoa were obtained by forced spawning induced by injection of either 0.5 M KCl or 0.1 M acetylcholine into the coelomic cavity. Semen was always freshly collected as "dry sperm" and kept undiluted on ice. The number of spermatozoa was counted with a hemocytometer.

Incubation of spermatozoa

Dry sperm were diluted 100-fold in artificial seawater (ASW) consisting of 458 mM NaCl, 9.6 mM KCl, 10 mM $CaCl_2$, 49 mM $MgSO_4$, 10 mM Tris-HCl at pH 8.2. Following dilution and incubation at 20°C, each sample was centrifuged at 3,000×*g* for 5 min at 0°C.

Analysis of lipids

Total lipids were extracted from spermatozoa by the method of Bligh and Dyer [13] and analyzed by high-performance thin-layer chromatography (HPTLC), according to the method of Macala *et al* [12] with some modifications as described in previous papers [18, 22]. The amounts of PC, phosphatidylserine (PS), phosphatidylethanolamine (PE), cardiolipin (DPG), cholesterol (Ch), and free fatty acid (FFA) in sea urchin spermatozoa were determined from the standard curves of the respective authentic lipids. The mass of PC in μg was converted to nmol using the relation, 1 μg= 1.27 nmol, based on 1-palmitoyl-2-arachidonyl-PC. Also, 1 μg of FFA was calculated to be 3.27 nmol as arachidonic acid.

Analysis of fatty acid composition of PC

Isolated PC on a thin-layer chromatography (TLC) plate was subjected to methanolysis by heating with 5% HCl-methanol at 85°C for 2 hr, as described previously [16, 17]. The fatty acid methylesters were extracted with *n*-hexane, followed by evaporation under a stream of N_2. The residues were dissolved in a small amount of *n*-hexane and analyzed using a GC-R1A gas-liquid chromatograph (GLC) (Shimadzu Instruments, Kyoto, Japan).

Assays of glycogen and glucose

Before and after incubation of dry sperm in ASW, spermatozoa were homogenized with 0.6 M perchloric acid. The homogenate was used for determination of glycogen by the enzymatic method [8]. The acidified homogenate was centrifuged at 10,000×*g* for 10 min at 4°C, and the supernatant was used for estimation of glucose after neutralization to pH 6.5–7.0 with KOH. Glucose was measured enzymatically according to Kunst *et al* [9]. Readings were made at 340 nm at 20°C using a UVIDEC 430B spectrophotometer (Japan Spectroscopic Co., Tokyo, Japan).

Oxygen consumption

Oxygen consumption in a sperm suspension was measured polarographically with an oxygen consumption recorder (MD-1000, Iijima Electronics MFG Co., Aichi, Japan). Twenty-five microliters of dry sperm was incubated in 2.5 ml ASW in a closed vessel of the oximeter at 20°C. The diluted spermatozoa were left exposed to air until determination of oxygen consumption at the desired time. Total oxygen consumption was calculated from the respiratory rate and incubation period, as described previously [20].

Estimation of phospholipase and lipase activity

Dry sperm were homogenized with 10 mM

$CaCl_2$, 10 mM $MgCl_2$, 1 mM dithiothreitol and 50 mM Tris-HCl at pH 7.5. The homogenate was incubated with 4.75 kBq 1-palmitoyl-2-[1-^{14}C]arachidonyl-PC (1.9 GBq/mmol), 4.75 kBq 1-palmitoyl-2-[1-^{14}C]arachidonyl-PE (1.9 GBq/mmol) or 9.25 kBq [carboxyl-^{14}C]-triolein (4.1 GBq/mmol) for 1 hr at 20°C in a total volume of 0.4 ml. The lipids were extracted according to Bligh and Dyer [3]. The radioactivity in the FFA fraction separated by TLC was measured by liquid scintillation spectrometry. The protein content of the homogenate was measured by the method of Lowry *et al* [11], using bovine serum albumin as the standard.

Electron microscopic observation

Before and after dilution of dry sperm in ASW and incubation for 30 min at 20°C, the spermatozoa were prefixed in 2.5% glutaraldehyde-ASW solution for 40–60 min at 4°C; a volume of sperm suspension was mixed with the same volume of cold 5% glutaraldehyde in 80% ASW. The fixed spermatozoa were rinsed with cold ASW and post-fixed with 1% OsO_4 in ASW for 2 hr at 4°C. Samples were washed in distilled water, and then immersed in saturated aqueous uranyl acetate for 4 hr for block staining. After dehydration in a graded series of ethanol solutions, the specimens were embedded in epoxy resin and ultrathin-sections were cut on a Reichert Ultracut ultramicrotome. After staining with lead citrate, they were observed using a Hitachi 7000 electron microscope.

Reagents

The phospholipid, Ch and FFA standards were purchased from Sigma Chemical. 1-Palmitoyl-2-[1-^{14}C]arachidonyl-PC, 1-palmitoyl-[1-^{14}C]-arachidonyl-PE and [carboxyl-^{14}C]triolein were obtained from Du Pont-New England Nuclear. All reagents and solvents were of analytical grade. HPTLC and TLC plates (silica gel 60) were obtained from E. Merck (Darmstadt, Germany).

