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## [REVIEW]

# Energy Metabolism of Sea Urchin Spermatozoa: An Approach Based on Echinoid Phylogeny

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## INTRODUCTION

Spermatozoa have the biologically important roles of egg activation and transmission of the male genetic message to offspring. For this purpose, spermatozoa must swim towards the egg. Since flagellar movement in spermatozoa occurs partly through reactions catalyzed by dynein ATPase (Gibbons and Gibbons, 1972; Christen *et al.*, 1982, 1983; Evans and Gibbons, 1986), energy metabolism for production of ATP by mitochondrial respiration is indispensable for swimming. Sea urchin spermatozoa stored in the testis or in undiluted semen are immotile (Gray, 1928; Rothschild, 1956). Upon spawning in seawater, these spermatozoa begin flagellar movement, and respiration is activated. The mechanism responsible for their flagellar movement has been discussed in several reviews (Mohri *et al.*, 1979; Brokaw, 1987, 1990; Shapiro, 1990). The initiation of sea urchin sperm motility requires external Na<sup>+</sup> and is associated with Na<sup>+</sup>-dependent acid release (Nishioka and Cross, 1978). Following dilution in seawater, the intracellular pH (pH<sub>i</sub>) of sea urchin spermatozoa rises from 6.8 to 7.4 (Christen *et al.*, 1982, 1983; Lee *et al.*, 1983; Bibring *et al.*, 1984). CO<sub>2</sub> is responsible for the immobility of sea urchin spermatozoa in semen, because CO<sub>2</sub> lowers the pH<sub>i</sub> of spermatozoa by entering the cells as a neutral weak acid (Mohri and Yasumasu, 1963; Johnson *et al.*, 1983). Internal alkalization following dilution in seawater leads to activation of dynein ATPase and to initiation of motility (Christen *et al.*, 1982, 1983). The ADP molecule derived from ATP by hydrolysis is used as a substrate for mitochondrial respiration. However, it is unlikely that an exogenous substrate can be used for energy metabolism in sea urchin spermatozoa, because they swim in seawater where hardly any nutrients are present.

In earlier studies by Rothschild and Cleland (1952) and Mohri (1957a, 1964), the endogenous phospholipid content of sea urchin spermatozoa was shown to decrease following the initiation of flagellar movement. Our previous work has also confirmed it (Mita and Yasumasu, 1983a; Mita and Ueta, 1988). These findings strongly suggest that sea urchin spermatozoa obtain energy for swimming through the oxidation of endogenous phospholipids. It is of considerable interest that phospholipids are usable for energy production, because most cells generally use glycogen or triglyceride (TG) to produce ATP.

Recently, we reported that among phospholipids, phosphatidylcholine (PC) was a preferred substrate in spermatozoa of sea urchins belonging to the order Echinoida (Mita and Ueta, 1988, 1990; Mita and Nakamura, 1993b; Mita *et al.*, 1994c). Ultrastructural studies also showed that PC available for utilization in energy metabolism was contained in lipid bodies in the intramembrane space of sperm mitochondria (Mita *et al.*, 1991, 1994d; Mita and Nakamura, 1992, 1993b).

It is important to determine whether phospholipid is a common preferred substrate for energy metabolism in sea urchin spermatozoa. With regard to the taxonomy of the echinoids (Shigei, 1974), in addition to the order Echinoida including *Hemicentrotus pulcherrimus* and *Strongylocentrotus purpuratus*, the orders Arbacioida, Clypeasteroida, Diadematoida and Cidaroida are included in the class Echinoidea. It is generally considered that the Cidaroida are a primitive group, from which the Diadematoida and Arbacioida have evolved, and that the Echinoida are the newest group (Fig. 1). Clypeasteroida of the Irregularia became separated from the Regularia during evolution from the Cidaroida to the Arbacioida after the Diadematoida had branched off. In this review, we describe energy metabolism using an endogenous substrate stored in the spermatozoa of sea urchins which belong not only to the Echinoida but also to the Arbacioida, Clypeasteroida and Diadematoida. In contrast to the Echinoida, spermatozoa in the orders Arbacioida, Clypeasteroida and Diadematoida have been found to use TG instead of PC as a substrate for

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This paper is dedicated to Professor Hideo Mohri on the occasion of his award of the Purple Ribbon Medal from the Emperor of Japan.

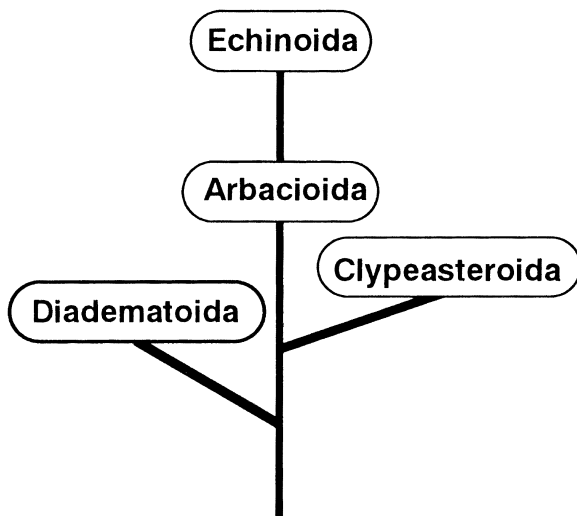


Fig. 1. Phylogeny of echinoids.

energy metabolism. Thus it seems that the energy production system in sea urchin spermatozoa is related to the phylogeny of the class Echinoidea.

#### High-energy phosphate compounds in sea urchin spermatozoa

In sea urchin spermatozoa, a creatine-phosphate shuttle apparently establishes a pool of ATP used primarily for motility (Moriwaki, 1958; Yanagisawa, 1967; Tombes and Shapiro, 1985; Tombes *et al.*, 1987). At the junction of the mitochondria and tail, a creatine kinase isozyme forms creatine phosphate, which then diffuses down the tail, where ATP is formed by another creatine kinase isozyme, presumably in close proximity to dynein ATPases (Tombes and Shapiro, 1985).

