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Inhibition of Bovine Spermatozoa by Caudal Epididymal Fluid: II. Interaction of pH and a Quiescence Factor[1]

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ABSTRACT

Previous studies (Carr and Acott, 1984) indicate that bovine sperm are maintained in a quiescent state in the caudal epididymis (CE) by a pH-dependent inhibitory factor. Here, we have determined that the pH of bovine CE fluid and of CE semen is approximately 5.8, and that the motility of CE sperm in undiluted CE fluid increases as the pH is elevated. Therefore, the acidity of CE fluid may play a physiological role in the maintenance of sperm quiescence. The changes in sperm motility, in response to changes in the pH of CE fluid, are reversible and rapid. Dilution of CE fluid with buffers at either pH 5.5 or 7.6 produces a much slower initiation of motility.

In buffer a significantly lower pH is required to inhibit sperm motility than is required in CE fluid. The apparent pKs for inhibition are 5.3 in buffer and 6.6 in CE fluid. However, the motility of sperm in buffers that contain lactate, shows a pH dependence similar to sperm in CE fluid. That is, lactate inactivates sperm in buffer at pH 5.5 but not at pH 7.6. Lactate, and several other permeant weak acids, have previously been shown to reduce the intracellular pH of bovine sperm and many other types of cells. We show that these permeant weak acids, but not impermeant weak acids, reversibly reduce CE sperm motility in buffer at pH 5.5 but not at pH 7.6. Weak bases, which have previously been shown to elevate intracellular pH, initiate sperm motility in CE fluid. These results suggest that intracellular pH can regulate CE sperm motility and may be the intracellular messenger for the pH-dependent quiescene factor. Although sperm cyclic AMP levels have been previously correlated with motility stimulation, cyclic AMP levels do not change when the pH of CE fluid is elevated, even though full motility is initiated.

INTRODUCTION

Mammalian sperm from the caudal epididymis (CE) are essentially mature, in that they are capable of fertilizing eggs (Bedford, 1975; Orgebin-Christ et al., 1975) and of exhibiting progressive motility (Acott and Hoskins, 1983; Acott et al., 1983). Bovine sperm are stored in the caudal epididymis in a quiescent state (Carr and Acott, 1984; Cascieri et al., 1976), but become motile upon dilution into seminal plasma or into osmotically balanced buffers (Acott et al., 1983). The quiescence of bovine caudal sperm in CE fluid is maintained by an unidentified factor in CE fluid, which is active at pH 5.5 but is not active at pH 7.6 (Carr and Acott, 1984).

The motility of diluted caudal or ejaculated mammalian sperm is not strongly affected by pH within the range from 5.5 to 8.5 (Bishop and Walton, 1960; Chang and Thorsteinsson, 1958; Blackshaw and Emmens, 1951; Lardy and Phillips, 1943; van Duijn and Rikmenspoel, 1960). Motility is reduced by 20–100% at pH values below 6 (Blackshaw and Emmens, 1951; Chulavatnatol and Haesungcharern, 1977; Emmens, 1948; Laing, 1945; Makler et al., 1981; Peterson and Freund, 1973). We could find no reports of the effects of pH upon mammalian sperm motility in neat CE semen.

In mammals the pH of the female reproductive tract fluids varies considerably. The vagina is acidic, around pH 4.0 (Zaneveld and Chatterton, 1982), cervical mucus is basic, pH 8.4 (Lardy et al., 1940) and the uterus is intermediate, pH 7.8 (Zaneveld and Chatterton, 1982). The pH of primate oviductal fluid is around 7.1 to 7.3 in follicular phase and 7.5 to 7.8 in luteal phase (Mass et al., 1977). Therefore, it seems essential that sperm maintain good motility over a relatively wide range of extracellular pH values.

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[1] Brief accounts of a portion of this work have been presented previously (Acott and Carr, 1983; Usselman et al., 1983).