RESULTS

Lipid content

Previous studies have shown that the lipids in *H. pulcherrimus* spermatozoa are composed of several kinds of phospholipid and Ch [16, 17]. Similar phospholipids and Ch were also detected in *A. crassispina, E. mathaei, P. depressus, S. intermedius, S. nudus* and *T. hardwickii* (Fig. 1). Among the phospholipids, PC, PS, PE and DPG were identified in these spermatozoa. PC was present at high concentrations. Triglyceride and cholesterol ester were present at extremely low levels (less than 1 μg/10^9 sperm).

When dry sperm of *A. crassispina, E. mathaei, P. depressus, S. intermedius, S. nudus* and *T. hardwickii* were diluted 100-fold in ASW and incubated for 1 hr at 20°C, the PC content decreased significantly following the initiation of flagellar movement (Figs. 1 and 2). Although a slight increase in FFA was also observed during incubation, the levels of other phospholipids and Ch remained almost constant. During incubation in ASW for 1 hr, about 11, 9 and 5 nmol PC were consumed by 10^9 spermatozoa of *A. crassispina, E. mathaei* and *P. depressus,* respectively (Fig. 2a). Amounts of FFA accumulated were about 5, 5 and 2 nmol in 10^9 spermatozoa of these species (Fig. 2b).

Fatty acid composition of PC

Fatty acid components of PC in dry sperm of *A. crassispina, E. mathaei, P. depressus, S. intermedius, S. nudus* and *T. hardwickii* were of the unsaturated type for the most part, such as oleic (18:1), eicosamonoenoic (20:1), arachidonic (20:4) and eicosapentaenoic acid (20:5) (Table 1). Polyenoic fatty acids constituted more than 50% of the total fatty acid moiety of PC in these spermatozoa, except that the PC in *T. hardwickii* spermatozoa contained about 35% polyenoic fatty acids. In contrast, the saturated fatty acids in PC were present at only 20–30%, palmitic (16:0) and stearic (18:0) acids being predominant.

Glycogen and glucose content

Glycogen was present in spermatozoa of the six

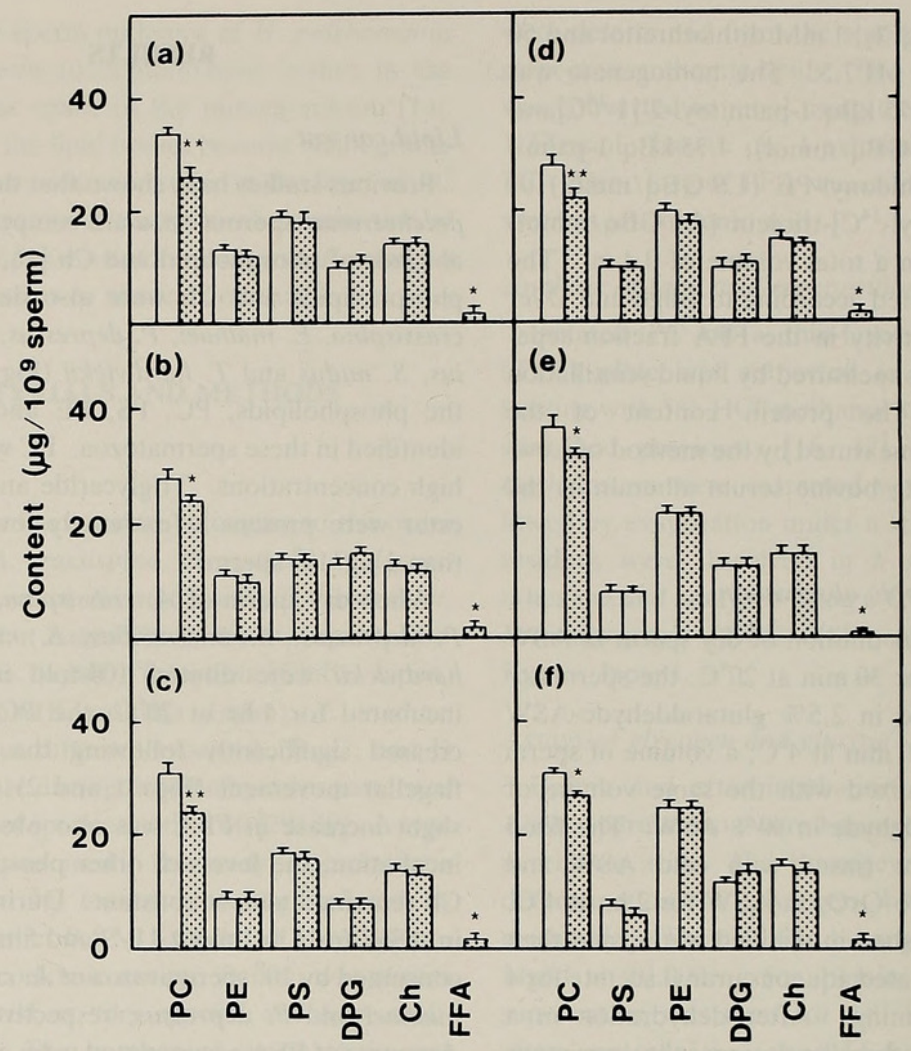


FIG. 1. Changes in lipid levels after incubation of spermatozoa of *A. crassispina* (a), *E. mathaei* (b), *P. depressus* (c), *S. intermedius* (d), *S. nudus* (e) and *T. hardwickii* (f). Before (clear) and after (dotted) 100-fold dilution and incubation of dry sperm in ASW for 1 hr at 20°C, lipids were extracted and analyzed by HPTLC. Each value is the mean of four separate experiments. Vertical bars show S.E.M. *P* values are compared with those prior to incubation by means of Student's *t* test. $^{*}P<0.1$; $^{**}P<0.05$.

