BioOne COMPLETE

Degeneration of Respiratory System in Sea Urchin Spermatozoa during Incubation in Seawater for Long Duration

Authors: Ohtake, Tateru, Mita, Masatoshi, Fujiwara, Akiko, Tazawa, Eigoro, and Yasumasu, Ikuo

Source: Zoological Science, 13(6) : 857-863

Published By: Zoological Society of Japan

URL: https://doi.org/10.2108/zsj.13.857

BioOne Complete (complete.BioOne.org) is a full-text database of 200 subscribed and open-access titles in the biological, ecological, and environmental sciences published by nonprofit societies, associations, museums, institutions, and presses.

Your use of this PDF, the BioOne Complete website, and all posted and associated content indicates your acceptance of BioOne's Terms of Use, available at www.bioone.org/terms-of-use.

Usage of BioOne Complete content is strictly limited to personal, educational, and non - commercial use. Commercial inquiries or rights and permissions requests should be directed to the individual publisher as copyright holder.

BioOne sees sustainable scholarly publishing as an inherently collaborative enterprise connecting authors, nonprofit publishers, academic institutions, research libraries, and research funders in the common goal of maximizing access to critical research.

Downloaded From: https://bioone.org/journals/Zoological-Science on 11 Sep 2024
Terms of Use: https://bioone.org/terms-of-use

ZOOLOGICAL SCIENCE **13**: 857–863 (1996) © 1996 Zoological Society of Japan

Degeneration of Respiratory System in Sea Urchin Spermatozoa during Incubation in Seawater for Long Duration

Tateru Ohtake[1], Masatoshi Mita[1,2], Akiko Fujiwara[1], Eigoro Tazawa[3] and Ikuo Yasumasu[1,*]

[1]*Department of Biology, School of Education, Waseda University, Shinjuku-ku, Tokyo 169-50, Japan*
[2]*Teikyo Junior College, Shibuya-ku, Tokyo 151, Japan*
[3]*Biological Institute, Faculty of Literature and Science, Yokohama City University, Yokohama, Kanagawa 236, Japan*

ABSTRACT—Motility and respiration were examined in spermatozoa of the sea urchin *Hemicentrotus pulcherrimus* after dilution and incubation in seawater at pH 8.2 at 20°C. Almost all spermatozoa were motile during 12 hr of incubation, but their respiratory rate decreased gradually. The acrosome reaction was also induced by egg jelly solution during 12 hr of incubation in seawater. However, the ratio of spontaneous acrosome reacted spermatozoa was quite low during the same period. An intracellular pH (pH*i*) of spermatozoa was about 7.5 just after dilution in seawater and was almost constant during 12 hr of incubation. Upon dilution and incubation in seawater, activity of NADH-cytochrome *c* reductase decreased in propotion to the decrease in the respiration in spermatozoa, whereas cytochrome *c* oxidase activity was hardly changed. These suggest that the degeneration of respiratory system during 12 hr of incubation in seawater is due to the decrease in the NADH-cytochrome *c* reductase activity. In energy production system, phosphatidylcholine as a preferred substrate was efficiently hydrolyzed during 4 hr of incubation and then the activity of the energy metabolism decreased gradually. Beyond 12 hr incubation in seawater, the number of immotile spermatozoa increased and respiratory rate declined rapidly. Also, the percentage of the acrosome reaction induced by the egg jelly solution decreased. These are probably due to the increase in the number of dead spermatozoa after 12 hr of incubation in seawater. It is thus concluded that the life-span of *H. pulcherrimus* spermatozoa is about 12 hr after dilution in seawater.

INTRODUCTION

It is known that sea urchin spermatozoa in undiluted semen are immotile (Gray, 1928; Rothschild, 1956). Upon spawning in seawater, these spermatozoa begin flagellatory movement and respiration is activated, in close association with Na^+-dependent acid extraction (Nishioka and Cross, 1978; Christen *et al.*, 1982; Lee *et al.*, 1983; Bibring *et al.*, 1984). Internal alkalization leads to activation of dynein ATPase, resulting in the initiation of motility (Christen *et al.*, 1983).

The energy for flagellatory motility of spermatozoa of the sea urchin *Hemicentrotus pulcherrimus* is produced by the oxidation of endogenous phospholipids (Mohri, 1957; Mita and Yasumasu, 1983). The spermatozoa of *H. pulcherrimus* are generally composed of various phospholipids and cholesterol (Mita and Ueta, 1988, 1989). Triacylglycerol and glycogen are present in trace amounts (Mita and Yasumasu, 1983; Mita and Ueta, 1988). The phospholipids include phosphatidylcholine (PC), phosphatidylserine (PS), phosphatidylethanolamine (PE), and cardiolipin (CL). Following incubation with seawater, the level of PC decreases, with no change in the levels of other phospholipids (Mita, 1992; Mita and Ueta, 1988, 1989; Mita *et al.*, 1990), indicating that PC may be a substrate for energy metabolism in *H. pulcherrimus* spermatozoa.