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The mammalian epididymis is reported to be relatively acidic, i.e., pH 5.5–6.8 (Jones and Glover, 1975; Levine and Kelly, 1978; Mann and Lutwak-Mann, 1981; Milovanov, 1934; Wales et al., 1966; Zaneveld and Chatterton, 1982). The acidity of the epididymis may be maintained by the sperm's metabolic activity, i.e., by the release of lactic acid, and by transport of weak electrolytes into the epididymis by the epithelial cells lining the lumen (Anderson, 1942; Berstein and Slovohotov, 1933; Jones and Glover, 1973; Malinavov, 1934; Sergin, 1935). The ratio of the volumes of epididymal to accessory gland fluids is thought to be important in determining the pH of seminal plasma (Anderson, 1942; Milovanov, 1934). However, the pH of semen is also inversely related to the concentration of sperm (Anderson, 1942; Davis and Williams, 1939; Schneerson, 1936). The pH of mammalian seminal plasma is reported to be between 6.4 and 7.8, with an average value of 7.2 (Zaneveld and Chatterton, 1982). The pH of bovine seminal plasma is reported to be 6.7 (Laing, 1945; Anderson, 1942). In several species, the fluid pH is significantly lower in the epididymis than in the testis, but is elevated again in the ejaculate (Levine and Kelly, 1978; Zaneveld and Chatterton, 1982). Levine and Kelly (1978) have postulated, based on the increased stability of sperm at slightly acidic pH (Anderson, 1942) that reduced pH aids in the maintenance of sperm viability during epididymal storage.

The roles of extracellular and intracellular pH in the initiation of motility has been studied in several invertebrates, especially sea urchins (Christen et al., 1981, 1982a,b; Lee et al., 1983; Schackmann et al., 1981). Sea urchin sperm remain quiescent in the testes for months, but become motile upon dilution into pH 8.0 seawater containing sodium ions. At pH 6.0 or in the presence of elevated potassium ion concentration, they remain quiescent. At low external pH, the addition of an egg peptide, speract, can overcome this inhibition (Garbers and Kopf, 1980; Garbers et al., 1978; Hansbrough and Garbers, 1981a,b). The initiation of motility, respiration and the acrosome reaction have been correlated with an increase in the intracellular pH (Christen et al., 1981, 1982a,b; Lee et al., 1983; Schackmann et al., 1981). This internal alkalinization appears to be due to a rapid, sodium-dependent proton efflux.

Wong et al. (1981) observed a release of acid, induced by sodium ions, that correlated with the initiation of rat CE sperm motility. The intracellular pH of washed bovine CE sperm was recently determined to be 6.5–6.6, its elevation to 6.8–6.9 was associated with increases in both motility and metabolic activity (Babcock, 1983; Babcock et al., 1983). The addition of ammonium chloride, a weak base, or the elevation of the extracellular pH to 7.5–7.8 in the presence of high potassium (100–200 mM) increased the intracellular pH and stimulated motility and metabolism. The addition of weak acids, e.g., lactic, pyruvic or β-hydroxybutyric, reduced the intracellular pH, motility and metabolism. However, the addition of fructose or the impermeant weak acid, succinate, had negligible effects. Ouabain did not inhibit the process and sodium would not substitute for potassium at high pH, suggesting that a potassium-proton exchange mechanism is active in this species. The elevation in intracellular pH did not increase the ratio of activated to inactivated cyclic AMP-dependent protein kinase and presumably, therefore did not elevate the cyclic AMP levels. This observation is in accord with the results of Garbers et al. (1982), who found that permeant weak acids and bases do not change the calcium-induced elevation of guinea pig sperm cyclic AMP levels.

We have reported studies of the regulation of bovine sperm quiescence in the cauda epididymidis and of the initiation of motility as it occurs upon ejaculation (Acott and Carr, 1983; Carr and Acott, 1984). While characterizing the quiescence induced by CE fluid, we observed that it was strongly dependent upon pH, i.e., sperm are quiescent at pH 5.5 but not at pH 7.6 (Carr and Acott, 1984). In this report we present studies intended to clarify the role of pH in the maintenance of sperm quiescence by bovine CE fluid.

MATERIALS AND METHODS

Materials

The majority of the materials and methods employed here have been described previously (Carr and Acott, 1984, ***companion paper***). The buffer, unless otherwise designated, was buffer II. Cyclic AMP was measured by radioimmunoassay using a commercial assay kit (New England Nuclear, Boston, MA); assay methods were as previously reported (Carr and Acott, 1984). Biochemicals not previously mentioned were purchased from Sigma Chemical Co., (St. Louis, MO).