When sea urchin spermatozoa are diluted and incubated in seawater, a rapid decline in the level of ATP is observed (Mohri, 1958; Mita and Yasumasu, 1983a; Mita *et al.*, 1994b). The energy charge of the adenylate pool is also known to decrease rapidly in spermatozoa of *Hemicentrotus pulcherrimus* (Mita and Yasumasu, 1983a), *Paracentrotus lividus* (Mita *et al.*, 1994b) and *Arbacia lixula* (Mita *et al.*, 1994b). These findings suggest that the amount of ATP hydrolyzed by dynein ATPase is much greater than that of ATP produced by mitochondrial respiration. It has also been demonstrated that creatine phosphate levels decrease rapidly following incubation in seawater (Mita and Yasumasu, 1983a; Mita *et al.*, 1994b), indicating that the creatine phosphate pool is discharged immediately upon initiation of swimming. It is unlikely that the creatine phosphate shuttle can sustain sperm motility. In contrast to the decrease in ATP, the level of AMP increases markedly following dilution and incubation of sea urchin spermatozoa in seawater, whereas the ADP level shows only a slight change (Mita and Yasumasu, 1983a; Mita *et al.*, 1994b). Since adenylate kinase is distributed in sea urchin flagella (Yanagisawa *et al.*, 1968; Brokaw and Gibbons, 1973; Tombes and Shapiro, 1985; Mita *et al.*, 1994b), the enzyme is

considered to catalyze the conversion of ATP and AMP, as soon as ADP is produced from ATP, suggesting that ATP produced in the midpiece can diffuse down the tail directly. It is also possible that not only the creatine phosphate shuttle but also adenylate kinase plays an important role in the supply of ATP for flagellar motility.

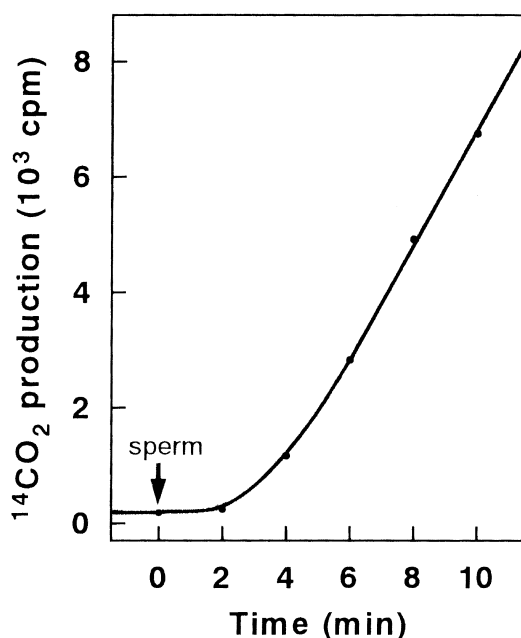
#### Metabolism of carbohydrate in sea urchin spermatozoa

In vertebrate cells, glucose is a good candidate for the energy metabolism substrate, because it is easy to obtain from blood fluid. Indeed, it has been demonstrated that [ $^{14}\text{C}$ ]glucose is converted to  $^{14}\text{CO}_2$  in sea urchin spermatozoa under hypotonic conditions (Mohri and Yasumasu, 1966). Both glycolysis and the Krebs cycle also occur in sea urchin spermatozoa (Mita and Yasumasu, 1983a, b). It is possible that the spermatozoa use glucose for energy metabolism. However, sea urchin spermatozoa would not be capable of using an exogenous substrate such as glucose, since they swim in seawater, where glucose and other carbohydrates are virtually absent. Thus, sea urchin spermatozoa must use an endogenous substrate to produce ATP. It has also been shown that endogenous glycogen and glucose are present in very small quantities in spermatozoa of most species of sea urchin belonging to the orders Echinoida (Mita and Yasumasu, 1983a; Mita and Nakamura, 1993b), Arbacioida (Mita, 1991; Mita *et al.*, 1994c), Clypeasteroida (Mita *et al.*, 1994e) and Diadematoida (Mita *et al.*, 1995). There is no significant change in the levels of glucose and glycogen in these spermatozoa before and after swimming. These findings suggest that sea urchin spermatozoa do not use sugar as a substrate for energy metabolism.

#### PC as a preferred substrate for energy metabolism in Echinoida spermatozoa

Following dilution of *Hemicentrotus* spermatozoa (order Echinoida) in artificial seawater at pH 8.2 (ASW) containing [ $^{14}\text{C}$ ]oleic acid and carbonic anhydrase,  $^{14}\text{CO}_2$  is released within 2 min (Fig. 2). This is in accord with earlier work (Mohri, 1957a, b) indicating that a fatty acid oxidizing system is present in sea urchin spermatozoa. Thus, it is considered that sea urchin spermatozoa obtain energy for movement through oxidation of fatty acids.

