Changes of Elemental Concentrations Around and on the Surface of Fowl Sperm Membrane During Maturation in the Male Reproductive Tract and After In Vitro Storage

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X-ray microprobe analysis was performed to investigate the changes of elemental concentrations around or on the membrane of the head, midpiece, and principal piece regions of individual fowl spermatozoa during maturation in the male reproductive tract and after storage in vitro at 4°C. The pattern of change of elemental concentrations during maturation and postejaculation was, in general, similar in the three different subcellular regions; i.e., concentrations of sodium, potassium, chlorine, and calcium decreased gradually during sperm passage through the male reproductive tract and after storage. Phosphorus concentration remained almost constant in the male tract and decreased gradually after storage. In contrast, magnesium, zinc, and copper concentrations showed an interesting pattern: concentrations increased significantly during maturation to a maximum at ejaculation and decreased again after storage. The ratios of sodium to potassium in the midpiece region showed patterns similar to those of magnesium, zinc, and copper concentrations.

Key words: spermatozoa, elements, maturation, storage, x-ray microanalysis, fowl

INTRODUCTION

Changes in the chemical nature of mammalian sperm membrane during post-testicular maturation in the epididymis have been demonstrated in numerous studies [Bedford and Cooper, 1978]. Generally, these studies have focused on changes at the membrane exterior; alterations in specific antigenic sites and carbohydrate moieties have been demonstrated representing either additions to the plasma membrane by secretions of the epididymal epithelium or a modification of preexisting surface components [Olson, 1980]. Nevertheless, little data is available

concerning the changes of elemental concentrations during sperm maturation in the male reproductive tract [Horiuchi et al., 1982; Ozawa et al., 1987]. Around or on the surface of rat spermatozoa, concentrations of sodium, potassium, chlorine, and calcium decreased gradually during sperm passage through the male reproductive tract. In contrast, magnesium concentration increased significantly through the process of maturation [Ozawa et al., 1987].

On the other hand, no morphological modifications were evident, except a thickening of the inner mitochondrial membranes, in fowl spermatozoa during the maturation process and movement down the excurrent ducts of the testis [Nicander and Hellström, 1967; Tingari, 1973]. It also has been shown that in contrast to mammals, no changes occur in certain surface properties of the spermatozoa during their passage from the upper to the lower part of the male tract [Bedford, 1979]. However, there is no information about the changes of elemental concentrations around or on the fowl sperm membrane during maturation in the male reproductive tract. Furthermore, little information is available regarding the quantitative analyses of the elements of the different subcellular regions of individual fowl spermatozoa after ejaculation.

The present study focuses on the changes of elemental concentrations around or on the membrane surface of the different subcellular regions of individual fowl spermatozoa during maturation in the male reproductive tract and after storage in vitro, using x-ray microprobe analysis with scanning electron microscopy.

MATERIALS AND METHODS

Animals

White Leghorn roosters (Babcock strain) obtained from the Akagi Poultry Breeding Farm (Miyazaki, Japan) were housed in cages and fed ad libitum on a proprietary breeder diet. They were exposed to 14 hours of light per 24 hours.

Sperm Preparation and Analyses of Elements

Reproductive organs were removed from the roosters and washed with 0.1M cacodylate buffer (pH 7.4). Spermatozoa were collected from five regions of these organs: testis, epididymis, upper ductus deferens, middle ductus deferens, and lower ductus deferens. Immediately after collection from these regions, spermatozoa were washed once with 0.1M cacodylate buffer at 110 g for 10 minutes and three times at 280 g for 10 minutes. These spermatozoa were prepared for x-ray microprobe analysis as described previously [Ashizawa et al., 1987]. Semen was collected for storage from several roosters by the method of Bogdonoff and Shaffner [1954]. A fourfold dilution of the pooled semen was made in phosphate buffer [Wilcox and Shaffner, 1958] and stored in a test tube at 4°C. After storage for 0, 24, and 48 hours, spermatozoa were washed and prepared as described above. The spermatozoa were analyzed by using a, JEOL scanning electron microscope (JSM-35C) fitted with wavelength-dispersive x-ray microanalyzer (35-SDS, DDS, JEOL). The instrument was operated at a beam current of 2×10^{-8} A and an accelerating voltage of 25kV.