In general, the biological role of spermatozoon is the transportation of the genetical information (genome) to an egg. Sea urchin spermatozoa swim in seawater and travel toward an egg. However, it is unknown how long sea urchin spermatozoa can swim in seawater. To elucidate this, the present study was undertaken to measure motility and respiratory rate of spermatozoa of the sea urchin *H. pulcherrimus* during incubation in seawater of long duration. Furthermore, changes in the levels of phospholipids available for utilization in energy metabolism were examined in this study.

* To whom all correspondence should be addressed.

Downloaded From: https://bioone.org/journals/Zoological-Science on 11 Sep 2024
Terms of Use: https://bioone.org/terms-of-use

MATERIALS AND METHODS

Materials

Gametes of the sea urchin, *H. pulcherrimus*, were obtained by forced spawning induced by 0.5M KCl injection into the coelomic cavity. Semen was always freshly collected as "dry sperm" and kept undiluted on ice. The number of spermatozoa was calculated on the basis of protein concentration, as determined by the methods of Lowry *et al.*, (1951), using bovine serum albumin as the standard. The protein content per 10^9 spermatozoa was 0.5 mg.

Preparation of egg jelly solution

Egg jelly solution was prepared by acidifying a 20% egg suspension with HCl to pH 5.5 for 4 to 5 min. The eggs were removed by a hand-driven centrifuge, and the supernatant was adjusted to pH 8.2 with NaOH. Undissolved matter was removed by centrifugation at 12,000 × g for 20 min at 4°C. The concentration of egg jelly solution was estimated by carbohydrate determination using the method of Dische and Shettles (1951) with fucose as the standard. The egg jelly solution per ml was equivalent to 3 nmol fucose.

Incubation of spermatozoa

One ml of dry sperm were diluted and incubated in 100 ml artificial seawater (ASW, Jamarin U, Jamarin Lab., Osaka) at pH 8.2 at 20°C. The diluted sperm were left exposed to air with stirring slowly until desired time. Sperm motility was scored qualitatively in five categories as follows: +++, almost all spermatozoa briskly moving; ++, almost spermatozoa moving; +, almost 50% of spermatozoa moving; ±, a few spermatozoa still moving; –, all spermatozoa immotile.

Oxygen consumption assay

Oxygen consumption in a sperm suspension was measured polarographically with an oxygen consumption recorder (MD-1000, Iijima Electronics MFG, Aichi). At the desired time after incubation, 2.5 ml of sperm suspension were transferred to a closed vessel of the oximeter and measured the oxygen consumption.

Measurement of the acrosome reaction

After incubation in ASW, 0.1 ml of sperm suspension was incubated with either seawater or egg jelly solution in a total volume of 0.5 ml for 5 min at 20°C and each sample was fixed by glutaraldehyde at a final concentration of 2%. The percentage of acrosome reacted spermatozoa was determined from electromicroscopic observation (JEM 100CX, JEOL).

Measurement of fertilizing capacity

After incubation in ASW, 0.05 ml of sperm suspension was added to unfertilized egg suspension (100 - 200 eggs/0.2 ml). After 10 min of incubation, the percentage of fertilized eggs based on formation of fertilization membrane was calculated.

Assays of NADH-cytochrome c reductase and cytochrome c oxidase

After sperm suspension was centrifuged at 3,000 × g for 5 min at 4°C, the precipitate was homogenized with 50 mM phosphate buffer, pH 7.2 and 1 mM EDTA. The homogenate was used for assay of NADH-cytochrome *c* reductase (Mahler, 1955) and cytochrome *c* oxidase (Rafael, 1983).

Measurement of intracellular pH (pHi)

The pH*i* of spermatozoa was calculated using a quenching of 9-aminoacridine (9AA) as described previously (Christen *et al.*, 1983). After incubation of desired time, 2.5 ml of sperm suspension was transferred to a cuvette for a fluorescence spectrophotometer (Hitachi F-3000). The quenching was measured by adding 9AA.

Analysis of lipids

Total lipids were extracted from spermatozoa by the method of Bligh and Dyer (1959) and analyzed by high-performance thin-layer chromatography, according to the method of Macala *et al.*, (1983) with some modification as described in previous papers (Mita and Ueta, 1988, 1989). The amount of PC, PS, PE, CL, cholesterol (CH) and free fatty acid (FA) in spermatozoa were determined from the standard curves of the respective authentic lipids.

Analysis of fatty acid composition

Isolated PC on a thin-layer chromatograph plate was subjected to methanolysis by heating with 5% HCl in methanol at 85°C for 2 hr, as previously described (Mita and Ueta, 1988, 1989). Fatty acid methylesters were extracted with *n*-hexane, followed by N_2-blow evaporation. The residues were dissolved in a small amount of *n*-hexane and analyzed using a GC-8A gas-liquid chromatograph (Shimadzu Instruments, Kyoto).