Lipids extracted from dry sperm of *Hemicentrotus* consist primarily of phospholipids and cholesterol (CH) (Mita and Ueta, 1988, 1989). TG and cholesterol-ester (CE) are also present, but at extremely low levels. Similar results have been reported for other species of Echinoida spermatozoa (Mita and Nakamura, 1993b; Mita *et al.*, 1994a, c). When dry sperm is diluted and incubated in seawater, the phospholipid content decreases rapidly (Mita and Ueta, 1988; Mita and Nakamura, 1993b; Mita *et al.*, 1994a, c). These findings confirm the observations made by Rothschild and Cleand (1952) and Mohri (1957a), suggesting that sea urchin spermatozoa use phospholipids as the source of energy for metabolism. Among phospholipids, PC, phosphatidylserine (PS), phosphatidylethanolamine (PE) and cardiolipin (CL) are found in



**Fig. 2.** Oxidation of fatty acid in *Hemicentrotus pulcherrimus* spermatozoa. Dry sperm were diluted 100-fold in seawater containing [1-<sup>14</sup>C]oleic acid and carbonic anhydrase in a total volume of 10 ml at 20°C. Radioactivity in the <sup>14</sup>CO<sub>2</sub> produced was counted by the thin-window gas flow counter.

spermatozoa of the order Echinoida (Fig. 3a), but not phosphatidic acid (PA) or sphingophospholipid. It is notable that during incubation in seawater, only the level of PC decreases rapidly (Mita and Ueta, 1988; Mita and Nakamura, 1993b; Mita *et al.*, 1994a, c). In contrast to PC, the levels of other phospholipids remain almost constant.

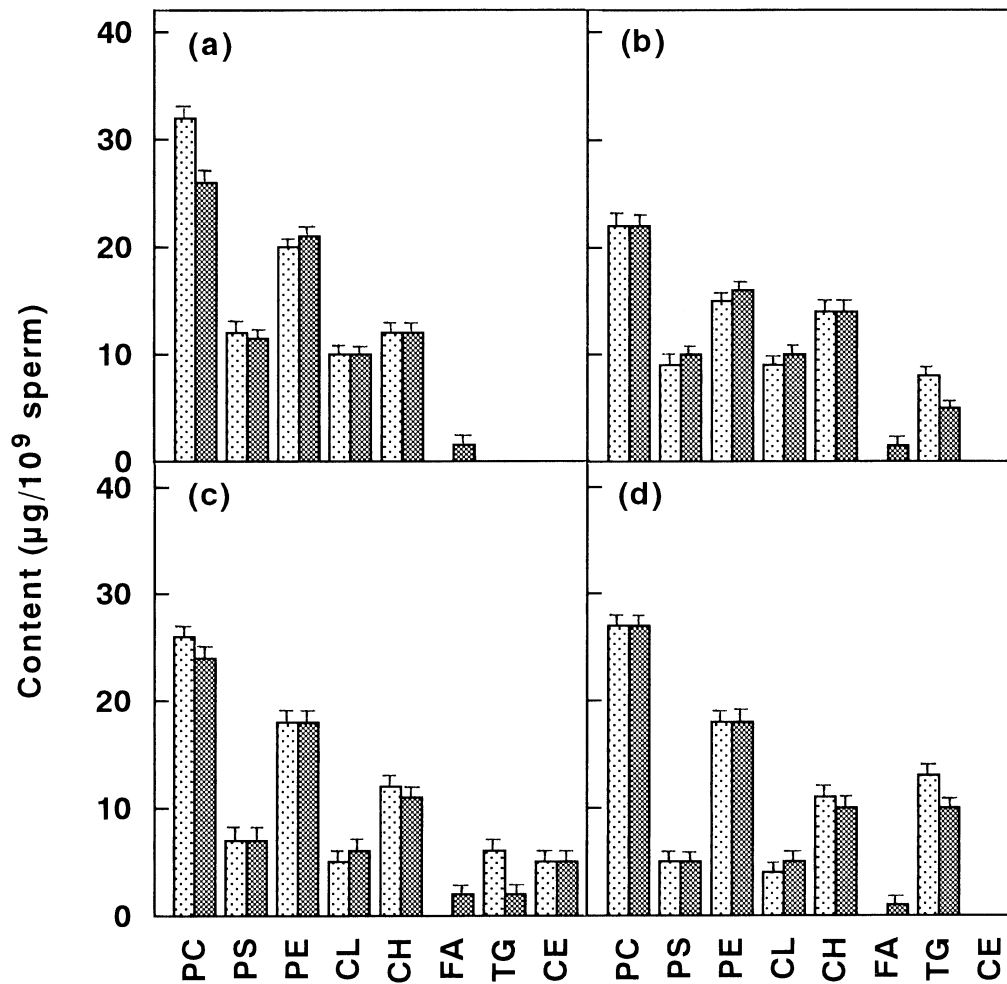
Fatty acids composed of PC in *Hemicentrotus* spermatozoa are mostly of the unsaturated type, such as eicosamonoenoic (20:1), arachidonic (20:4) and eicosapentaenoic acids (20:5) (Mita and Ueta, 1988, 1989). During incubation, unsaturated fatty acids in PC are oxidized by *Hemicentrotus* spermatozoa to a greater extent than saturated fatty acids. Indeed, <sup>14</sup>CO<sub>2</sub> has been shown to be produced more effectively from [1-<sup>14</sup>C]oleic acid than from [1-<sup>14</sup>C]palmitic acid, and the use of [1-<sup>14</sup>C]arachidonic acid leads to maximum production (Mita and Ueta, 1990). In a cell-free system, it has also been found that radiolabeled PC is oxidized to <sup>14</sup>CO<sub>2</sub>, but not PE (Mita and Ueta, 1990). This is good evidence indicating that only PC is consumed during incubation to provide the energy in *Hemicentrotus* spermatozoa.

In addition to diacyl phospholipids, alkenyl and alkyl derivatives are known to be present in sea urchin spermatozoa (Mohri, 1959a, 1961). In *Hemicentrotus* spermatozoa, PC contains alkylacyl (19%) and diacyl (81%) components (Mita, 1992; Mita and Ueta, 1992). After incubation of these spermatozoa in seawater, only the diacyl PC content decreases significantly, and no changes are detectable in the other phospholipids (Mita, 1992). Palmitic (16:0), stearic (18:0), eicosamonoenoic (20:1), arachidonic (20:4) and eicosapen-

taenoic (20:5) acids at the 1-position and arachidonic (20:4) and eicosapentaenoic (20:5) acids at the 2-position of diacyl PC also decrease during incubation. These observations suggest that the diacyl PC composed of unsaturated fatty acid is available for utilization in energy production by *Hemicentrotus* spermatozoa.