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Measurements of pH

The pH values were measured with a Model 25 pH meter and a type GK2321 C combination electrode (Radiometer). This calomel-saturated electrode has combined glass and reference cells and is of the type recommended by the National Bureau of Standards (NBS). The meter and electrode were calibrated, at 23 and 37°C, with a phosphate buffer secondary standard, both concentrated and diluted 1:10, purchased from the NBS and as a generous gift from Dr. Ann Keisling. On a daily basis, standardizations were achieved with commercial buffers (Am. Sci. and Chem., Inc.; Radiometer and VWR, Inc.). Several ranges of pH papers were calibrated against meter-adjusted solutions and were used to estimate the pH of small volumes of solutions and fluids.

The pH of CE fluid, obtained by retrograde flush of the vas deferens or by micropuncture of the CE, and of seminal plasma was determined by both methods. Measurements of pH were made on CE semen; CE fluid after a gentle centrifugation, 10 min at 700 × *g*; after an additional centrifugation for 10 min at 1000 × *g*; after high-speed centrifugation at 100,000 × *g*; before and after freezing; and after various dilutions.

The pH was also measured before and after gentle degassing of the fluid. The pH of CE fluid, from a wide variety of breeds and ages of bulls, was measured over a period of approximately 1 yr. Most of the values for the pH of CE fluid were determined approximately 1 h postmortem. In one set of experiments, the measurements were completed on five epididymides within 5 min of death and were repeated on the same tissues at 5-min intervals for 30 min and again at 1 h.

These measurements were made with pH paper by extruding CE semen from small punctures of the tubules and were compared to the color guide on the package and to pH meter calibrated buffers on the same paper. We also collected CE semen in micropuncture capillaries with and without oil and extruded it directly, or after centrifugal separation of sperm and fluid, onto pH paper. The pH of incubation solutions was measured at the beginning and end of each motility experiment. In addition, those samples containing quiescent sperm were diluted and/or their pH was adjusted to check for the reversibility of the inhibition; the sperm in all samples reactivated unless specified otherwise in the text.

RESULTS

Characterization of the pH Effect - pH Profiles in Buffer and in CE Fluid

To elucidate the effects of pH upon the sperm quiescence activity of bovine CE fluid, we compared the motility of CE sperm in buffer and in CE fluid between pH 4.0 and 8.5 (Fig. 1). In buffer, lower pH's were required to depress motility, than were required in CE fluid. The apparent pKs for the response of motility to changes in pH, were 5.25 and 6.55 for buffer and CE fluid, respectively. The inhibition that was observed in CE fluid was completely reversible. By contrast, in buffer below pH 5.0, the inhibition became increasingly irreversible with longer incubation times (data not shown).

Measurements of the pH of Epididymal Fluid and Semen

The possible physiological relevance of CE fluid pH was studied by measuring the pH of bull CE fluid and seminal plasma. The pH of bovine seminal plasma is approximately 6.8, as reported previously (Zaneveld and Chatterton, 1982). The pH of neat CE semen using pH paper or the pH meter was 5.77 + 0.13 (n=48); the pH of CE fluid was 5.86 + 0.10 (n=110). These measurements were made from 30 min to several hours postmortem. The pH of CE semen, measured less than 5 min postmortem, was 5.88 + 0.13 (triplicate measurements on five separate animals). No changes were detected in the pH of CE semen for up to 6 h postmortem. The pH was not significantly different for samples protected from air, degassed or after prolonged exposure to air.

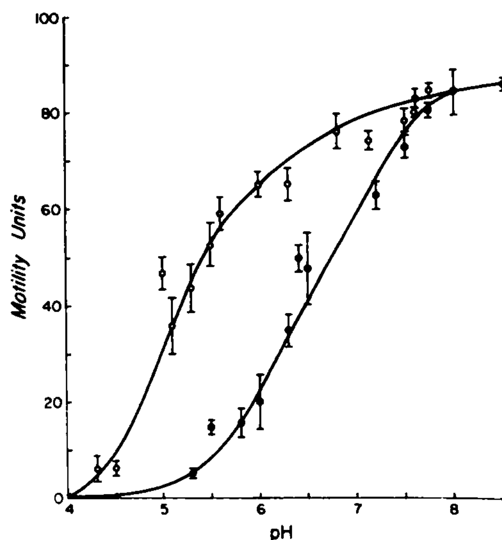

FIG. 1. Dependence of caudal sperm motility on pH in buffer and in neat caudal fluid. Caudal sperm were diluted (1:100) into buffer *(open circles)* or neat caudal fluid *(closed circles)*, both of which had been adjusted to the indicated pH. Motility was assessed after a 20-min incubation and pH was measured before and after the experiment and was invariant to 0.1 unit. Values are means (± SEM) of triplicate determinations in five separate experiments.