Assay of urea and ammonia

After dilution and incubation in ASW at a desired time, the sperm suspension was centrifuged at 3,000 × g for 5 min at 4°C. The supernatant obtained was used for assays of urea and ammonia by Determiner UN-E kit (Kyowa Medical Co., Tokyo).

Reagents

The phospholipids, CH, and FA standards and 9AA were purchased from Sigma Chemical Co. (St. Louis, MD, USA). Plates for thin-layer chromatography were obtained from E. Merck (Darmstadt, Germany).

RESULTS

When dry sperm of the sea urchin *H. pulcherrimus* were diluted 100-fold in ASW at pH 8.2 at 20°C, the briskly flagellatory movement of spermatozoa was observed. The respiratory rate was about 15 nmol O_2 uptake/min in 10^9 spermatozoa (Fig. 1). The motility and respiration decreased gradually as incubation time elongated. After incubation for 12 hr, the respiratory rate in 10^9 spermatozoa decreased to about 11 nmol O_2 uptake/min, although more than 90% spermatozoa were motile. This suggests that some physiological change brings about the decrease in respiratory activity during 12 hr of incubation. In contrast, further incubation of spermatozoa in ASW, number of immotile spermatozoa increased and the oxygen consumption in the sperm suspension declined rapidly (Fig. 1). At 20 hr of incubation, only 40% spermatozoa were motile and the oxygen consumption was 6 nmol O_2/min in 10^9 spermatozoa. It is probable that the abrupt decrease in respiratory rate beyond 12 hr of incubation is caused by the increase in number of dead spermatozoa. Thus, we focused on the respiration and motility in *H. pulcherrimus* spermatozoa during 12 hr of incubation.

Previous studies have shown that the motility and the respiration in sea urchin spermatozoa are regulated by the pH*i* (Nishioka and Cross, 1978; Christen *et al.*, 1982, 1983; Lee *et al.*, 1983; Bibring *et al.*, 1984). First, the pH*i* of spermatozoa was examined during incubation in ASW for long duration. The pH*i* was between 7.4 and 7.6 just after dilution and incubation in ASW (Table 1). The value was almost constant, except of a slight decrease after 12 hr of incubation (Fig. 2).

Downloaded From: https://bioone.org/journals/Zoological-Science on 11 Sep 2024
Terms of Use: https://bioone.org/terms-of-use

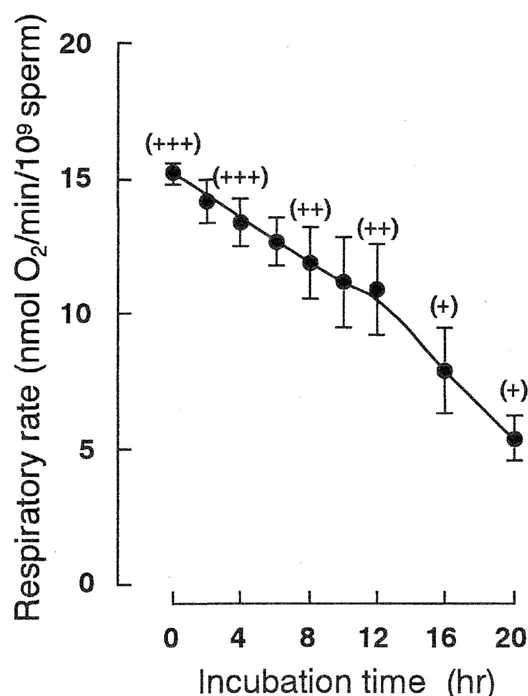

Fig. 1. Change in the rate of oxygen consumption during incubation of sea urchin spermatozoa with seawater. Dry sperm were diluted 100-fold and incubated in ASW at 20°C. Each value is the mean of three separate experiments. Vertical bars show SEM. Symbols in parentheses are sperm motility as shown in "Materials and Methods".

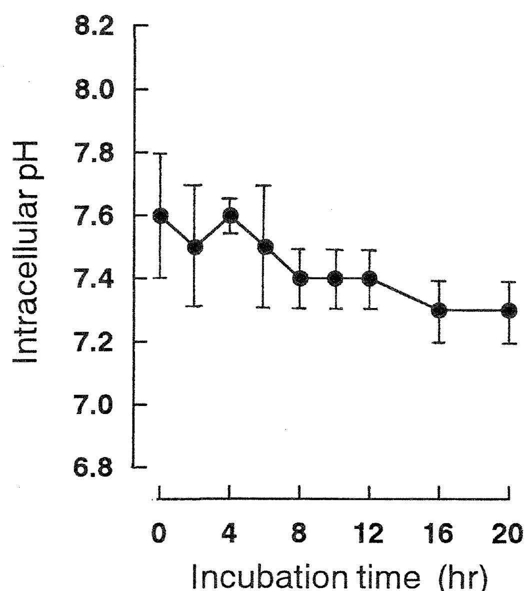

Fig. 2. Change in the value of intracellular pH in sea urchin spermatozoa during incubation in seawater. Dry sperm were diluted 100-fold and incubated in ASW at 20°C. Each value is the mean of three separete experiments. Vertical bars show SEM.