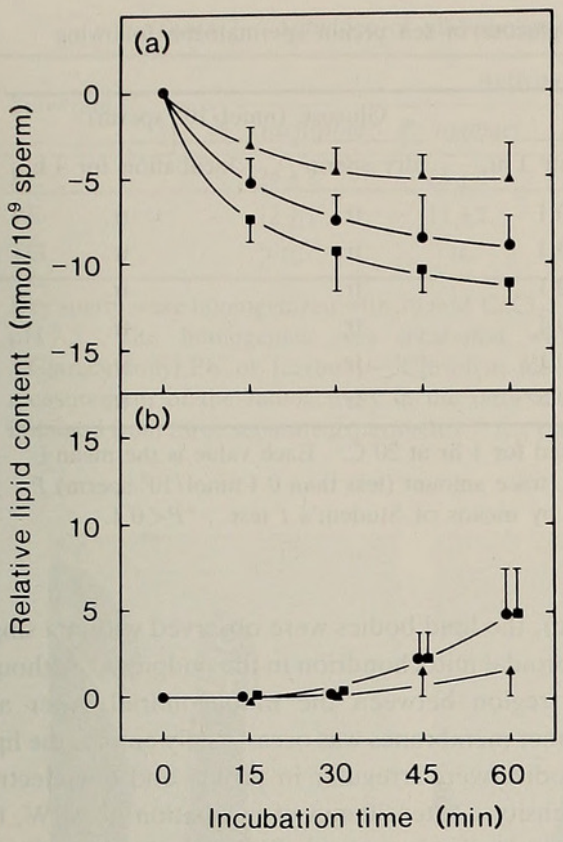
species, but at extremely low levels (Table 2). Glucose was present in a trace amount (Table 2). After incubation in ASW for 1 hr, the glycogen content of *T. hardwickii* spermatozoa decreased significantly, but there was little change in spermatozoa of the other species.

Oxygen consumption

Since oxygen is required for oxidation of the lipid, the amount of O_2 consumed by the spermatozoa was measured at various intervals after dilution in ASW (Fig. 3). The rate of O_2 consumption decreased gradually during long-term incubation. About 0.55, 0.40 and 0.20 μmol O_2 was consumed during incubation of 10^9 spermatozoa of *A. crassispina*, *E. mathaei* and *P. depressus* for 1 hr, respectively.

Phospholipase activity

Since hydrolysis of PC in *H. pulcherrimus* spermatozoa occurs *via* the action of phospholipase A_2 [13, 16, 18], an experiment was conducted to examine the properties of the phospholipase A_2 in spermatozoa of the other species of sea urchin. The homogenates of dry sperm from *A. crassispina*, *E. mathaei*, *P. depressus*, *S. intermedius*, *S. nudus* and *T. hardwickii* were incubated with 1-palmitoyl-2-[1-^{14}C]arachidonyl-PC, 1-palmitoyl-



2-[1-^{14}C]-arachidonyl-PE or [carboxyl-^{14}C]-triolein for 1 hr at 20°C, followed by extraction and separation of FFA by TLC. The radioactivity of FFA from PC released by phospholipase A_2 in these species of spermatozoa was 2–3 times higher than that from PE (Table 3), suggesting that the phospholipase A_2 in sea urchin spermatozoa of the order Echinoidea has high substrate specificity for PC. In contrast to phospholipase, there was only a trace amount of radioactivity of FFA from TG released by lipase (Table 3).

Observation of sperm midpieces

The sea urchin spermatozoon consists of a head, a midpiece and a tail. Previous studies have shown

FIG. 2. Changes in levels of phosphatidylcholine (a) and free fatty acid (b) following incubation of sea urchin spermatozoa. Dry sperm of *A. crassispina* (■), *E. mathaei* (▲) and *P. depressus* (●) were diluted 100-fold and incubated in ASW at 20°C. Each value is the mean of four separate experiments. Vertical bars show S.E.M.

TABLE 1. Fatty acid composition of phosphatidylcholine in sea urchin spermatozoa

Fatty acid	Percentage					
	A. crassispina	*E. mathaei*	*P. depressus*	*S. intermedius*	*S. nudus*	*T. hardwickii*
14:0	2.3±0.9	1.1±0.1	1.3±0.2	0.3±0.1	2.1±0.2	2.1±0.4
15:0	n.d.	0.3±0.1	0.1	tr.	1.9±0.2	2.7±0.4
16:0	12.6±0.7	19.6±0.1	12.1±0.5	12.7±0.6	22.8±0.3	18.5±1.5
16:1	4.2±1.0	n.d.	3.4±0.3	2.4±0.3	1.5±0.1	4.1±0.5
18:0	7.9±0.8	9.7±1.1	3.1±0.1	1.1±0.2	6.6±0.7	13.5±2.5
18:1	9.8±0.4	5.1±0.3	8.3±0.9	7.8±0.6	6.7±0.4	15.8±2.5
18:2	1.5±0.2	1.5±0.1	1.1±0.3	0.8±0.1	1.3±0.2	2.3±0.7
18:3	0.3±0.1	1.2±0.3	0.5±0.1	n.d.	0.6±0.1	1.0±0.3
18:4	8.9±0.3	4.5±0.7	14.3±0.5	7.4±0.5	n.d.	n.d.
20:1	4.6±0.2	8.0±0.4	13.4±0.5	8.3±0.3	7.4±0.2	8.4±1.2
20:2	n.d.	2.4±0.7	n.d.	4.9±0.5	2.9±0.1	2.2±0.3
20:3	7.8±0.6	3.2±0.5	6.0±0.6	tr.	2.1±0.6	1.0±0.5
20:4 (n−6)	27.1±0.9	22.5±1.1	23.4±1.5	17.2±0.4	20.0±0.6	7.9±1.1
20:5 (n−3)	12.3±0.2	16.8±1.5	11.2±0.5	32.8±1.3	18.4±0.2	10.6±2.7
22:4	0.5±0.1	0.9±0.1	0.4±0.1	0.9±0.1	0.7±0.2	0.9±0.3
22:5	n.d.	0.7±0.1	0.1	0.2±0.1	1.3±0.2	2.2±0.2
22:6	tr.	2.3±0.6	1.4±0.8	3.2±0.7	3.7±0.3	7.0±0.5
Saturated	22.8±0.8	30.6±0.9	16.6±0.6	14.2±0.7	33.5±0.7	36.6±2.8
Monoenoic	18.6±1.0	13.1±0.2	25.1±1.6	18.4±0.9	15.5±0.4	28.3±2.1
Polyenoic	58.5±1.1	56.2±0.9	58.3±2.2	67.4±1.5	50.8±0.4	35.1±3.2