The surface of the head, midpiece, and principal piece regions of each sperm cell were analyzed randomly by spot pulse analysis. Elemental analysis of only apparently morphologically normal spermatozoa was recorded. The elements analyzed were sodium, potassium, chlorine, calcium, phosphorus, magnesium, copper,

TABLE 1. Changes of Elemental Concentrations on the Fowl Sperm Head Region During Maturation in the Male Reproductive Tract[+]

Sites	Concentrations of elements ($\mu g/cm^3$)									
	Na	K	Cl	Ca	P	Mg	Cu	Zn	Fe	Mn
Testis	818 ± 19[a]*	751 ± 35[a]*	1919 ± 88[a]	524 ± 61[a]	787 ± 18[a]	33 ± 4[a]	n.d.	29 ± 3[a]	n.d.	19 ± 4[a]
Epididymis	706 ± 96[b]	659 ± 49[b]	1637 ± 93[ab]	417 ± 19[b]	788 ± 25[a]	46 ± 6[b]	n.d.	40 ± 3[b]	n.d.	24 ± 2[ab]
Ductus deferens										
Upper	696 ± 79[b]	577 ± 76[c]	1301 ± 128[bc]	342 ± 25[bc]	766 ± 55[a]	65 ± 3[c]	n.d.	37 ± 4[b]	n.d.	21 ± 3[ab]
Middle	664 ± 70[b]	464 ± 57[d]	1184 ± 142[cd]	294 ± 9[cd]	777 ± 44[a]	78 ± 2[d]	21 ± 2[a]	39 ± 8[b]	n.d.	27 ± 4[b]
Lower	641 ± 35[b]	376 ± 54[e]	1045 ± 50[d]	256 ± 26[d]	789 ± 40[a]	121 ± 6[e]	29 ± 7[a]	45 ± 4[b]	n.d.	30 ± 6[b]

[+]Each figure represents an average of 18 trials ± SEM. n.d., below limits of detection.
*Within columns, values with different superscripts differ significantly ($P < 0.05$) from each other.

zinc, iron, and manganese. Calcium and chlorine were contained in the cacodylate buffer; therefore, phosphate buffer (pH 7.5) was used for preparations when calcium and chlorine were analyzed. Under these experimental conditions, it is assumed that primarily membrane-bound elements, which probably were trapped and/or released in the surface glycoprotein, were measured rather than the free element. Therefore, the concentration of each element was calculated and expressed as $\mu g/cm^3$ by the method of Hirata and Okumura [1977, 1978]. All data were subjected to statistical analysis by the method of Duncan [1955].

RESULTS

Changes of Elemental Concentrations During Maturation

Table 1 shows the concentrations of elements on the head region of fowl sperm membrane. Of the bulk elements, sodium, potassium, chlorine, and calcium concentrations were highest in the testis. These decreased gradually during maturation. Concentrations of potassium, chlorine, and calcium on spermatozoa from the lower part of ductus deferens were about half those from the testis. In contrast, magnesium concentration increased significantly during sperm passage through the male reproductive tract, and the concentration in the lower part of the ductus deferens was about four times higher than that in the testis. Phosphorus concentrations were almost constant during maturation. Of the trace elements, zinc and manganese showed a pattern similar to that of magnesium; i.e., the concentrations of both elements on spermatozoa from the lower part of ductus deferens were about twice those from the testis. Copper concentrations also increased with sperm maturation.

Table 2 shows the concentrations of elements on the midpiece region. As on the head region, sodium, potassium, chlorine, and calcium concentrations were highest in the testis and decreased gradually during transit in the reproductive tract. In contrast, magnesium, zinc, and copper concentrations increased gradually, and phosphorus concentration remained similar throughout the reproductive tract, as found for those elements on the head region.