It has been reported that the sperm respiration decrease upon formation of acrosome process (Kinsey *et al.*, 1979). It may be possible that number of spermatozoa reacted acrosome increases following incubation in seawater. Upon incubation of spermatozoa in egg jelly solution, about 72% spermatozoa underwent acrosome reaction (Table 1). Their respiratory rate decreased to 2 nmol O_2/min 10^9 spermatozoa, whereas that in normal seawater was about 10 nmol O_2/min. However, just after dilution in ASW, the ratio of the acrosome reaction was about 10% and the low percentage maintained during incubation for long duration (Fig. 3). This suggests that the decrease in respiratory rate of spermatozoa during 12 hr of incubation is not related to formation of acrosome reaction. On the other hand, the egg jelly solution was capable of inducing the acrosome reaction in about 70% of spermatozoa during incubation in ASW for 10 hr (Fig. 3). The percentage declined gradually beyond 12 hr of incubation: only 20% of spermatozoa underwent the acrosome reaction upon 20 hr of incubation. Nevertheless, the spermatozoa obtained from 20 hr of incubation could completely fertilize eggs (Fig. 3).

Next, we noticed the respiratory system in sperm mitochondria, because the decrease in the reaspiratory rate is not directly caused by the acrosome reaction. Since NADH-cytochrome *c* reductase and cytochrome *c* oxidase is known to play important roles for the electron transport system of mitochondria, activities of these enzymes were examined during incubation in ASW. The NADH-cytochrome *c* reductase activity in *H. pulcherrimus* spermatozoa decreased gradually following incubation in ASW, whereas cytochrome *c* oxidase activity was almost constant until 10 hr of incubation (Fig. 4). These results suggest that the respiratory system in mitochondria is degenerated by causing the decrease in the NADH-cytochrome *c* reductase activity following initiation sperm motility.

Previous studies have shown that *H. pulcherrimus* spermatozoa use endogenous phospholipids to produce energy for moving (Mita and Ueta, 1988; Mita and Nakamura, 1993). Upon incubation in ASW, the content of PC significantly decreased (Fig. 5a), although other lipids, PS (Fig. 5b), PE (FIG. 5c), CL (Fig. 5d) and CH (Fig. 5e) remained constant, except a slight increase in level of FA (Fig. 5f). The level of PC decreased markedly during 4 hr of incubation and then the PC consumption became slow down gradually.

Fatty acids of PC in *H. pulcherrimus* spermatozoa were mostly palmtic (16:0) (12%), eicosamonoenoic (20:1) (11%), arachidonic (20:4) (28%), and eicosapentaenoic acids (20:5) (23%) (Table 2). These accord with the previous studies (Mita and Ueta, 1988, 1989) The percentage of each fatty acid moiety in PC was almost constant during incubation for 8 hr, 12 hr and 16 hr. Based on the net content of PC as shown Fig. 5a, the amount of each principal fatty acid moiety in PC was calculated. Amounts of arachidonic and eicosapentaenoic acids consumed by 10^9 spermatozoa during incubation for 16 hr were about 10 and 11 nmol, respectively (Fig. 6). In contrast, palmitic acid content showed no change, whereas stearic acid content changed a little.

Since endogenous glycogen and glucose contents have already shown to be quite low in *H. pulcherrimus* spermatozoa (Mita and Yasumasu, 1983), it is unlikely that carbohydrate is

Downloaded From: https://bioone.org/journals/Zoological-Science on 11 Sep 2024
Terms of Use: https://bioone.org/terms-of-use

Table 1. Effect of egg-jelly on respiration, acrosome reaction and pH*i* of sea urchin spermatozoa

Conditions	Respiratory rate (nmol O_2/min/10^9 sperm)	Acrosome reaction (%)	pH*i*
ASW	10.1 ± 0.3	9 ± 1	7.5 ± 0.1
Egg-jelly solution	2.2 ± 0.1	72 ± 5	7.8 ± 0.1*

The value is the mean ± SEM obtained from four separate experiments. *P* value is compared with that in ASW. $^*P < 0.1$.