Previous studies have shown that phospholipase activity is present in sea urchin spermatozoa (Mohri, 1959b, 1964; Mita and Yasumasu, 1983a). The hydrolysis of phospholipid is generally catalyzed by phospholipases, which are classified into A<sub>1</sub>, A<sub>2</sub>, C and D based on the site of phospholipid hydrolysis. To determine the phospholipase associated with energy metabolism in sea urchin spermatozoa, the conversion of radioactivity from 1-palmitoyl-2-[1-<sup>14</sup>C]linoleoyl-PC has been examined. After incubation with the homogenate of *Hemicentrotus* spermatozoa, about 80% of the radioactivity was recovered the free fatty acid (FA) fraction (Mita and Ueta, 1988, 1990). Only 4% and 7% of the radioactivity was observed in lysophosphatidylcholine (LysoPC) and PS, respectively. There was only a trace of radioactivity in PE, PA, CL and diacylglycerol (DG). Thus it is concluded that the hydrolysis of PC takes place through the action of phospholipase A<sub>2</sub>. On the other hand, incubation in the presence of 1-palmitoyl-2-[1-<sup>14</sup>C]linoleoyl-PE shows that only 20% of the radioactivity is distributed in FA (Mita and Ueta, 1990). More than 50% of the radioactivity from PE is converted to PS. This suggests that phospholipase A<sub>2</sub> in *Hemicentrotus* spermatozoa has strict substrate specificity for PC, thus explaining the selective hydrolysis of PC. As shown in Table 1, similar results have been obtained from other Echinoida spermatozoa, such as *Anthocidaris crassispina* (Mita and Nakamura, 1993b), *Echinometra mathaei* (Mita and Nakamura, 1993b), *Paracentrotus lividus* (Mita *et al.*, 1994c), *Pseudocentrotus depressus* (Mita and Nakamura, 1993b), *Strongylocentrotus intermedius* (Mita and Nakamura, 1993b), *Strongylocentrotus nudus* (Mita and Nakamura, 1993b), and *Temnopleurus hardwickii* (Mita and Nakamura, 1993b). It is concluded that sea urchin spermatozoa of the order Echinoida commonly use PC as an endogenous substrate for energy metabolism.

Furthermore, accumulation of choline has been observed in *Hemicentrotus* spermatozoa following incubation in seawater (Mita and Ueta, 1990). Since the amount of PC consumed is essentially the same as that of choline accumulated (Fig. 4), it is suggested that PC is finally metabolized to choline in sea urchin spermatozoa. The major degradative pathway for PC in Echinoida spermatozoa is considered to be: PC → LysoPC → glycerophosphocholine → choline (Mita and Ueta, 1990). Fatty acids obtained by hydrolysis of PC are metabolized through β-oxidation and the Krebs cycle to CO<sub>2</sub> and H<sub>2</sub>O (Table 2). α-Glycerophosphate derived from PC is also metabolized by glycolysis and the Krebs cycle. Recently, it was reported that *Hemicentrotus* spermatozoa can maintain their motility in seawater for half a day (Ohtake *et al.*, 1996). It is likely that the endogenous PC is enough to support the motility through the oxidation of fatty acid to produce ATP.



**Fig. 3.** Changes in lipid levels after incubation of spermatozoa of *Hemicentrotus pulcherrimus* (a), *Glyptocidaris crenularis* (b), *Clypeaster japonicus* (c) and *Diadema setosum* (d). Before (clear) and after (dotted) 100-fold dilution and incubation of dry sperm in seawater for 1 hr at 20°C, lipids were extracted and analyzed. Each value is the mean  $\pm$  SEM of four separate experiments. PC, phosphatidylcholine; PS, phosphatidylserine; PE, phosphatidylethanolamine; CL, cardiolipin; CH, cholesterol; FA, fatty acid; TG, triglyceride; CE, cholesterol-ester.

**Table 1.** Comparison of preferred substrates for energy metabolism in sea urchin spermatozoa based on echinoid phylogeny