Each value is the mean±S.E.M. obtained in three separate experiments. tr., trace amount (less than 0.1%); n.d., not detectable.

TABLE 2. Changes in the levels of glycogen and glucose in sea urchin spermatozoa following incubation with seawater

Species	Glycogen (μg/10^9 sperm)		Glucose (nmol/10^9 sperm)	
	dry sperm	incubation for 1 hr	dry sperm	incubation for 1 hr
A. crassispina	0.5±0.1	0.5±0.1	tr.	tr.
E. mathaei	0.6±0.1	0.7±0.1	tr.	tr.
P. depressus	0.3±0.1	0.2±0.1	tr.	tr.
S. intermedius	0.5±0.2	0.4±0.1	tr.	tr.
S. nudus	0.4±0.1	0.3±0.1	tr.	tr.
T. hardwickii	3.7±0.4	2.1±0.5*	tr.	tr.

Dry sperm were diluted 100-fold in ASW and incubated for 1 hr at 20°C. Each value is the mean± S.E.M. obtained from three separate experiments. tr., trace amount (less than 0.1 nmol/10^9 sperm) *P* values are compared with those prior to incubation by means of Student's *t* test. $^*P<0.1$.

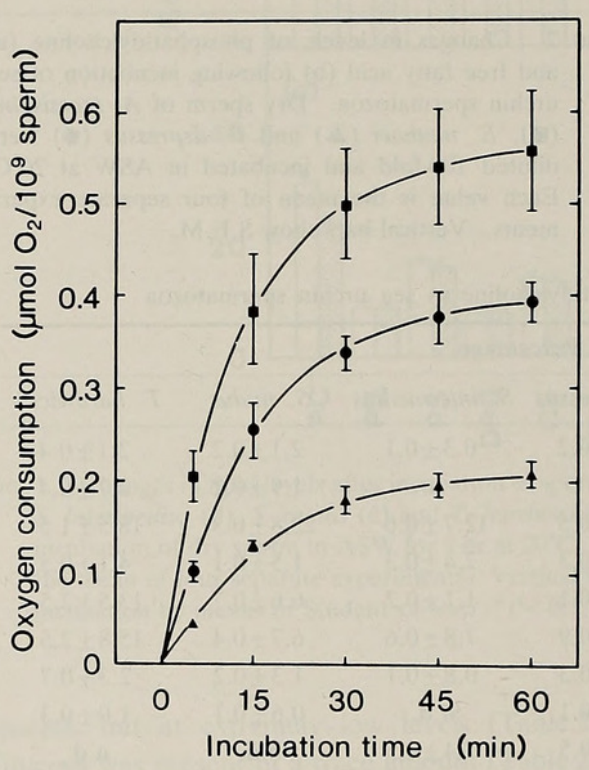


FIG. 3. Oxygen consumption in sea urchin spermatozoa. Dry sperm of *A. crassispina* (■), *E. mathaei* (▲) and *P. depressus* (●) were diluted 100-fold and incubated in ASW at 20°C. Each value is the mean of three separate experiments. Vertical bars show S.E.M.

that the sperm midpiece of *H. pulcherrimus* contains lipid bodies in the intramembrane space of the mitochondrion [14]. In longitudinal sections through the spermatozoa of *A. crassispina* (Fig. 4a), *E. mathaei* (Fig. 4b) and *P. depressus* (Fig. 4c), the lipid bodies were observed within a single toroidal mitochondrion in the midpiece. Although a region between the mitochondrial outer and inner membranes was occasionally empty, the lipid bodies were irregular in profile and low-electron density. After 30 min of incubation in ASW, the lipid bodies in sperm midpieces of *A. crassispina* (Fig. 5), *E. mathaei* (Fig. 6) and *P. depressus* (Fig. 7) had shrunk or disappeared. Although these spermatozoa did not possess the lipid globules which have observed in *Brissopsis lyrifera* [1], *Echinarachinus parma* [26] and *Glyptocidaris crenularis* [15], the midpiece of *A. crassispina* occasionally contained spherical inclusion bodies within mitochondrial matrix (data not shown). However, these inclusion bodies did not change during incubation.

