A $^{31}$P NMR Study of the Epididymis and Epididymal Sperm of the Bull and Hamster

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ABSTRACT

$^{31}$P NMR signals assigned to intracellular adenine nucleotides and to inorganic phosphate were detected in dense suspensions of epididymal sperm obtained from bulls or hamsters. Similar adenine nucleotide signals and an additional large resonance peak, attributable to extracellular glycerylphosphorylcholine, were observed with whole bovine cauda epididymides. Provision of the glycolytic substrate fructose to such sperm suspensions promoted apparent conversion of intracellular ADP to ATP with a concomitant decrease in cellular inorganic phosphate (Pi) content. Subsequent treatment with the methylxanthine caffeine resulted in diminution of the intracellular $\gamma$-P-ATP signal that was consistent with the decreased ATP and ADP contents previously demonstrated by chemical analyses of cellular extracts. Alternatively, treatment with fructose followed by the membrane-selective detergent digitonin produced loss of the nucleotide NMR signals, indicating release of ATP and Pi from the sperm cytosol with subsequent hydrolysis in the extracellular medium. Comparison of intracellular Pi and ATP resonance signals with those of ATP and Pi in vitro, in media of varied pH and cation composition, allowed calculation of a cytosolic pH of 6.5–6.6 and a cytosolic $\text{Mg}^{2+}$ concentration of 0.5 mM for fresh suspensions of bovine cauda epididymal sperm. Intracellular Pi of hamster epididymal sperm reported a similar cytosolic pH. Other, more acidic compartments were not detected in these experiments. However, during prolonged incubation, the pH of the bovine sperm interior slowly decreased as the extracellular medium was acidified by extensive production of lactate. Intracellular ATP was detectable until cytosolic pH declined to approximately 5.5. Rapid intracellular acidification, resulting from exchange of internal $K^+$ for $H^+$, was observed after treatment with the carboxylic acid ionophore nigericin. This lowering of internal pH was followed by a slower return toward initial internal pH values, probably as a consequence of secondary exchange of internal protons for other external monovalent cations, rather than as a result of the operation of a cellular homeostatic mechanism.

Together, these studies utilizing noninvasive NMR techniques provide evidence that within the bovine epididymis sperm utilize an unknown energy source to phosphorylate adeninenucleotides and maintain a slightly acidic cytosolic pH.

INTRODUCTION

This and other laboratories have attempted determination of the intracellular pH of spermatozoa by examining the pH of cell homogenates (Wong et al., 1981), or of the intra- and extracellular distribution of fluorescent or isotopically labeled amines (Meizel and Deamer, 1978; Schackmann et al., 1981; Christen et al., 1982; Lee et al., 1983), and by examination of the pH-dependent spectral properties of carboxyfluorescein generated intracellularly from a permeant precursor (Babcock, 1983; Babcock et al., 1983). These measurements indicated that an elevation of pH in the major cytosolic compartment increases motility and metabolism of invertebrate and mammalian sperm and suggested that such an increase in internal pH may function physiologically at motility initiation, following exit of mammalian sperm from the epididymis (Wong et al., 1981; Babcock et al., 1983; Carr and Acott, 1984). Considerable evidence, based upon measurements of distributions of weak acids and amines, also indicates that the sperm acrosome may be maintained at an acidic pH prior to the acrosome reaction. Such measurements sup-
ported the conclusions (Meizel and Deamer, 1978) that the acrosome of hamster epididymal sperm is maintained at several pH units below that of the rest of the cell, and that this apparent large pH gradient, observed in hamster sperm incubated in K+-deficient medium, disappeared when the acrosome reaction was induced by increased extracellular K+. (Working and Meizel, 1983) Lee et al. (1983) and Schackmann et al. (1981) also utilized distribution measurements and concluded that an increase in cellular pH accompanied the acrosome reaction of sea urchin sperm.