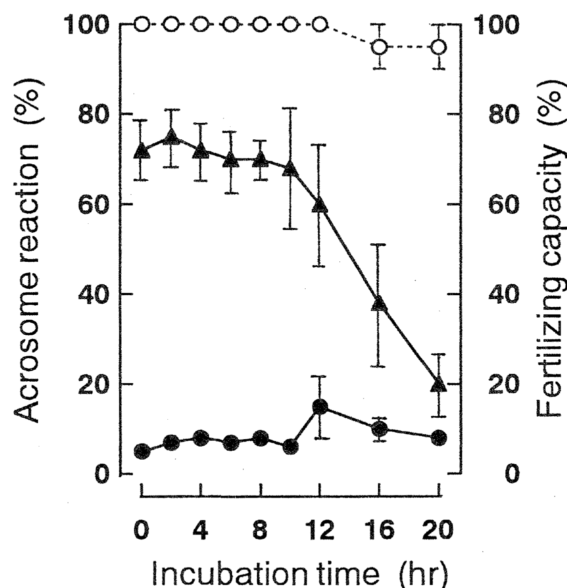

Fig. 3. Change in the percentage of acrosomal reacted spermatozoa during incubation in seawater. Dry sperm were diluted 100-fold and incubated in ASW at 20°C. After incubation at a desired time, each sample was treated with either ASW (●) or egg jelly solution (▲) and scored on acrosomal reacted spermatozoa by transmission electron microscope. In a assay for fertilizing capacity of spermatozoa (○), the sperm suspension was added to the unfertilized egg suspension and the fertilization membrane formation was measured. Each value is the mean of three separate experiments. Vertical bars show SEM.

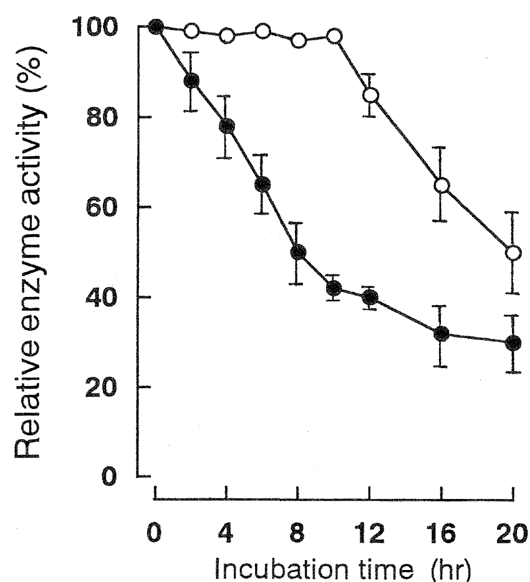

Fig. 4. Change in activities of NADH-cytochrome *c* reductase (●) and cytochrome *c* oxidase (○) in sea urchin spermatozoa during incubation in seawater. Dry sperm were diluted 100-fold and incubated in ASW at 20°C. After incubation at a desired time, sperm suspension was centrifuged. The spermatozoa obtained were homogenized with 50 mM phosphate buffer, pH 7.2 and 1 mM EDTA. The homogenate was used for these enzyme assays. Both activities were indicated as relative percentage with respect to those without incubation. Each value is the mean of three separate experiments. Vertical bars show SEM.

used for energy metabolism. However, it is unclear whether these spermatozoa use protein or amino acid to produce energy for movement. This study also measured amounts of urea and ammonia. However, they were present in trace amounts (less than 0.01 μmol/ml) in ASW during incubation for long duration (data not shown). Thus, this suggests that endogenous protein and amino acid are not metabolized to urea or ammonia for energy production in *H. pulcherrimus* spermatozoa.

DISCUSSION

The study showed that spermatozoa of the sea urchin *H. pulcherrimus* can continue swimming for 20 hr over in ASW at 20°C. Approximately 90% spermatozoa were motile during 12 hr incubation, though the respiratory rate decreased gradually following incubation. Beyond 12 hr of incubation, the respiratory rate in spermatozoa decreased rapidly. Since a marked increase in the number of immotile spermatozoa was observed beyond 12 hr of incubation, the decrease in respiratory rate is probably caused by dead spermatozoa in the suspension. This study also showed that about 70% of spermatozoa during 10 hr of incubation in ASW could undergo the acrosome reaction by the treatment with the egg jelly solution. The percentage decreased beyond 12 hr of incubation. After 20 hr of incubation, only 20% of spermatozoa underwent the acrosome reaction in the treatment with the egg jelly solution. Thus, the duration of motility and the life-span involving the acrosome reaction in *H. pulcherrimus* is estimated to be about 12 hr at 20°C.

However, it is interesting that sperm suspension upon 20 hr of incubation in ASW could fertilize the eggs. This result

Downloaded From: https://bioone.org/journals/Zoological-Science on 11 Sep 2024
Terms of Use: https://bioone.org/terms-of-use