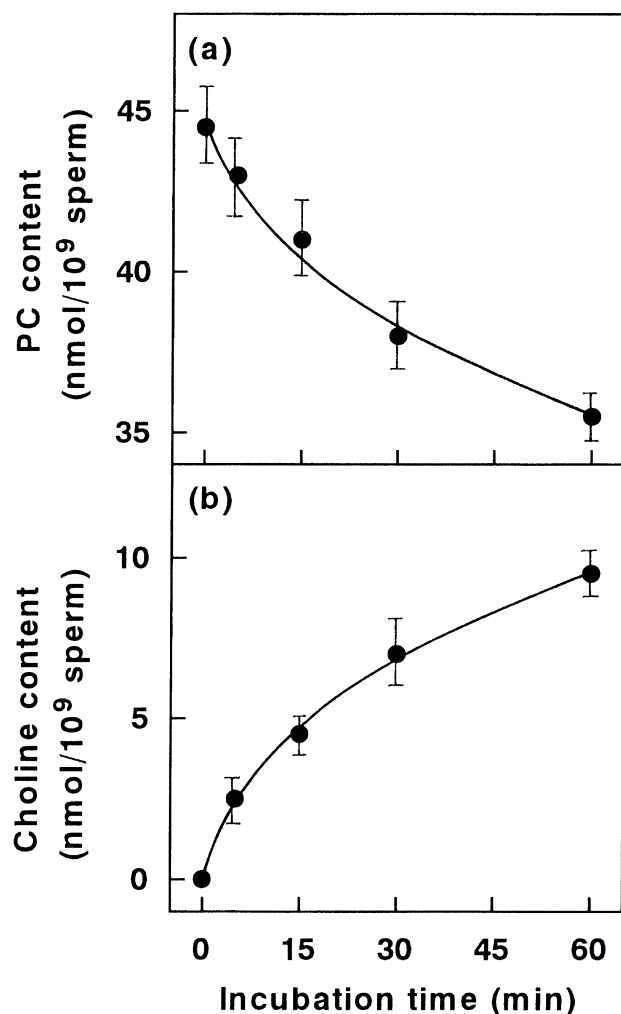
Order	Species	Substrate	References
Echinoida	<i>Anthocidaris crassispina</i>	Phosphatidylcholine	Mita and Nakamura (1993b)
	<i>Echinometra mathaei</i>	Phosphatidylcholine	Mita and Nakamura (1993b)
	<i>Hemicentrotus pulcherrimus</i>	Phosphatidylcholine	Mita and Ueta (1988)
	<i>Paracentrotus lividus</i>	Phosphatidylcholine	Mita <i>et al.</i> (1994c)
	<i>Pseudocentrotus depressus</i>	Phosphatidylcholine	Mita and Nakamura (1993b)
	<i>Strongylocentrotus intermedius</i>	Phosphatidylcholine	Mita and Nakamura (1993b)
	<i>Strongylocentrotus nudus</i>	Phosphatidylcholine	Mita and Nakamura (1993b)
	<i>Temnoplexus hardwickii</i>	Phosphatidylcholine	Mita and Nakamura (1993b)
Arbacioida	<i>Arbacia lixula</i>	Triglyceride	Mita <i>et al.</i> (1994c)
	<i>Glyptocidaris crenularis</i>	Triglyceride	Mita (1991)
Clypeasteroida	<i>Clypeaster japonicus</i>	Triglyceride	Miita <i>et al.</i> (1994e)
Diadematoida	<i>Diadema setosum</i>	Triglyceride	Mita <i>et al.</i> (1995)

#### Energy metabolism in spermatozoa of the orders Arbacioida, Clypeasteroida and Diadematoida

Similar to the Echinoida, sea urchin spermatozoa of the

orders Arbacioida, Clypeasteroida and Diadematoida are quiescent in undiluted semen. After dilution and incubation in seawater, spermatozoa begin flagellar movement and respi-





**Fig. 4.** Changes in levels of phosphatidylcholine (a) and choline (b) following incubation of *Hemicentrotus pulcherrimus* spermatozoa in seawater. Each value is the mean  $\pm$  SEM of four separate experiments.

ration is activated. Thus, spermatozoa of the orders Arbacioida, Clypeasteroida and Diadematoida also obtain energy for swimming through oxidation of an endogenous substrate. As described already, however, it is unlikely that a carbohydrate is the substrate for energy metabolism, since the endogenous glucose and glycogen contents are quite low in spermatozoa of the sea urchins *Arbacia lixula* (Mita *et al.*, 1994c), *Glyptocidaris crenularis* (Mita, 1991), *Clypeaster japonicus* (Mita *et al.*, 1994e) and *Diadema setosum* (Mita *et al.*, 1995). It is notable that the lipid composition of these spermatozoa is different from that in the Echinoida. The lipids in spermatozoa of *Glyptocidaris* (Fig. 3b) and *Diadema* (Fig. 3d) contain CH, TG and several kinds of phospholipids including PC. CE in addition to similar phospholipids, CH and TG, is present in *Clypeaster* spermatozoa (Fig. 3c). After incubation of these spermatozoa in seawater, the level of TG decreases without any change in the levels of phospholipids (Mita, 1991; Mita *et al.*, 1994c, e, 1995). This suggests that spermatozoa of sea

**Table 2.** Energy metabolism in sea urchin spermatozoa of the orders Echinoida, Arbacioida, Clypeasteroida and Diadematoida

Echinoida		
PC (Lipid bodies)	PLase A <sub>2</sub> → FA	β-Oxidation Krebs cycle → CO <sub>2</sub> + H <sub>2</sub> O + ATP
$  \begin{array}{c}  \text{CH}_2\text{OCOR}_1 \\    \\  \text{CH OCOR}_2 \\    \\  \text{CH}_2\text{O}-\text{P}-\text{OCH}_2\text{CH}_2\text{N}^+(\text{CH}_3)_3 \\    \\  \text{O}^-  \end{array}  $		
Arbacioida, Clypeasteroida, Diadematoida		
TG (Lipid globules)	Lipase → FA	β-Oxidation Krebs cycle → CO <sub>2</sub> + H <sub>2</sub> O + ATP
$  \begin{array}{c}  \text{CH}_2\text{OCOR}_1 \\    \\  \text{CHOCOR}_2 \\    \\  \text{CH}_2\text{OCOR}_3  \end{array}  $		