Examination of the NMR spectra of a variety of organs and tissues and of several types of isolated cells has provided unique information about cell viability, metabolic activity, and the intracellular ionic environment. Calculations of intracellular pH based on comparison of the inorganic phosphate (Pi) or sugar phosphate NMR signals in vivo and in vitro have proven particularly valuable because of the uniquely noninvasive nature of NMR determinations (see Roberts and Jardetzky, 1981; Roberts et al., 1981; Gadian, 1982; Iles et al., 1982). Analysis of sea urchin sperm by 31P NMR (Winkler et al., 1982; Christen et al., 1983; Lee et al., 1983) has shown that initiation of motility is associated with increased intracellular pH and results in extensive hydrolysis of the abundant phosphocreatine present in quiescent sperm.

Mammalian spermatozoa are especially well suited for examination by 31P NMR because sperm viability is supported by glycolysis under anaerobic conditions. However, previous studies (Arrata et al., 1978) failed to observed ATP phosphate resonance signals in human semen. Therefore we have reexamined, by nondisruptive, noninvasive NMR methodology, epididymal sperm of the bull and hamster with the goals of determining intracellular pH, of acquiring further evidence of localization of acidic intracellular compartments, and of documenting the changes in intracellular pH that are induced by ionophore-mediated transmembrane exchange of metal ions for protons.

**MATERIALS AND METHODS**

Chemicals were obtained from the following sources: morpholinopropane sulfonate (MOPS), N-[2-acetamid]ol]-2-aminoethane sulfonate (ACES), L-fructose, and digitonin from Sigma Chemical Co. (St. Louis, MO), and nigericin from Eli Lilly and Co. (Indianapolis, IN). Digitonin was recrystallized from ethanol prior to use. Bovine cauda epididymides were a gift of Oscar Mayer Co. (Madison, WI). Epididymal sperm were extruded from the distal cauda and vas deferens by retrograde flushing with medium NKM (120 mM NaCl/5 mM KCl/1 mM MgCl2/10 mM Na morpholinopropane sulfonate; pH 7.4).

After centrifugation (1000 x g, 6 min), the diluted supernatant plasma was saved and the collected sperm were washed by dilution in 15-20 vol of medium NKM. Cells were again collected by centrifugation (600 x g, 6 min) and sperm were gently resuspended in 1 vol of either medium NKM or medium in which NaCl replaced NaCl and KCl and ACES (at pH 6.50) replaced MOPS. Suspensions of washed sperm (8-10 ml, 2-4 X 109 cells/ml) were transferred to NKM sample tubes (20 mm diameter) that were fitted with a specially constructed Teflon vortex plug that assured alignment of a capillary containing the methylphosphonate external standard. The plug also prevented diffusional entry of oxygen, so that suspensions rapidly became anaerobic. All procedures were conducted at room temperature. The sample assembly containing the sperm suspension was transferred to a Nicolet NT-200 NMR spectrometer equipped with a broad band probe and an 85 MHz spinner for phosphorus. Spectra were collected without sample tube rotation (nonspinning). Due to the stability of the field produced by the superconducting magnet employed with this instrument, a field frequency lock was not required. Radio frequency pulse times were adjusted to produce a prescission angle of 55°, and an 0.1-s delay time between pulses was utilized. Phosphorus chemical shifts are reported relative to 85% phosphoric acid. The pH of standard solutions and the pH of cell suspension media were determined with a Beckman digital pH meter and calibrated phosphate standards (Fisher Chemical Co.). The pH reading before and after NMR measurement usually did not differ by more than 0.02 units. The chemical shift of Pi1, employed to estimate intracellular pH in these experiments, is a weighted average of fast exchange between protonated and unprotonated forms (Hoult et al., 1974). Comparison of chemical shifts of standard solutions and of biologic samples was facilitated by an iterative, nonlinear least-squares curve-fitting program provided by Dr. D. A. Kramp that allowed determination of the constants R1 (0.52 and 7.83), K2 (2.87 and 5.04), and K (6.52 and 5.5) for Pi and ATP standards, respectively, from the following modified form of the Henderson-Hasselbach equation:

$$\text{pH} = \text{pK}_a + \log \left( \frac{\Delta - \text{R}_1}{\text{R}_2 - \Delta} \right)$$

where A is the absolute value of the observed resonance frequency shift (for the constants above, expressed relative to the resonant position of phosphocreatine for the purpose of the calculations). Application of this equation with these determined constants and the A values observed in cellular preparations provided an estimate of intracellular pH. Intracellular Mg2+ was calculated by the methods of Gupta and Yushok (1979) and Gupta and Wasley (1980).

**RESULTS**

The ability of 31P NMR signals to report the pH of the environment of partially protonated phosphates is demonstrated by the experiments summarized below. Fig. 1a shows the shifts in
the resonance signal of $P_i$ examined in medium NKM without NaMOPS in which the ionic strength is comparable to that of the intracellular environment, but adjusted to the indicated pH values. The downfield shift in the resonance signal that occurs with increasing pH is fit smoothly by application of Eq. 1 with apparent pK$_a$ values of 6.52 and 6.82, respectively, in the presence and absence of Mg$^{2+}$ ions. The slight lowering in pK$_a$ and small decrease in extent of the shifts observed in the presence of Mg$^{2+}$ resemble those reported by Roberts et al. (1981), who examined extensively the effects of environment on $P_i$ NMR signals.

The NMR signals from the $\gamma$-phosphate (but not those from $\alpha$- or $\beta$-phosphate) of free ATP also similarly depend upon the pH of the surrounding medium, with an apparent pK$_a$ of 6.45 (Fig. 1b). However, the additional presence of Mg$^{2+}$ affects the response of the $\gamma$-P-ATP NMR signal to pH much more than it affects the pH-dependent shifts of the $P_i$ NMR signal.

With equimolar Mg$^{2+}$, the apparent pK$_a$ is reduced by 1.0 unit, to 5.53. Also shown is a curve calculated, by interpolative methods, for ATP that is complexed with Mg$^{2+}$ to the extent of 90%—conditions expected to prevail intracellularly (see below).

Resonance signals at $-5.2$ ppm, derived from the intracellular $\gamma$-P-ATP (with a small contribution from $\beta$-P-ADP), also are observed in the $^{31}$P NMR spectrum of an intact bovine cauda epididymis (Fig. 2a), and in dense suspensions of bull (Fig. 2b) and hamster (Fig. 2c) cauda epididymal sperm. In addition, resonance peaks characteristic of $\beta$-P-ATP and the $\alpha$-phosphates of adenine nucleotides (primarily ATP) also are observed in each spectrum, at $-19$ ppm and $-10.3$ ppm, respectively. A $P_i$ resonance signal at $+1.8$ ppm is obtained from the sperm suspensions, but in the spectrum obtained from the intact epididymis, an intense resonance signal centered at $+0.6$ ppm prevents accurate determination of the $P_i$ resonance frequency.

**FIG. 1.** Resonance shifts in the in vitro titration of $P_i$ and $\gamma$-P-ATP. (a) Sodium phosphate (5 mM) in 100 mM NaCl and 5 mM KCl ($\bullet$) and with the additional presence of 5 mM MgCl$_2$ (●). The pH was adjusted with NaOH or HCl. (b) Sodium ATP (5 mM) in the above medium in the absence ($\bullet$) or presence (●) of 5 mM MgCl$_2$. Also shown is a calculated curve (solid line) for 0.90 molar ratio of Mg-ATP.
FIG. 2. The 80.96 MHz $^{31}$P NMR spectra of a bovine cauda epididymis and of bovine and hamster epididymal spermatozoa. The caudal portion of a selected bovine epididymis was carefully prepared by removal of the tunica and gently forced into the sample tube. Spectra were collected in the subsequent 11 min interval (a). Washed bovine sperm in medium NKM with 10 mM fructose were prepared as described in Materials and Methods and spectra were collected over an 11-min interval (b). Hamster sperm were prepared in a similar manner and examined under comparable conditions (c).