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Time Course of Changes in Motility Following Changes in the pH of CE Fluid

Changes in the pH of CE fluid produced rapid changes in motility with half-times of less than 20 sec (Fig. 2A). In contrast, the rate of initiation of motility in response to dilution at either pH 5.5 or 7.6, has a half-time of from 2 to 5 min (Fig. 2B). The inhibition of motility that results from resuspension of motile sperm in CE fluid is also rapid, with a half-time of less than 20 sec (Carr and Acott, 1984). These short half-times are approximate; rapid mixing devices would be required to obtain more precise values. These experiments also demonstrated that both the initiation of motility and the induction of quiescence by alteration of the pH of CE fluid were reversible.

Dependence of Motility Upon CE Fluid Concentration

The effect of CE fluid concentration on CE sperm motility at pH 7.6 and at pH 6.0 was determined after a 20-min incubation; the results are shown in Fig. 3. At pH 6.0, decreasing the concentration of CE fluid from 100% to 0% allowed an increase in motility to over 70 motility units. At pH 7.6, the motility measured was between 82 and 85 motility units, and was independent of the concentration of CE fluid.

Inhibition of Sperm Motility in Buffer by Weak Acids

The intracellular pH of bovine CE sperm in buffer is reduced by incubation with permeant weak acids (Babcock, 1983; Babcock et al., 1983). To determine the effect of weak acids upon motility under our conditions, CE sperm were first diluted into buffer to initiate motility. They were then incubated at pH 5.5 (Fig. 4, *open bars*), in buffer containing 0, 1, 5, 10 or 50 mM L-lactate or pyruvate (both permeant acids), or containing 0 or 50 mM L-glutamate (an impermeant acid). The *hatched bars* in Fig. 4 show the motility that was observed when