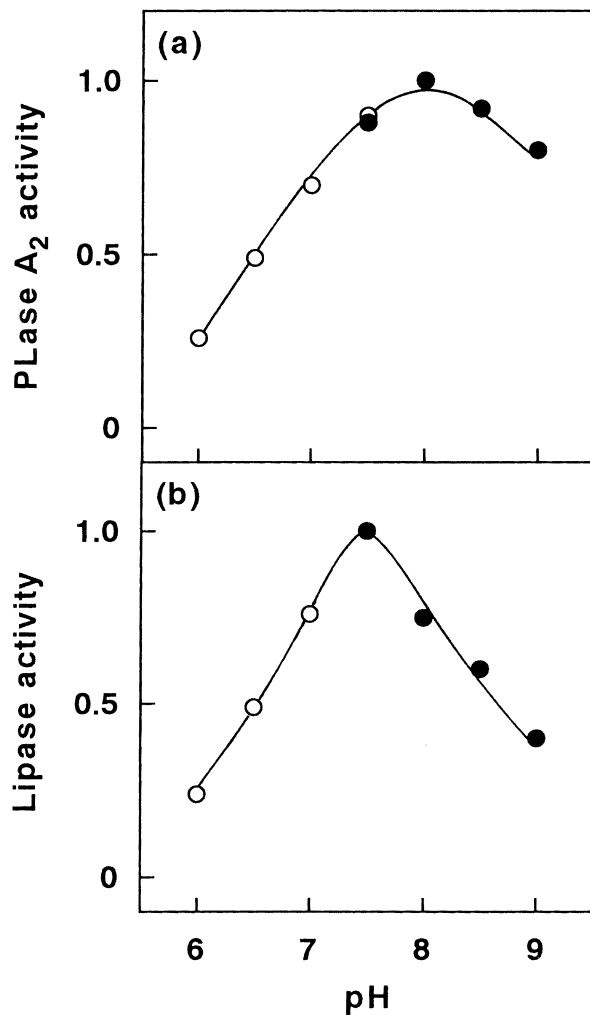
urchins in the orders Arbacioida, Diadematoida and Clypeasteroida use TG as a substrate for energy metabolism (Table 1).

Although PC is present in these spermatozoa, it is unclear why they do not use it for energy metabolism. In the spermatozoa of *Arbacia* (Mita *et al.*, 1994c), *Glyptocidaris* (Mita, 1991), *Clypeaster* (Mita *et al.*, 1994e) and *Diadema* (Mita *et al.*, 1995), the activity of lipase is fairly high, but that of phospholipase A<sub>2</sub> is extremely low. Since Echinoida spermatozoa have high phospholipase A<sub>2</sub> activity, the lack of change in the level of PC appears to be due to the low activity of phospholipase in Arbacioida, Clypeasteroida and Diadematoida spermatozoa. Thus, it is considered that the preferential hydrolysis of PC and TG is related to the properties of phospholipase A<sub>2</sub> with respect to the Echinoida and of lipase with respect to the Arbacioida, Clypeasteroida and Diadematoida, respectively.

### Regulation of energy metabolism

Since it is known that a specific pH<sub>i</sub> value is essential for the activation of sperm respiration and motility (Christen *et al.*, 1982, 1983; Lee *et al.*, 1983; Bibring *et al.*, 1984), sperm metabolism is likely to be regulated through the alteration of pH<sub>i</sub>. To confirm this possibility, the effect of pH on the activities of phospholipase A<sub>2</sub> and fatty acid oxidation in *Hemicentrotus* spermatozoa has been examined (Mita *et al.*, 1990). The activity of phospholipase A<sub>2</sub> is indeed extremely low at pH 6.0, and it increases gradually as the pH increases, reaching maximal activity near pH 8.

In addition, the oxidation of [1-<sup>14</sup>C]palmitic, [1-<sup>14</sup>C]oleic,



**Fig. 5.** Effect of pH on activities of phospholipase A<sub>2</sub> in *Hemicentrotus pulcherrimus* (a) and lipase in *Glyptocidaris crenularis* spermatozoa (b). The enzyme activities were measured at the pH indicated by Aces (pH 6.0-7.5) (○) and Tris (pH 7.5-9.0) (●).

and [<sup>14</sup>C]arachidonic acids to <sup>14</sup>CO<sub>2</sub> in a cell-free system of *Hemicentrotus* spermatozoa is also pH-dependent (Mita *et al.*, 1990). It is relatively low under acidic (pH 6.5) and alkaline (pH 8) conditions and is maximal at about pH 7.5. It has also been demonstrated that a sperm-activating peptide (SAP) isolated from the egg jelly (Hansbrough and Garbers, 1981; Suzuki *et al.*, 1981; Garbers *et al.*, 1982; Suzuki and Garbers, 1984; Suzuki, 1990) stimulates sperm respiration and motility in slightly acidic seawater (pH 6.6). SAP causes an increase of PC consumption and fatty acid oxidation in *Hemicentrotus* spermatozoa at pH 6.6 (Mita *et al.*, 1990).

The lipase activity in *Glyptocidaris* (order Arbacioida) is also influenced by pH (Mita, 1991). The activity is relatively low under acidic (pH 6.0) and alkaline (pH 9.0) conditions, and maximal activity is attained at pH 7.5 (Fig. 5b). Thus, these findings suggest that the activity of phospholipase A<sub>2</sub> or lipase and fatty acid oxidation are enhanced by a rise in pH, from 6.5 to 7.5 following sperm dilution in seawater, accom-