Table 3 shows the concentrations of elements on the principal piece region. Although the pattern of changes of bulk elemental concentrations except magnesium was generally similar to those on the head and midpiece regions, it was difficult to identify magnesium and the trace elements on the principal piece region.

The ratios of sodium to potassium on the midpiece region increased signifi-

TABLE 2. Changes of Elemental Concentrations on the Fowl Sperm Midpiece Region During Maturation in the Male Reproductive Tract+

	Concentrations of elements (μg/cm³)									
Sites	Na	K	Cl	Ca	P	Mg	Cu	Zn	Fe	Mn
Testis	793 ± 25[a]*	644 ± 60[a]	1803 ± 99[a]	402 ± 67[a]	715 ± 21[a]	24 ± 2[a]	n.d.	26 ± 3[a]	n.d.	n.d.
Epididymis	729 ± 42[ab]	606 ± 41[a]	1580 ± 112[ab]	352 ± 45[b]	713 ± 14[a]	35 ± 4[b]	n.d.	27 ± 8[a]	n.d.	n.d.
Ductus deferens										
Upper	698 ± 30[bc]	485 ± 31[b]	1303 ± 67[bc]	305 ± 41[c]	689 ± 16[a]	41 ± 10[bc]	n.d.	28 ± 7[a]	n.d.	4 ± 2[a]
Middle	679 ± 21[bc]	400 ± 13[b]	1106 ± 82[cd]	261 ± 31[d]	689 ± 46[a]	56 ± 8[c]	18 ± 4[a]	36 ± 8[b]	n.d.	6 ± 3[a]
Lower	644 ± 32[c]	332 ± 23[c]	890 ± 23[d]	218 ± 15[e]	721 ± 24[a]	78 ± 8[d]	25 ± 9[a]	43 ± 8[b]	n.d.	7 ± 3[a]

+Each figure represents an average of 18 trials ± SEM. n.d., below limits of detection.
*Within columns, values with different superscripts differ significantly ($P < 0.05$) from each other.

TABLE 3. Changes of Elemental Concentrations on the Fowl Sperm Principal Piece Region During Maturation in the Male Reproductive Tract+

	Concentrations of elements (μg/cm³)									
Sites	Na	K	Cl	Ca	P	Mg	Cu	Zn	Fe	Mn
Testis	766 ± 23[a]*	554 ± 92[a]	1712 ± 10[a]	327 ± 26[a]	701 ± 2[a]	n.d.	n.d.	n.d.	n.d.	n.d.
Epididymis	693 ± 22[b]	507 ± 12[a]	1490 ± 50[b]	319 ± 42[a]	694 ± 21[a]	n.d.	n.d.	n.d.	n.d.	n.d.
Ductus deferens										
Upper	680 ± 26[b]	457 ± 33[a]	1171 ± 78[bc]	259 ± 34[b]	679 ± 19[a]	n.d.	n.d.	n.d.	n.d.	n.d.
Middle	649 ± 14[bc]	378 ± 6[b]	971 ± 33[cd]	229 ± 18[b]	670 ± 21[a]	n.d.	n.d.	n.d.	n.d.	n.d.
Lower	610 ± 15[c]	328 ± 7[b]	873 ± 43[d]	207 ± 14[b]	692 ± 19[a]	n.d.	n.d.	n.d.	n.d.	n.d.

+Each figure represents an average of 18 trials ± SEM. n.d., below limits of detection.
*Within columns, values with different superscripts differ significantly ($P < 0.05$) from each other.

cantly during maturation, and the highest value (1.94) was obtained in the lower part of ductus deferens (Table 4).