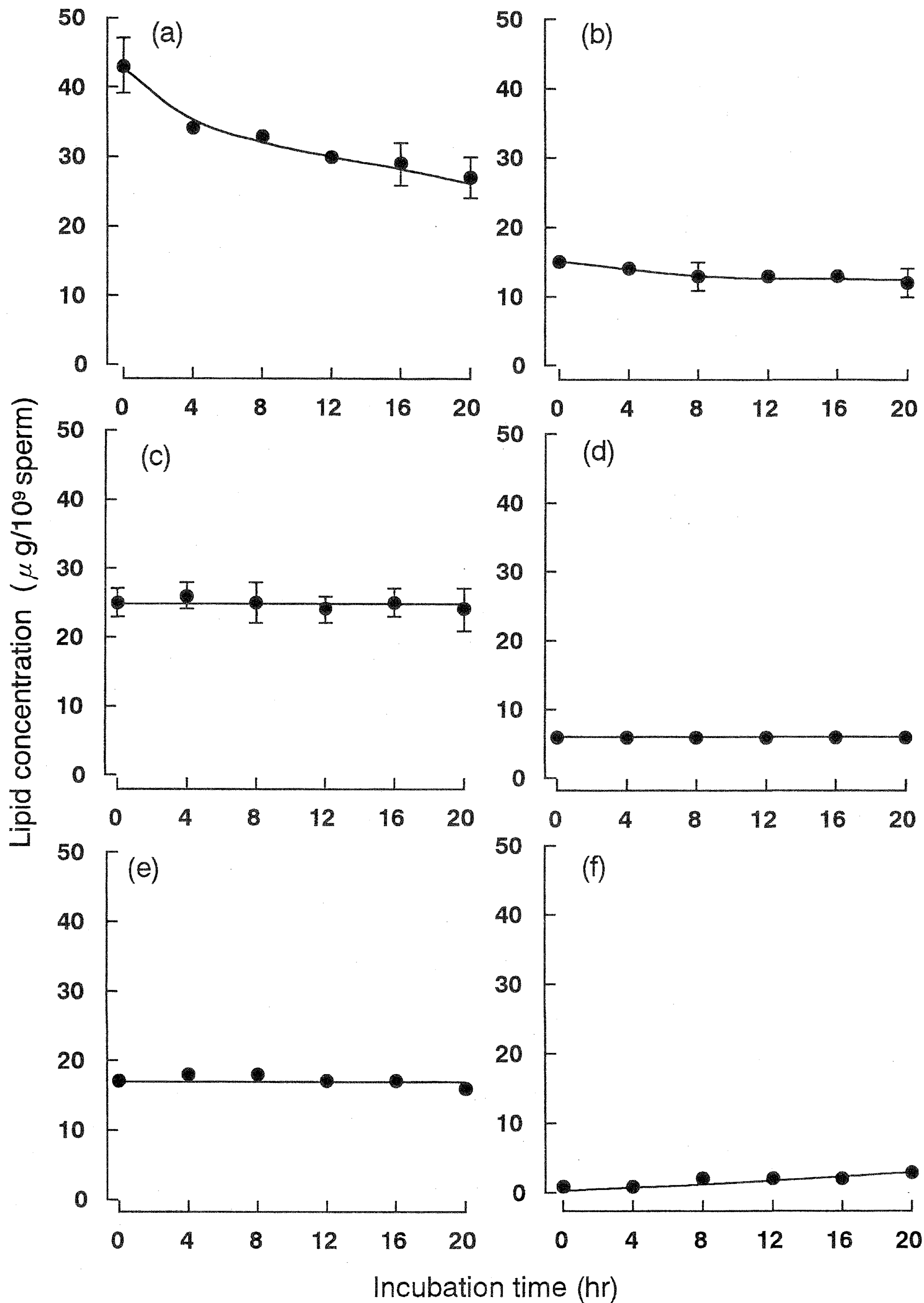

Fig. 5. Change in levels of phosphatidylcholine (a), phosphatidylserine (b), phosphatidylethanolamine (c), cardiolipin (d), cholesterol (e), and free fatty acid (f) in sea urchin spermatozoa during incubation in seawater. Dry sperm were diluted 100-fold and incubated in ASW at 20°C. After incubation at a desired time, total lipids were extracted from spermatozoa and each lipid content was analyzed by high-performance thin-layer chromatography. Each value is the mean of three separate experiments. Vertical bars show SEM.

Downloaded From: https://bioone.org/journals/Zoological-Science on 11 Sep 2024
Terms of Use: https://bioone.org/terms-of-use

Table 2. Fatty acid composition of phosphatidylcholine of sea urchin spermatozoa

Fatty acid	Percentage			
	Incubation time (hr)			
	0	8	12	16
14:0	0.9 ± 0.6	0.8 ± 0.2	4.6 ± 1.2	2.3 ± 1.0
16:0	12.5 ± 0.9	14.2 ± 0.5	12.6 ± 0.4	16.7 ± 0.7
16:1	4.2 ± 1.1	4.9 ± 1.3	5.2 ± 1.0	3.8 ± 0.6
18:0	4.6 ± 0.5	6.9 ± 1.1	5.4 ± 0.8	8.1 ± 3.7
18:1	7.5 ± 0.7	7.2 ± 1.1	6.4 ± 1.0	6.3 ± 1.2
18:4	7.5 ± 2.2	6.9 ± 0.8	8.2 ± 1.8	6.0 ± 1.0
20:1	10.8 ± 1.8	8.9 ± 1.0	10.0 ± 1.5	10.5 ± 1.6
20:4 (n-6)	28.5 ± 1.4	26.8 ± 1.8	30.3 ± 3.3	27.7 ± 2.7
20:5 (n-3)	23.3 ± 0.9	20.2 ± 2.0	20.3 ± 1.5	19.5 ± 2.2

Each value is the percentage of the total and the mean ± SEM obtained from three separate experiments.