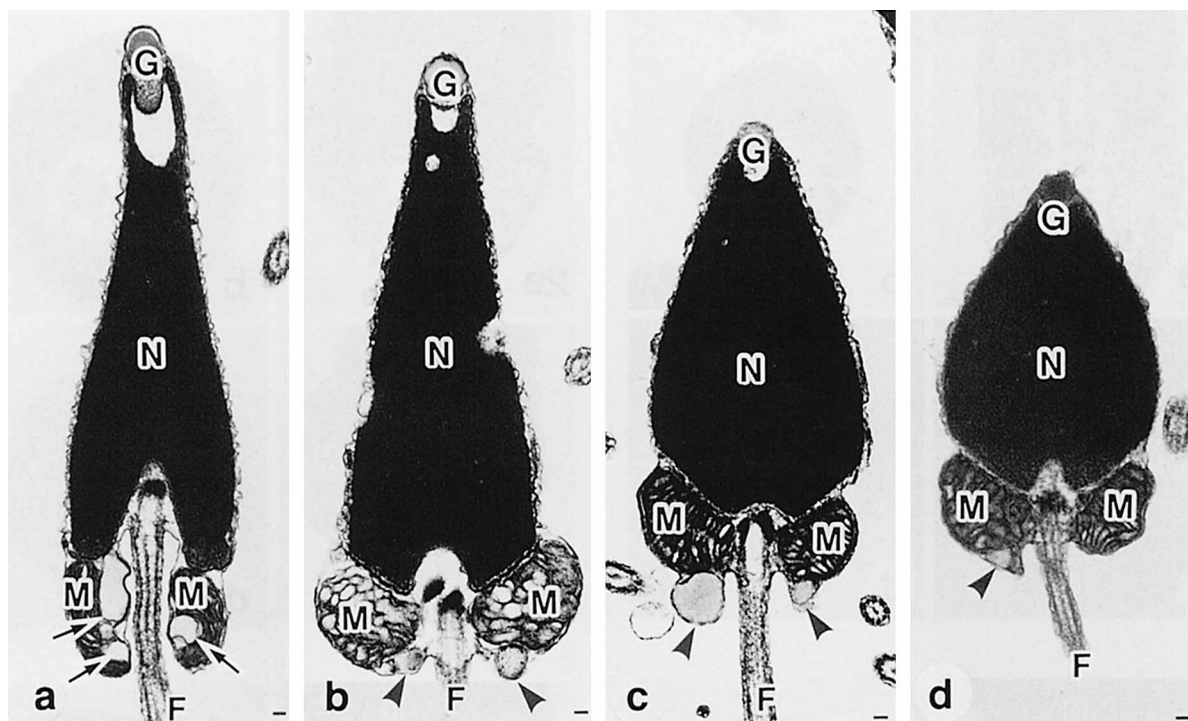
panied by initiation of motility and activation of respiration.

#### Localization of endogenous substrates available for energy metabolism

In general, phospholipids including PC are components of the plasma membrane. If PC located in the plasma membrane is digested during swimming, the membrane will be destroyed, causing the spermatozoa to die. Thus, it seems that a special kind of PC reservoir is available for utilization in energy metabolism. Analysis of the distribution of PC in *Hemicentrotus* sperm heads, tails and midpieces showed that about 85% of the PC was distributed in the midpiece (Mita *et al.*, 1991), whereas the head, tail and cell membrane contained only 16%, 4% and 13%, respectively. It is notable that the decrease in PC content during incubation in seawater is due to a change in the level of PC in the head and midpiece, without any change in the level of tail PC (Mita *et al.*, 1991). In contrast, the levels of PS, PE, CL and CH in the head and midpiece and the tail remain almost constant. Since PC is abundant in the midpiece, these findings suggest that midpiece PC is available for utilization for energy metabolism in *Hemicentrotus* spermatozoa.

Previously, Afzelius and Mohri (1966) have reported that the sperm mitochondrial matrix is damaged after prolonged incubation in seawater. To confirm this, we have ultrastructurally studied the endogenous substrate in sea urchin spermatozoa (Mita and Nakamura, 1992). Figure 6a shows a longitudinal section through spermatozoa of *Hemicentrotus*. It is evident that the intramembrane space, i.e. the region between the mitochondrial outer and inner membranes, has a band-like dilation nearest the flagellum and contains lipid bodies of low electron density. These lipid bodies are irregular in profile and about 0.1-0.2 μm in diameter (Fig. 7-1a and b). When spermatozoa are diluted and incubated in seawater, changes are observed in the structure of the inner ring of the mitochondrion. After 30 min of incubation, the lipid bodies and the inner ring of the mitochondrion disappear (Fig. 7-1c and d). Thus, the disappearance of the lipid bodies is correlated with the decrease in the level of PC (Mita and Nakamura, 1992). However, various structural features of the mitochondrion, such as the number of cristae and the thickness of the membranes, do not change during incubation of spermatozoa in seawater. Similar morphological changes in lipid bodies have been observed in sperm midpieces in other species of Echinoida (Mita and Nakamura, 1993b; Mita *et al.*, 1994d). In contrast, during incubation of *Hemicentrotus* spermatozoa in 100 mM K<sup>+</sup>-seawater, in which spermatozoa are immotile (Mita and Yasumasu, 1983b, 1984), there is no decrease in the level of PC, and the lipid bodies remain intact (Mita and Nakamura, 1992). These observations strongly suggest that PC available for use in Echinoida sperm energy metabolism is related to the lipid bodies within the mitochondria of the midpiece (Table 2).

In contrast, the spermatozoa of *Glyptocidaris* (Fig. 6b), *Clypeaster* (Fig. 6c) and *Diadema* (Fig. 6d) contain several lipid globules in the midpiece, although there are no lipid bod-



**Fig. 6.** Longitudinal sections of spermatozoa of *Hemicentrotus pulcherrimus* (a), *Glyptocidaris crenularis* (b), *Clypeaster japonicus* (c), and *Diadema setosum* (d). Arrows and arrow heads show lipid bodies and lipid globules, respectively. F, flagellum; G, acrosomal granule; M, mitochondrion; N, nucleus. Bar shows 0.1  $\mu$ m.

ies. The lipid globules differ from the lipid bodies in that the former are spherical and located in the posterior region between the basis of the mitochondrion and the plasma membrane (Mita and Nakamura, 1993a). The long and short axes of the globules measure 0.23 and 0.21  $\mu$ m, respectively. After incubation of these spermatozoa in seawater, morphological changes in the lipid globules occur (Fig. 7-2, 7-3 and 7-4). Some lipid globules lose their smooth spherical shape and their surface becomes irregular and uneven. In other cases, the lipid globules have a bilobed appearance. Vacuoles of various sizes and forms also appear near the lipid globules. The volume of lipid globules in the spermatozoa decreases as the TG content declines following incubation in seawater (Mita and Nakamura, 1993a; Mita *et al.*, 1994d, e, 1995). Thus, these findings strongly suggest that the spermatozoa of Arbacioida, Clypeasteroida and Diadematoida obtain energy through oxidation of fatty acid from TG stored in the lipid globules within their midpieces (Table 2).