DISCUSSION

This study showed that spermatozoa of *A. crassispina, E. mathaei, P. depressus, S. intermedius, S. nudus* and *T. hardwickii,* sea urchins of the order Echinoidea, contained PC at high concentrations (Table 1). The PC content decreased without any change in the content of other phospholipids following initiation of flagellar movement (Figs. 1 and 2). These spermatozoa contained triglyceride (Table 1) [16, 17], glycogen (Table 2) [20] and glucose (Table 2) [20] at very low levels. Thus it appears that PC is a common endogenous substrate for energy metabolism in spermatozoa of

TABLE 3. Activities of phospholipase A_2 and lipase in sea urchin spermatozoa

Substrate	Activity (nmol hydrolyzed/hr/mg protein)					
	A. crassispina	*E. mathaei*	*P. depressus*	*S. intermedius*	*S. nudus*	*T. hardwickii*
PC	24±3	25±3	21±3	23±2	21±2	29±2
PE	12±1	11±1	6±1	8±1	8±1	15±2
TG	tr.	tr.	tr.	tr.	tr.	tr.

Dry sperm were homogenized with 10 mM $CaCl_2$, 10 mM $MgCl_2$, 1 mM dithiothreitol and 50 mM Tris-HCl at pH 7.5. The homogenate was incubated with 1-palmitoyl-2-[1-^{14}C]arachidonyl-PC, 1-palmitoyl-2-[1-^{14}C]arachidonyl-PE or [carboxyl-^{14}C]triolein for 1 hr at 20°C. Total lipids were extracted following by measurement of the radioactivity in the fatty acid separated by TLC. Each value is the mean±S.E.M. obtained from three separate experiments. tr., trace amount (less than 0.1 nmol hydrolyzed/hr/mg protein).

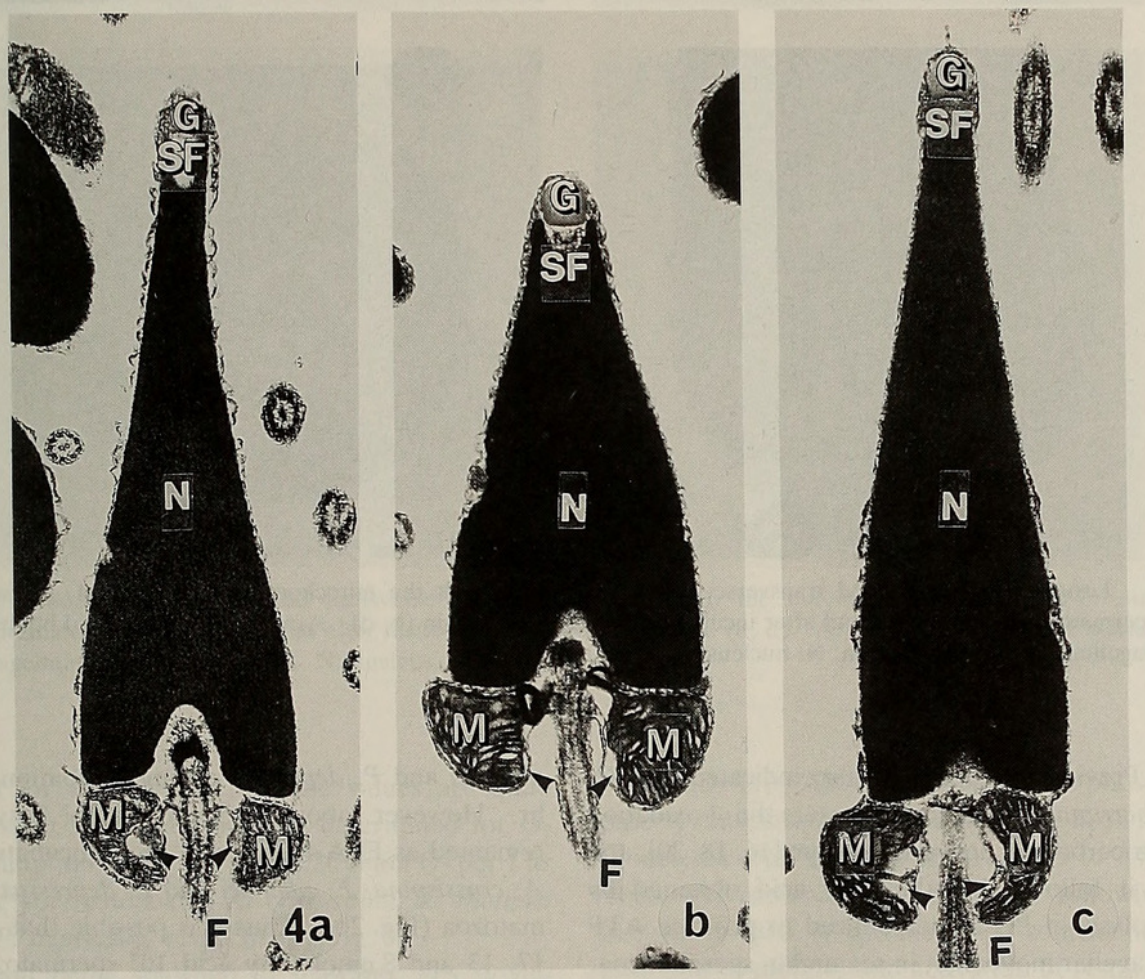


FIG. 4. Longitudinal section through a spermatozoon of *A. crassispina* (a), *E. mathaei* (b) and *P. depressus* (c). Arrow heads show lipid bodies. F: flagullum, G: acrosomal granule, M: mitochondrion, N: nucleus, SF: submitochondrial fossa. ×24,000.

urchins belonging to the order Echinoidea.