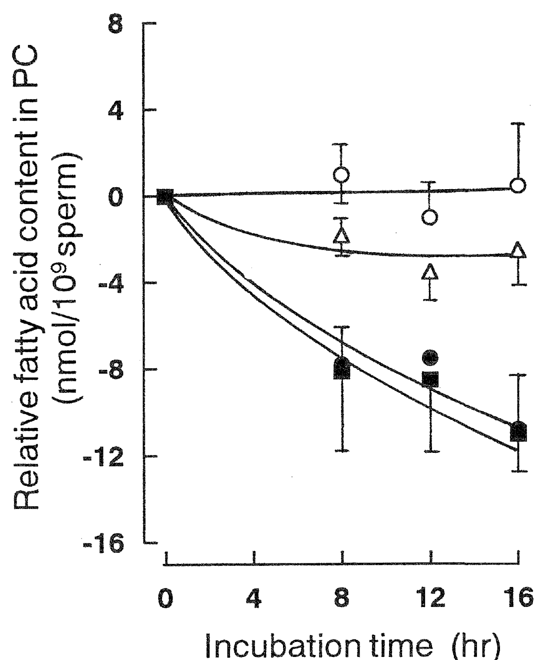

Fig. 6. Change in levels of fatty acid moieties in phosphatidylcholine during incubation of sea urchin spermatozoa in seawater. The fatty acid composition was calculated from the absolute value of PC (Fig. 5a) and its fatty acid composition (Table 2). Each value is the mean of three separate experiments. Vertical bars show SEM. (○), 16:0; (△), 18:0; (●), 20:4; (■), 20:5.

suggests that *H. pulcherrimus* spermatozoa during incubation for 20 hr maintain their fertilizing capacity. This is why all spermatozoa in the suspension are not required for fertilization. If a spermatozoon is still alive among the suspension during incubation for long duration, the spermatozoon can undergo the acrosome reaction and can fertilize the egg.

The initiation of sea urchin sperm motility requires external Na^+ and is associated with Na^+-dependent acid extraction (Nishioka and Cross, 1978). Following dilution in seawater, the pH*i* of sea urchin spermatozoa rises from 6.8 to 7.4 (Christen *et al.*, 1982; Lee *et al.*, 1983; Bibring *et al.*, 1984). Internal alkalization leads to activation of motility (Christen *et al.*, 1983). In this study, the pH*i* was found to remain about 7.5 during incubation in ASW for 12 hr. This suggests that the motility and the respiration is continuously activated during the incubation. However, the motility and respiratory rate were shown to degradate following initiation at sperm movement.

It has been demonstrated that the respiratory rate in sea urchin spermatozoa is rapidly decreased following the acrosome reaction (Kinsey *et al.*, 1979). However, about 10% spermatozoa were found to undergo the acrosome reaction spontaneously during incubation in ASW of long duration. This suggests that the decrease in respiratory rate is not due to the increase in the number of acrosomal reacted spermatozoa.

The oxygen consumption in mitochondria is regulated by NADH-cytochrome *c* reductase and/or cytochrome *c* oxidase. Since the activity of NADH-cytochrome *c* reductase was found to decrease gradually during incubation in ASW, the respiratory system in sperm mitochondria appears to be inflicted following incubation in ASW. But, it is still unclear why the NADH-cytochrome *c* reductase activity decreases upon initiation of sperm motility.

Previous studies have shown that PC is a preferred substrate for energy metabolism in *H. pulcherrimus* spermatozoa (Mita and Ueta, 1988, 1989, 1990; Mita *et al.*, 1990). The results obtained in the present study confirmed this. Following incubation in ASW, PC content decreased significantly without no changes in other phospholipids and cholesterol. The consumption of PC continued during incubation of long duration, but the FA level increased slightly. The fatty acid moieties of PC consumed by spermatozoa were mainly unsaturated fatty acids, such as arachidonic and eicosapentaenoic acids.

The observations in the present study also showed that urea and ammonia contents which are metabolites of protein and amino acids were low in the media after incubation of spermatozoa for long duration. The glycogen and glucose are also present at trace amounts (Mita and Yasumasu, 1983). These strongly suggest that the spermatozoa of *H. pulcherrimus* do not use amino acids and carbohydrates to obtain energy for swimming. Recently, the PC available for use in energy metabolism is located in the lipid bodies within mitochondria in the midpieces of *H. pulcherrimus* spermatozoa (Mita and Nakamura, 1992). It is also possible that the life-span (about 12 hr) of *H. pulcherrimus* spermatozoa is supported by the PC content stored in the lipid bodies.