## Conclusion

In mammals, sugar in seminal plasma and the female reproductive tract is known to be responsible for sperm motility (Peterson and Freund, 1976). Without seminal plasma, however, mammalian spermatozoa can maintain their motility under aerobic conditions (Lardy and Phillips, 1941a). During incubation, the endogenous phospholipid content used for metabolism diminishes (Lardy and Phillips, 1941b). Thus, mammalian spermatozoa may also be capable of using en-

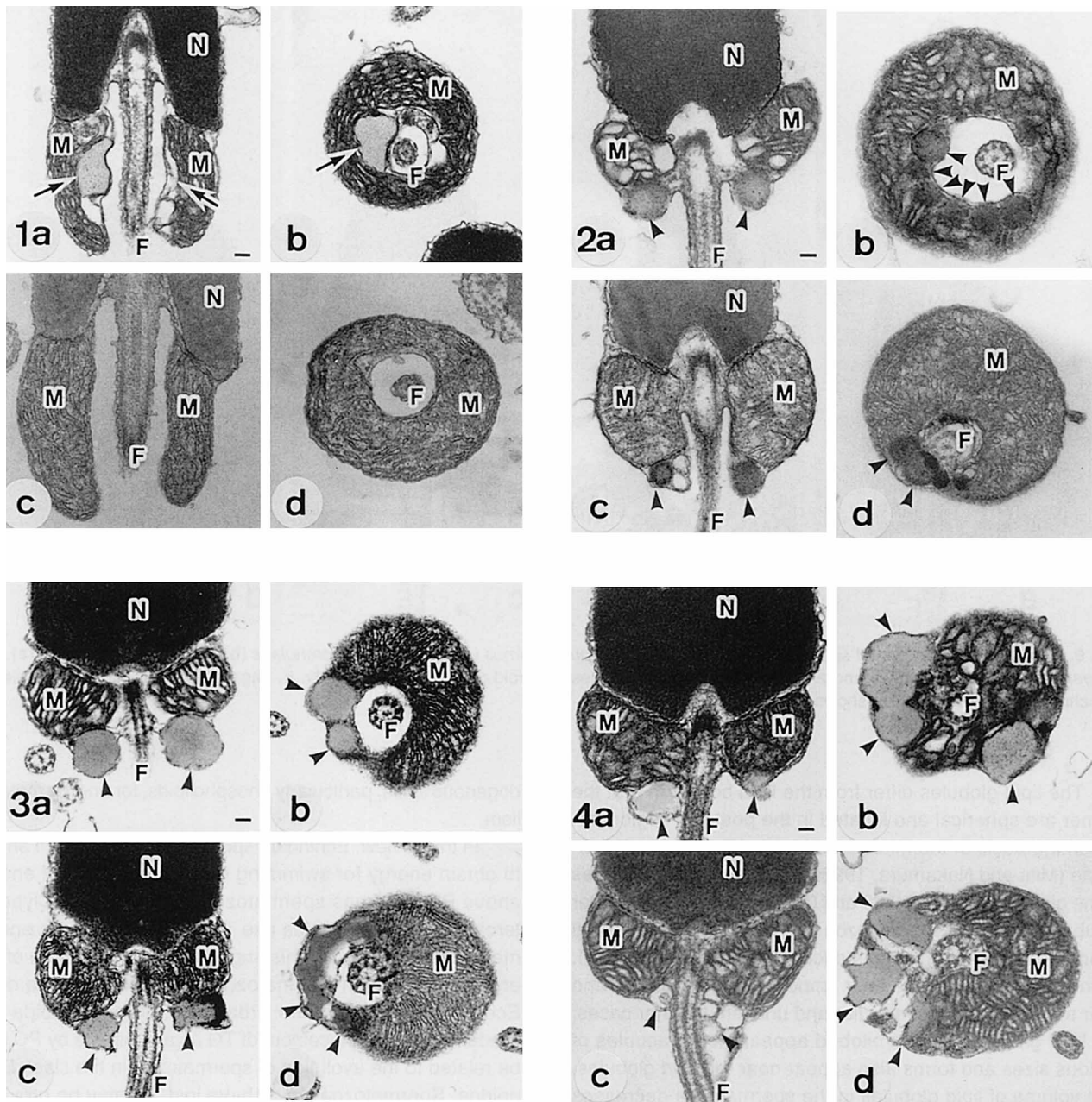
dogenous lipids, particularly phospholipids, for energy metabolism.

In this review, Echinoida spermatozoa have been shown to obtain energy for swimming through oxidation of endogenous PC, whereas spermatozoa of Arbacioida, Clypeasteroida and Diadematoida use TG as a substrate for energy metabolism (Table 1). This suggests that the system of energy metabolism in spermatozoa differs between the order Echinoida and the orders Arbacioida, Clypeasteroida and Diadematoida. Replacement of TG as a substrate by PC may be related to the evolution of spermatozoa in the class Echinoidea. Spermatozoa which have lost TG may be provided with a system for metabolism of phospholipids, particularly PC.

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**Fig. 7.** Longitudinal (a,c) and transverse (b,d) sections through the mitochondrial region of *Hemicentrotus pulcherrimus* (1), *Glyptocidaris crenularis* (2), *Clypeaster japonicus* (3) and *Diadema setosum* (4) spermatozoa before (a,b) and after incubation in seawater for 30 min (c,d). Arrows and arrow heads show lipid bodies and lipid globules, respectively. F, flagellum; M, mitochondrion; N, nucleus. Bars show 0.1  $\mu\text{m}$ .

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