The data also showed the presence of phospholipase A_2 activity in spermatozoa of these sea urchin species, but lipase activity was very low (Table 3). The phospholipase A_2 was found to have strict substrate specificity for PC. These results suggest that the preferential hydrolysis of PC among phospholipids is due to the properties of phospholipase

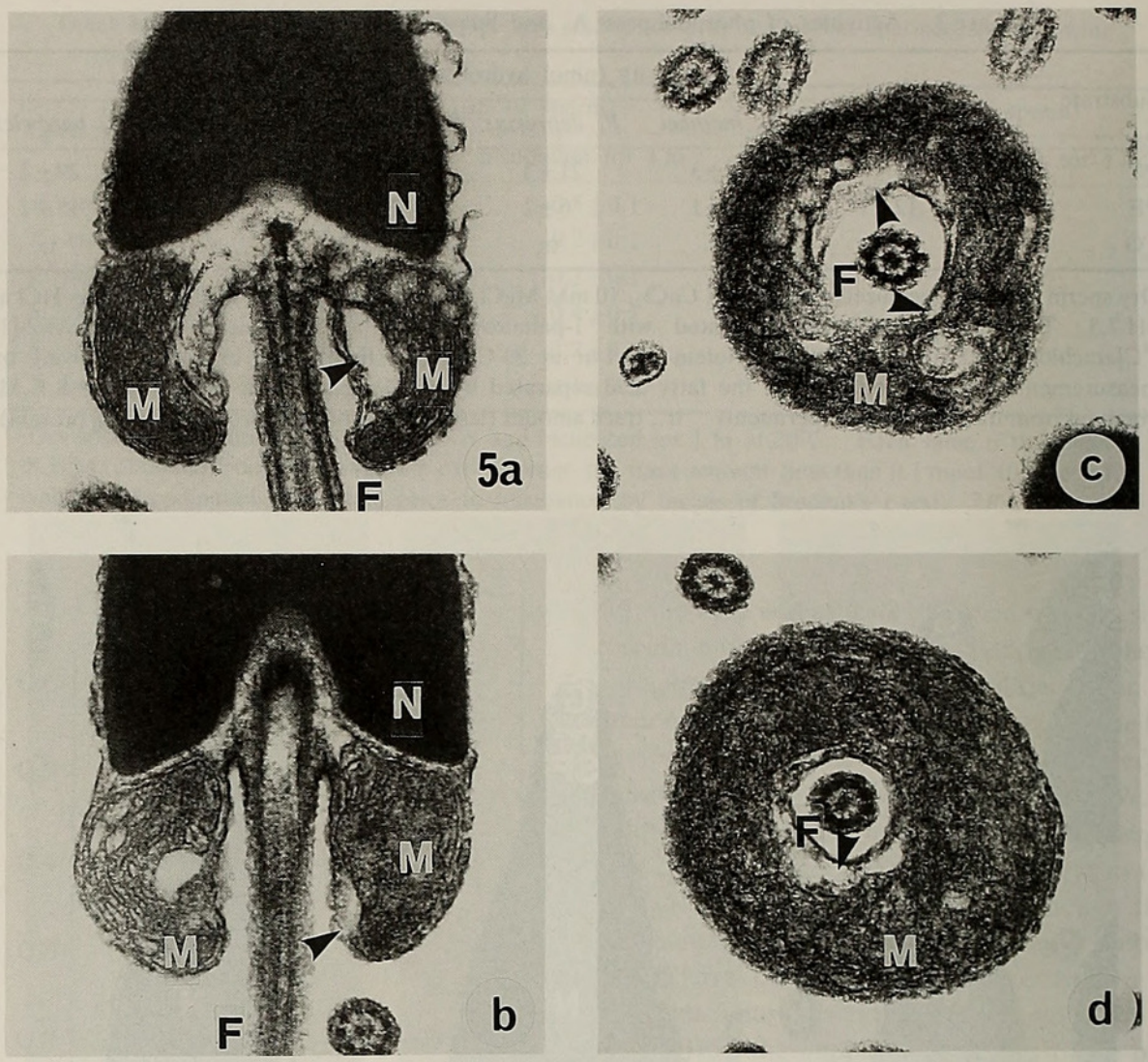


FIG. 5. Longitudinal (a, b) and transverse (c, d) sections through the mitochondrial region of *A. crassispina* spermatozoa before (a, c) and after incubation in ASW for 30 min (b, d). Arrow heads show lipid bodies. F: flagellum, M: mitochondrion, N: nucleus. ×42,600.

A_2. Previous studies have also indicated that *H. pulcherrimus* spermatozoa possess the β-oxidation and tricarboxylic acid cycle system [16, 18, 20], and thus it follows that the fatty acid obtained by hydrolysis of PC is metabolized to produce ATP for flagellar movement in sea urchin spermatozoa.