Comparison of the $P_i$ resonance frequency shifts of the sperm samples with those for $P_i$ in vitro (Fig. 1a) allow estimation of an intracellular pH of 6.5–6.6 for both bull and hamster epididymal sperm. A similar estimate of intracellular pH is obtained from comparison of cellular $\gamma$-P-ATP resonance signals with those for the $Mg^{2+}$-ATP complex in vitro (Fig. 1b), but it should be noted that the $^{31}$P NMR signal of the $Mg^{2+}$-ATP complex is not a precise indicator in this pH range.

However, additional information about the intracellular ionic environment is contained in the separation of the $\alpha$-P and $\beta$-P nucleotide
resonance peaks. Application of the equations of Gupta and coworkers (Gupta and Yushok, 1979; Gupta and Wasley, 1980) allows calculation that 88% of cellular ATP is bound to Mg\(^{2+}\) and that, with a reported ATP content of 25 nmol/10\(^8\) cells (Garbers et al., 1973), this extent of complex formation corresponds to a free Mg\(^{2+}\) concentration of approximately 0.5 mM for the bovine spermatozoa examined in the experiments summarized in Fig. 2.

The most prominent resonance signal in the spectrum from the intact bovine epididymis also was apparent in the spectrum (Fig. 3) of the supernatant obtained after centrifugal fractionation of epididymal semen was examined as in Fig. 2.

Spectra of washed bull epididymal sperm examined in the absence of a glycolytic substrate (Fig. 4a) show a reduced \(\gamma\)-P-ATP NMR signal. Resonance signals in the \(-5\) and \(-10\) ppm region, under these conditions, are therefore derived from the \(\alpha\)- and \(\beta\)-phosphates of ADP. A peak characteristic of (intracellular) Pi at pH 6.5–6.6 also is present. Additionally, a resonance signal at +3 ppm probably represents AMP. Spectra collected after the addition of fructose (Fig. 4b) show increased resonance signals in the \(-6\), \(-11\), and \(-19\) ppm regions, and decreased amplitude of the +1.6 ppm resonance signal (Pi), consistent with conversion of Pi, AMP, and ADP to ATP. Because phosphorylated metabolic intermediates in the glycolytic pathway produce NMR signals in the +3.0 to +4.5 ppm region (Evans and Kaplan, 1977), the changes in signal intensity in this region, observed after addition of fructose, probably represent the combined effects of the appearance of phosphorylated sugars and the disappearance of AMP.

When the NMR spectra of such anaerobic, glycolyzing sperm suspensions were monitored for longer periods of time (data not shown) a progressive upfield shift of the Pi NMR signal reflected the acidification of the medium and the intracellular environment that resulted from production of lactate (Babcock et al., 1983; Carr and Acott, 1984). It was noted, however, that \(\gamma\)-P-ATP resonance signals were still present when the internal pH had fallen to 5.5. Under no conditions, in these and other experiments (Figs. 2, 4, 5, 6, and 7), were NMR signals observed that could be assigned to arginine phosphate or creatine phosphate or to pyridine nucleotides, in agreement with the absence or low content of these compounds as determined by chemical analyses (Bistocchi et al., 1968; Brooks, 1971).