Changes of Elemental Concentrations After In Vitro Storage

The pattern of changes of elemental concentrations after storage at 4°C was, in general, similar for the three different subcellular regions; i.e., the concentrations of all elements—including magnesium, zinc, and copper—decreased gradually with storage. In the head region, mean concentrations of sodium, potassium, chlorine, calcium, phosphorus, and magnesium before storage were 579 ± 12, 333 ± 8, 860 ± 6, 194 ± 2, 769 ± 99, and 157 ± 4 μg/cm³ (± SEM, n = 18), compared with 558 ± 10, 278 ± 5, 790 ± 11, 166 ± 6, 697 ± 5, and 125 ± 6 μg/cm³ (± SEM, n = 18) after storage for 24 hours, and 389 ± 5, 263 ± 4, 578 ± 11, 141 ± 11, 600 ± 6, and 125 ± 6 μg/cm³ (± SEM, n = 18) after storage for 48 hours, respectively. The trace elements were not identified after storage for 48 hours. The pattern of changes of elemental concentrations on the midpiece and principal piece were similar to those on the head region, although the absolute concentrations were slightly different from that of the head region.

The sodium-potassium ratios on the midpiece region also decreased after storage (1.72 ± 0.09 after storage for 0 hours; 1.60 ± 0.04 after storage for 24 hours; 1.36 ± 0.06 after storage for 48 hours, ± SEM, n = 18).

DISCUSSION

It has been shown that, unlike mammals, no changes occur in certain surface properties of fowl spermatozoa during their passage from the upper to the lower part

TABLE 4. Changes of the Na-to-K Ratios on the Midpiece of Fowl Spermatozoa During Maturation in the Male Reproductive Tract[+]

			Ductus deferens		
	Testis	Epididymis	Upper	Middle	Lower
Na/K	1.24 ± 0.10^{a}*	1.21 ± 0.04^{a}	1.45 ± 0.13^{b}	1.70 ± 0.11^{c}	1.94 ± 0.06^{d}

[+]Each figure represents an average of 18 trials ± SEM.
*Values with different superscripts differ significantly ($P < 0.05$) from each other.

of the male reproductive tract, [Bedford, 1979]. However, the present data indicate that the elemental concentrations around or on the surface of fowl spermatozoa do change during maturation, some decreasing, some increasing, and some remaining constant. Ozawa et al. [1987] obtained similar results in rat spermatozoa; i.e., magnesium concentration increased significantly during maturation in the male reproductive tract, although sodium, potassium, chlorine, and calcium concentrations decreased gradually and phosphorus concentrations remained nearly constant. However, the source of these elements has not been determined. They may be ions from the testicular or epididymal fluids that are ''trapped'' and/or ''released'' on the surface glycoprotein (Dr. G. J. Wishart, personal communication).

In addition to changes of elemental concentrations in the male reproductive tract, changes during postejaculatory storage in vitro at 4°C were recorded. Battersby and Chandler [1977] reported that, except for phosphorus, intracellular concentrations of elements increased during storage at 20°C in human spermatozoa. In the present study, however, concentrations of most elements around or on the fowl sperm membrane were highest in the testis, decreasing gradually with the maturation and aging of spermatozoa. In contrast, magnesium, zinc, and copper concentrations showed an interesting pattern: concentrations were maximum in the lower part of the ductus deferens and immediately after ejaculation. The sodium-potassium ratios showed also a similar pattern. The biological or physiological significance of the high concentrations of magnesium and zinc for sperm function is little understood, although magnesium is believed to be important for membrane stability [Teraoka, 1966] and for the maintenance of full viability of fowl spermatozoa [Wales and White, 1958]; zinc is involved in membrane and chromatin stability and possibly also in the mechanical properties of the accessory fibers, sperm motility, and fertilizing ability [Arver and Eliasson, 1980]. Moreover, high sodium-potassium ratios have been shown to contribute to the marked increase in sperm motility [Nelson, 1975], although Battersby and Chandler [1977] reported that there was no relationship between sodium-potassium ratios and motility in human spermatozoa. The present work, together with the suggestion described above, suggest the possibility that high concentrations of magnesium and zinc and the high sodium-potassium ratios may be involved in sperm stability and motility processes.

ACKNOWLEDGMENTS

We wish to express our gratitude to Dr. G. J. Wishart, Dundee College of Technology, Scotland, United Kingdom, for his kind criticism in preparing the

manuscript and Dr. K. Mamba, St. Marianna University School of Medicine, Kanagawa, Japan, Ms. M. Kuroki, and Dr. T. Fukumori, Miyazaki University, for their skillful technical advice.

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