In this study, about 11 nmol PC was consumed in 10^9 spermatozoa of *A. crassispina* after incubation for 1 hr (Fig. 2a). In *E. mathaei* and *P. depressus*, PC consumption was about 9 and 5 nmol/10^9 spermatozoa, respectively. Since PC is composed of two fatty acid moieties, about 22, 18 and 10 nmol fatty acids/10^9 spermatozoa are produced respectively from PC in *A. crassispina*, *E. mathaei* and *P. depressus* during incubation for 1 hr. However, about 5, 5 and 2 nmol fatty acid remained as FFA following 1 hr of incubation of *A. crassispina*, *E. mathaei* and *P. depressus* spermatozoa (Fig. 2b). Thus it is possible that about 17, 13 and 8 nmol fatty acid/10^9 spermatozoa is utilized, respectively, to produce energy in these species. The chain length of fatty acid in PC of these spermatozoa was generally 20 carbons, as in the case of arachidonic and eicosapentaenoic acids (Table 1). From this, the amount of O_2 required for PC metabolism during incubation for 1 hr was determined to be 0.50 μmol in 10^9 spermatozoa for *A. crassispina*, 0.38 μmol for *E. mathaei* and 0.24

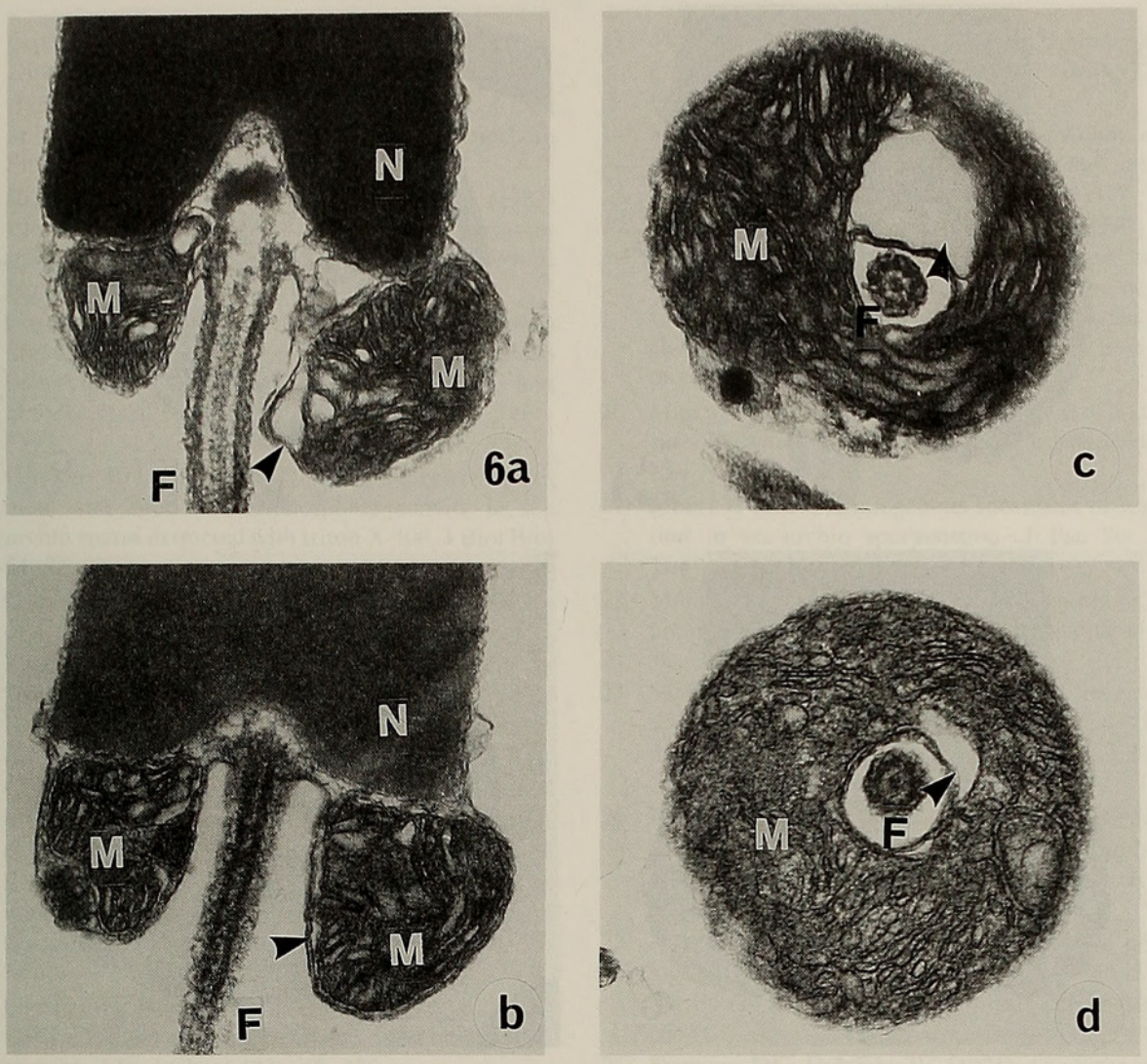


FIG. 6. Longitudinal (a, b) and transverse (c, d) sections through the mitochondrial region of *E. mathaei* spermatozoa before (a, c) and after incubation in ASW for 30 min (b, d). Arrow heads show lipid bodies. F: flagellum, M: mitochondrion, N: nucleus. ×42,600.

μmol for *P. depressus*. These values are quite consistent with those actually determined for O_2 consumption; 0.55, 0.40 and 0.20 μmol O_2 per 1 hr in 10^9 spermatozoa of *A. crassispina, E. mathaei* and *P. depressus,* respectively (Fig. 3).