Treatment of bovine sperm with digitonin results in selective disruption of the plasma membrane, but leaves the sperm mitochondria functionally intact (Singh et al., 1983). The upfield shift in the Pi NMR signal that follows treatment of sperm suspensions with digitonin (Figs. 5a,b) indicates an increase in pH from 6.5–6.6 to 7.3–7.5 that is consistent with the change expected upon release of cytosolic Pi to the extracellular medium. The magnitude of the Pi signal also increases after addition of digitonin concomitant with decreases in nucleotide phosphate signal intensities (see Fig. 6b,c), presumably as result of enzymatic hydrolysis following cellular disruption.
FIG. 4. Fructose alters the $^{31}\text{P}$ NMR spectra of bovine sperm. Sperm were prepared and examined as in Fig. 2, but without added fructose. After spectra were collected for the initial 11-min period (a), fructose (10 mM) was added with thorough mixing and additional spectra were collected (b) in the successive interval of the same duration. For comparison, the derived difference spectrum (c) is presented also.

Under the nearly anaerobic conditions prevailing in this experiment, mitochondrial ATP production would not be expected. Furthermore, the low signal intensity of the nucleotide $\alpha$- and $\beta$-$^{31}\text{P}$ NMR signals and their relative insensitivity to pH precludes assessment of the localization of these species. Thus it was not possible to make NMR signal assignments for the portion of adenine nucleotides that presumably still remain in the sperm mitochondria after treatment with digitonin.

Treatment with caffeine is another pharmacologic manipulation known to alter sperm adenine nucleotide composition as a result of caffeine's stimulation of motility. Garbers et al. (1973) demonstrated that the ATP content of cellular extracts decreased and the ADP content increased within minutes after addition of caffeine to sperm suspensions. Examination of the NMR spectra collected prior to and following caffeine treatment (Fig. 6) confirmed, by this nondisruptive method, the rapid conversion of ATP to ADP that was reported previously. In addition, these experiments document an increase in cellular $\text{P}_i$ content expected to accompany net ATP hydrolysis and allow determination of the effect of caffeine on intracellular pH. Because the $\text{P}_i$ NMR peaks indicate that the cells are becoming acidic during this experiment, we conclude that caffeine treatment acidifies the cytosol due to lactate formation as glycolysis is stimulated by
FIG. 5. Treatment with digitonin alters the $^{31}$P NMR spectrum of bovine sperm. Sperm were prepared and examined as in Fig. 2 in the presence of 10 mM fructose. After spectra were collected for the initial 11-min period (a), digitonin (100 µg/108 cells) was added with thorough mixing and additional spectra were collected (b and c) in subsequent intervals of the same duration.

ADP and Pi. Utilizing a spectral probe that allowed continuous monitoring of cytosolic pH, Babcock et al. (1983) saw no change in the cytosolic pH of bovine sperm treated with caffeine in the absence of fructose and examined on a somewhat shorter time scale. Thus cytosolic acidification is not obligatory for the stimulatory action of caffeine.

The monovalent cation-selective ionophore nigericin (Graven et al., 1966) promotes the selective exchange of K$^+$ for H$^+$ across the membranes of spermatozoa (Babcock, 1983). Nigericin, and the related Na$^+$-selective ionophore monensin (Estrado-O et al., 1967), therefore have been employed to examine the effects of altering sperm intracellular pH and cation content (Tilney et al., 1978; Mrsny and Meizel, 1981; Hansbrough and Garbers, 1981; Babcock et al., 1983; Lee et al., 1983). However, most previous experiments have not examined directly either the direction, magnitude, or time course of the effect of these agents on intracellular pH. The experiments summarized in Figs. 7 and 8 document the changes in the NMR spectra and in the calculated intracellular and measured extracellular pH that follow treatment of bovine sperm suspensions with nigericin.

These experiments utilized a medium in which LiCl replaced NaCl (to promote the selective exchange of K$^+$ for H$^+$) and that was buffered at a pH equivalent to the intracellular...
FIG. 6. Treatment with caffeine alters the $^3^1$$P$ NMR spectrum of bovine sperm. Sperm were prepared and examined as in Fig. 2 in the presence of 10 mM fructose. After spectra were collected for the initial 11-min period (a), caffeine (7.5 mM) was added with thorough mixing and additional spectra were collected in subsequent intervals of the same duration.