ACKNOWLEDGMENTS

The authors are grateful to Dr. S. Nemoto and the staff of Tateyama Marine Laboratory, Ochanomizu University, and Dr. K. Inaba and the staff of Misaki Marine Biological Station, University of Tokyo, for affording us the opportunity to utilize their facilities and for their kind assistance in collecting the sea urchin. This study was supported in part by a Grant-in-Aid (No.07640891 to M.M.) from the Ministry of Education, Science, Sports and Culture of Japan.

Downloaded From: https://bioone.org/journals/Zoological-Science on 11 Sep 2024
Terms of Use: https://bioone.org/terms-of-use

REFERENCES

Bibring TJ, Baxandall J, Harter CC (1984) Sodium-dependent pH regulation in active sea urchin sperm. Dev Biol 101: 425-435

Bligh EG, Dyer WJ (1959) A rapid method of total lipid extraction and purification. Can J Biochem Physiol 37: 911-917

Christen R, Schackmann RW, Shapiro BM (1982) Elevation of intracellular pH activates sperm respiration and motility of sperm of sea urchin *Strongylocentrotus purpuratus*. J Biol Chem 257: 14881-14890

Christen R, Schackmann RW, Shapiro BM (1983) Metabolism of sea urchin sperm. Interrelationships between intracellular pH, ATPase activity, and mitochondrial respiration. J Biol Chem 258: 5392-5399

Dische Z, Shettles LB (1951) A new spectrophotometric test for the detection of methyl pentose. J Biol Chem 192: 579-582

Gray J (1928) The effect of dilution of spermatozoa. J Exp Biol 5: 337-344

Kinsey WH, Segall GK, Lennarz W (1979) The effect of the acrosome reaction on the respiratory activity and fertilizing capacity of echinoid sperm. Dev Biol 71: 49-59

Lee HC, Johnson C, Epel D (1983) Changes in internal pH associated with initiation of motility and acrosome reaction of sea urchin sperm. Dev Biol 95: 31-45

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin phenol reagent. J Biol Chem 193: 265-275

Macala LJ, Yu RK, Ando S (1983) Analysis of brain lipids by high performance thin-layer chromatography and densitometry. J Lipid Res 24: 1243-1250

Mahler HR (1955) DPHN cytochrome *c* reductase. In “Method in Enzymology, Vol II”, Academic Press, New York, pp 266-273

Mita M (1992) Diacyl choline phosphoglycerides: The endogenous substrate for energy metabolism in sea urchin spermatozoa. Zool Sci 9: 563-568

Mita M, Nakamura M (1992) Ultrastructual study of an endogeous energy substrate in spermatozoa of sea urchin *Hemicentrotus pulcherrimus*. Biol Bull 182: 298-304

Mita M, Nakamura M (1993) Phosphatidylcholine is an endogenous substrate for energy metabolism in spermatozoa of sea urchins of the order Echinoidea. Zool Sci 10: 73-83

Mita M, Ueta N (1988) Energy metablism of sea urchin spermatozoa, with phosphatidylcholine as the preferred substrate. Biochim Biophys Acta 959: 361-369

Mita M, Ueta N (1989) Fatty chain composition of phospholipids in sea urchin spermatozoa. Comp Biochem Physiol 92B: 319-322

Mita M, Ueta N (1990) Phosphatidylcholine metabolism for energy production in sea urchin spermatozoa. Biochim Biophys Acta 1047: 175-179

Mita M, Ueta N, Harumi T, Suzuki N (1990) The influence of egg associated peptide on energy metabolism in sea urchin spermatozoa: the peptide stimulates preferred hydrolysis of phosphatidylcholine and oxidation of fatty acid. Biochim Biophys Acta 1035: 175-181

Mita M, Yasumasu I (1983) Metabolism of lipid and carbohydrate in sea urchin spermatozoa. Gemete Res 7: 133-144

Mohri H (1957) Endogenous substrates of respiration in sea urchin spermatozoa. J Fac Sci Tokyo Univ IV 8: 51-63

Nishioka D, Cross N (1978) The role of external sodium in sea urchin fertilization. In “Cell Reproduction” Ed by ER Dirksen, DM Prescott, CF Fox, Academic Press, New York, pp 403-413

Rothschild L (1956) The physiology of sea urchin spermatozoa. Action of pH, dinitrophenol, dinitrophenol+versence, and usnic acid on O_2 uptake. J Exp Biol 41: 66-71

Rafael J (1983) Cytochrome *c* oxidase. In “Method of Enzymatic Analysis, Vol 3” Ed by Bergmeyer HU, Verlarg Chemie, Weinhein, pp 266-273

(Received March 6, 1996 / Accepted September 10, 1996)

Downloaded From: https://bioone.org/journals/Zoological-Science on 11 Sep 2024
Terms of Use: https://bioone.org/terms-of-use