Upon being spawned in seawater, sea urchin spermatozoa begin flagellar movement and respiration is activated. The initiation of sea urchin sperm motility requires external Na^+ and is associated with Na^+-dependent acid extraction [23]. Following dilution in seawater, the intracellular pH (pH*i*) of sea urchin spermatozoa rises from 6.8 to 7.4 [2, 5, 10]. Internal alkalization leads to activation of dynein ATPase, resulting in initiation of motility [5]. Also, the activation of phospholipase A_2 and fatty acid oxidation have been shown to increase with a rise in pH from 6.5 to 7.5 [19]. Thus, PC metabolism is activated following an increase in pH*i* of spermatozoa, coincident with initiation of motility and activation of respiration.

Our data also showed that several lipid bodies were present within the mitochondria of *A. crassispina, E. mathaei* and *P. depressus* spermatozoa (Fig. 4). Similar lipid bodies located between the mitochondrial outer and inner membrane have been observed in *H. pulcherrimus* spermatozoa [14]. These lipid bodies shrink and disappear after incubation [14], and the present data for spermato-

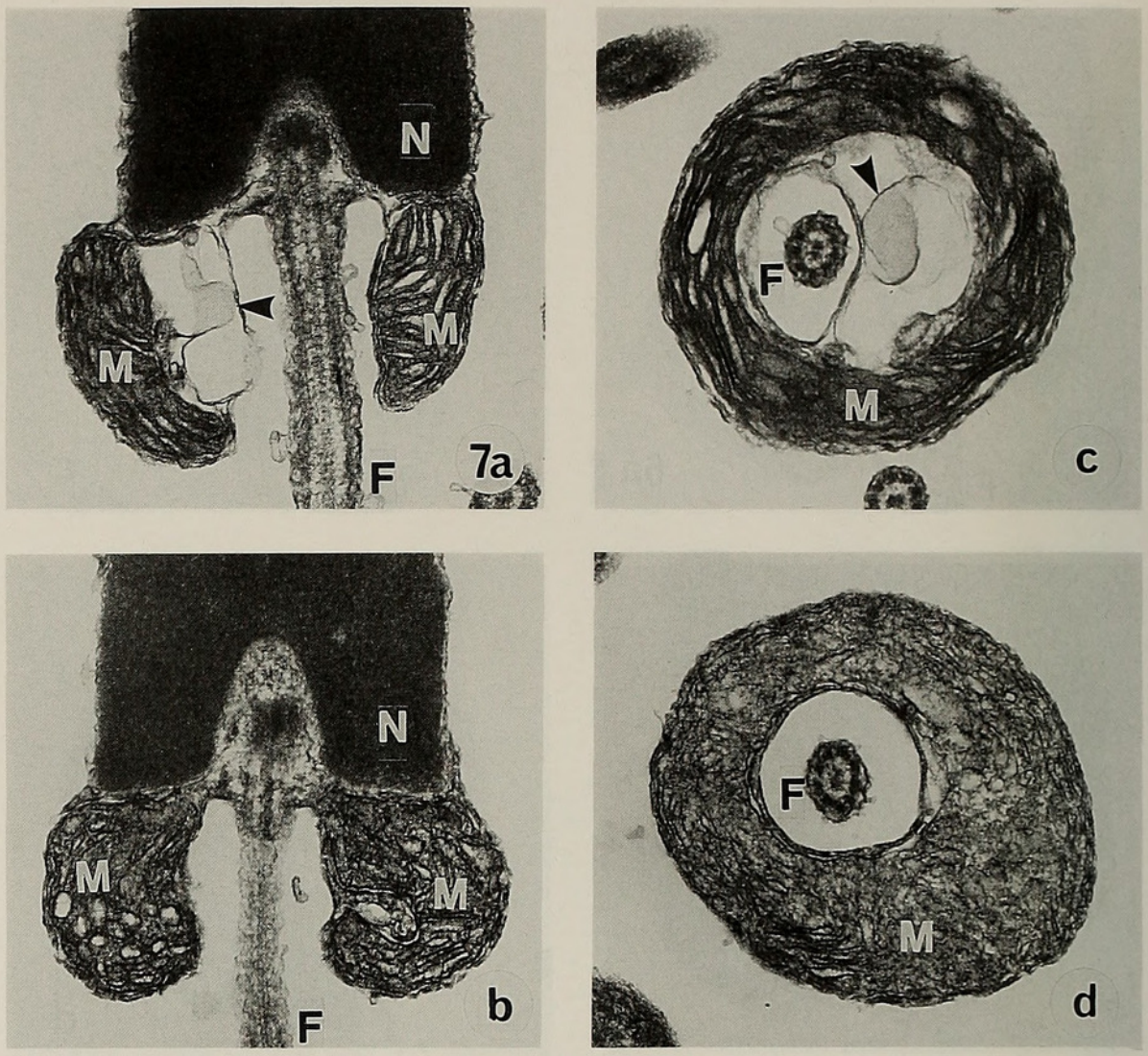


FIG. 7. Longitudinal (a, b) and transverse (c, d) sections through the mitochondrial region of *P. depressus* spermatozoa before (a, c) and after incubation in ASW for 30 min (b, d). Arrow heads show lipid bodies. F: flagullum, M: mitochondrion, N: nucleus. ×42,600.

zoa of *A. crassispina* (Fig. 5), *E. mathaei* (Fig. 6) and *P. depressus* (Fig. 7) confirm this. These findings suggest that the disappearance of the lipid bodies is correlated with the decrease in the level of PC. Presumably, the lipid bodies within mitochondria of echinoid spermatozoa are reservoirs of PC used as an endogenous substrate.

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