In the following intervals $P_i$ and $\gamma$$P$-ATP resonance signals shifted back upfield (Figs. 7b–7f) so that both the initial intracellular acidification and the initial extracellular alkalinization were reversed during subsequent incubation (Fig. 8).

Decay of the initial transmembrane pH gradient generated by ionophore treatment was

initial increase from pH 6.5 to pH 6.8 (Fig. 8). In the following intervals $P_i$ and $\gamma$$P$-ATP

FIG. 7. Treatment with nigericin alters the $^3^1$$P$ NMR spectrum of bovine sperm. Sperm were prepared and examined as in Fig. 2, except that LiCl medium was employed for the final washing and resuspension. After spectra were collected for the initial 11-min period (a), nigericin (100 nmol) was added with thorough mixing and additional spectra were collected (c–f) in subsequent intervals of the same duration.

pH of 6.5 (to eliminate transmembrane pH gradients). The $P_i$ and $\gamma$$P$-ATP NMR peak frequencies under these conditions, prior to the addition of nigericin (Fig. 7a), indicate an intracellular pH of 6.5, indistinguishable from that of cells in NaCl-based medium (Figs. 4–6). Addition of the ionophore (Fig. 7b) resulted in downfield shifts in the $P_i$ and $\gamma$$P$-NMR peaks that indicated an initial decrease in cytosolic pH to approximately 6.2. Measurement of the extracellular pH demonstrated a reciprocal
FIG. 8. Treatment with nigericin induces reciprocal effects on the \textit{intra}- and extracellular $\text{pH}$ of bovine sperm suspensions. During the experiment described in Fig. 8, the extracellular $\text{pH}$ ($\bullet$) was monitored with a glass electrode. The intracellular $\text{pH}$ was calculated from the resonance peaks of cellular $\text{Pi} (\bullet)$ and ATP ($\circ$).

**DISCUSSION**

Previous applications of $^{31}$P NMR determinations to mammalian sperm suspensions did not distinguish between signals arising from extra- and intracellular components, and did not detect nucleotide phosphate resonances (Arrata et al., 1978). The present study is thus the first application of $^{31}$P NMR to probe the intracellular contents of mammalian sperm preparations and of mammalian sperm within the epididymis. It is important, therefore, both to consider in detail the interpretation of the experiments that are reported here, and to recognize that the scope of this study is limited to bovine and hamster epididymal sperm and to the bovine epididymis and epididymal plasma. We cannot eliminate the possibility that the
preparation procedures employed may themselves alter the intracellular contents of the sperm. However, similar intracellular pH (and Mg$^{2+}$ concentrations; M. B. Smith, unpublished observations) were reported by examination of the inorganic or nucleotide phosphate NMR signals arising from preparations of washed bovine sperm and of the signals that presumably were derived primarily from densely packed sperm contained within the whole cauda epididymis. Moreover, the intracellular pH values derived from these experiments are in close agreement with previous determinations of cytosolic pH of bovine sperm (Babcock et al., 1983) and of estimates for the intracellular pH of rat sperm (Wong et al., 1981).

Multiple, partially resolved NMR peaks from P$_i$ and from $\gamma$-P-ATP have been observed in a limited number of other cell types (Navon et al., 1977; Griffiths et al., 1981; Garlick et al., 1983) and have been interpreted as evidence of the additional localization of P$_i$ and ATP in organelles maintained at a pH different than that of the major cytosolic compartment. Such multiple peaks were not observed in the spectra derived here from sperm of the hamster and bull. Any of several considerations may explain this observation of apparent homogeneity of the intracellular pH of sperm.

For instance, it is possible that the differences between the pH of the intracellular compartments was too small to produce resolvable spectral peaks. It is possible also that either low concentrations of P$_i$ and ATP or a small volume of the secondary compartment(s) limited the signal intensity to an undetectable level at the prevailing signal-to-noise ratios. In either case, the intracellular pH reported here is close to, or equivalent to, that of the major cytosolic compartment of the sperm. Thus this value differs in its significance from that of the weighted-average cytosolic pH that was derived previously by spectrophotometric examination of intracellular carboxyfluorescein (Babcock, 1983). The correspondence of the values derived by these different techniques may indicate that bulk spectral measurements of cell suspensions are heavily weighted by a preponderant localization of carboxyfluorescein in the major cytosolic compartment.

Thus bulk spectral determinations utilizing 31P NMR or carboxyfluorescein techniques presently appear incapable of examining the putative, strongly acidic acrosomal compartment reported by amine distribution measurements (Meizel and Deamer, 1978; Working and Meizel, 1983). This is unfortunate because the amine distribution techniques are also subject to limitations, including, but not limited to, requirements for accurate determination of intracellular volume and for adequate corrections for binding of the probe to cellular components. It is possible that the sperm examined in Figs. 2, 4, and 6 had lost their acrosomal contents due to occurrence of the acrosome reaction. However, because the simple incubation conditions employed closely resemble those shown previously not to promote loss of acrosomal hyaluronidase from bovine and hamster sperm (Triana et al., 1980), this is not a likely explanation.

Recent examination of the 31P NMR spectra of sea urchin spermatozoa (Winkler et al., 1982; Christen et al., 1983; Johnson et al., 1983) detected P$_i$ and nucleotide phosphate and the additional presence of high concentrations of creatine phosphate. The intracellular pH, calculated from the P$_i$ NMR signal, was approximately 7.4 for undiluted sperm preparations. An intracellular pH of 6.8 was reported for sperm diluted into a Na$^+$-free medium that suppressed motility. Subsequent addition of Na$^+$ increased internal pH (pH 7.4) and stimulated respiration and motility.

These and other studies of sea urchin (Hansbrough and Garbers, 1981; Schackman et al., 1981; Lee et al., 1983) and mammalian (Wong et al., 1981; Babcock et al., 1983; Tretiapsat and Chulavatnatol, 1983; Carr and Acott, 1984) sperm indicate that an increase in intracellular pH may play a universal role in the initiation of sperm motility. It has been further suggested that the mechanistic basis for this regulation is a direct consequence of the pH dependence of the dynein ATPase of the sea urchin sperm flagella (Christen et al., 1983). It is interesting that the more acidic intracellular pH reported here for hamster sperm correlates with a more acidic optimum of the pH dependence for the reactivation of motility of permeabilized hamster sperm (Morton, 1973).

Other differences noted in 31P NMR studies of bovine and sea urchin sperm include phosphocreatine content and the lesser effect of external pH on the internal pH of bovine sperm. Earlier work from this laboratory indicated that the energy storage function of phosphocreatine in invertebrate sperm may be replaced by acetylcarnitine of bovine sperm (Milkowski et al., 1976).
Other work from this laboratory (Babcock, 1983) demonstrated that the monovalent cation-selective ionophores nigericin and monensin alter intracellular pH and intracellular content. Most importantly, it was documented that these changes are obligatorily linked and that the resultant selective net fluxes cannot be predicted without knowledge of the initial transmembrane gradients of both pH and cations. However, it was not emphasized that less selective, secondary actions of the ionophores also must be considered in studies conducted under less controlled conditions or for longer duration.

We have shown in the present study that the intracellular acidification, induced by treatment with nigericin in a low K+ medium, disappears spontaneously during subsequent incubation. The time required for this secondary decay would be expected to decrease with increasing ionophore dosage and to decrease in medium in which Na+ replaced Li+. The internal pH achieved after complete equilibration would be expected to depend upon both initial cation composition and pH of the medium. These results indicate that considerable caution is required when the consequences of ionophore treatment are interpreted in terms of cellular regulatory mechanisms.

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REFERENCES