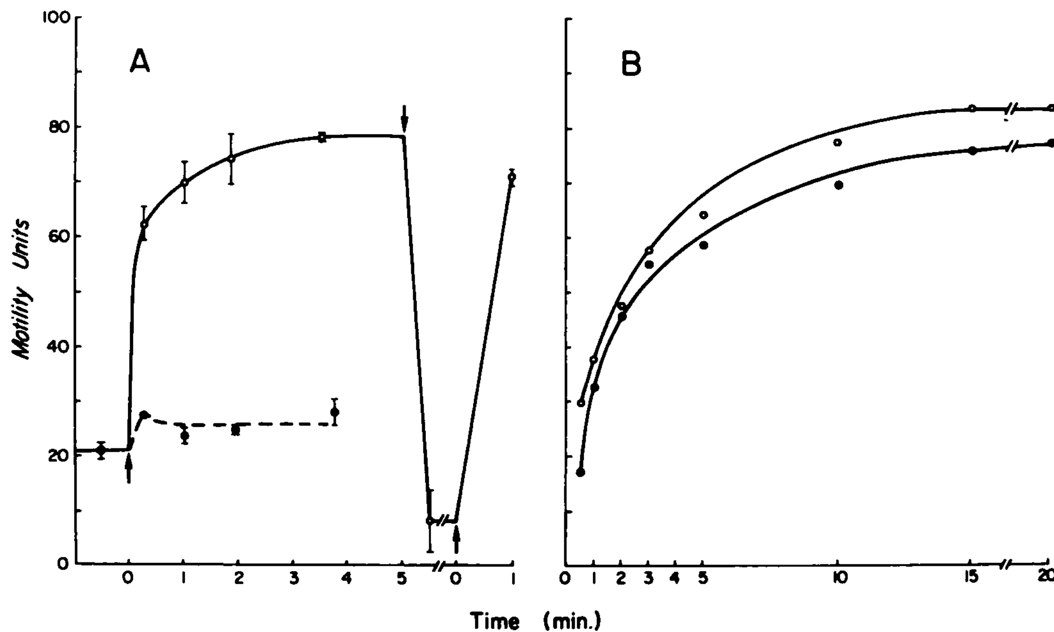

FIG. 2. Time courses of motility initiation after changes in pH. *A)* Neat caudal sperm were diluted (1:100) into neat caudal fluid and motility assessed in aliquots removed at the times indicated. At the time marked by the *first arrow*, NaOH *(open circles)* or NaCl *(closed circles)* was added. The NaOH concentration was selected to elevate the pH to 7.5; an equivalent amount of NaCl was added as a control. At the time indicated by the *second arrow*, sufficient HCl was added to return the caudal fluid to its original pH, 5.5. After the motility had stabilized, NaOH was again added *(third arrow)* and the response to increased extracellular pH was assessed. The pH was measured before and after each addition and was within 0.1 units. *B)* Neat caudal sperm were diluted 1:100 into buffer at pH 7.6 *(open circles)* or at pH 5.5 *(closed circles)* and aliquots removed for motility assessment at the times indicated. All assessments were completed within 20 sec of the time of removal. Values are means (± SEM) of triplicate determinations in two separate experiments.

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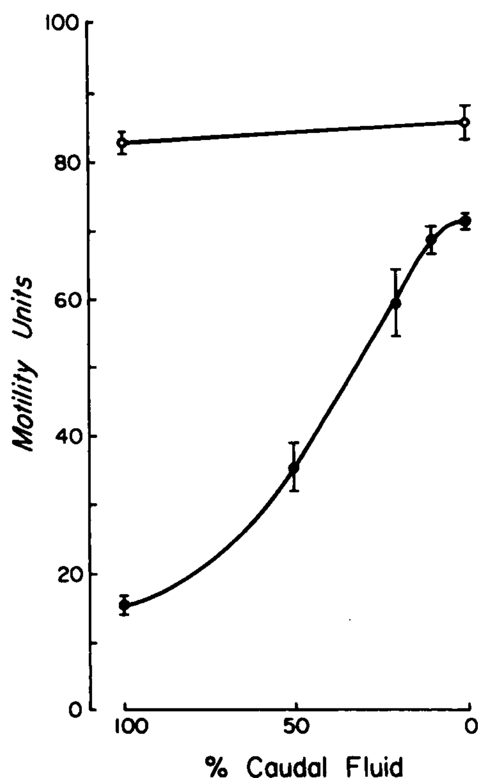

FIG. 3. Dependency of caudal sperm motility on the concentration of caudal fluid at pH 6.0 and 7.6. Caudal sperm were diluted 1:100 into various concentrations of caudal fluid diluted with buffer at pH 6.0 *(closed circles)* or pH 7.6 *(open circles)*. Aliquots were removed for motility assays at 20 min. The pH of all test solutions was measured before and after the experiment and was invariant to 0.1 unit. Values are means (± SEM) of triplicate determinations from two experiments.

sperm were incubated at pH 7.6 in 0 or 50 mM L-lactate (*L*), pyruvate (*P*) or L-glutamate (*G*). Both permeant weak acids reduced sperm motility in a dose-dependent manner at pH 5.5, while the impermeant acid did not. At pH 7.6, none of the acids inhibited motility. At very high concentrations, 50 mM and above, the permeant acids produced some loss of reversibility after prolonged exposure at pH 5.5.

Several weak acids were tested for effects on motility at their optimum reversible concentrations, in buffer at pH 5.5 (Fig. 5, *hatched bars*). The pH of each incubation solution was then raised to 7.6 (Fig. 5, *open bars*) for a second assessment of motility. The test solutions were CE fluid (CF), buffer (MES), 10 mM L-lactate, 10 mM D-lactate, 10 mM pyruvate, 5 mM propionate, 10 mM β-hydroxybutyrate, 50 mM L-glutamate and 2.5 mM succinate. All of the permeant acids inhibited motility at pH 5.5 but not at pH 7.6. The impermeant acids were ineffectual at either pH.

Initiation of Motility in CE Fluid by Weak Bases

Several weak bases were tested for their ability to initiate motility of quiescent sperm in CE fluid (Fig. 6). Ammonium chloride induced a marginally significant doubling of motility ($P<0.054$). Methylamine and dimethylamine caused approximately 2.8-fold increases in motility ($P<0.011$ and 0.005, respectively). Trimethylamine caused a doubling of motility that was not statistically significant ($P<0.212$). Even at concentrations of 50 mM, none of the weak bases were able to induce normal full motility. Interestingly, the percent motility was relatively high and the vigor low in these experiments. Concentration curves for the effects of all of these weak bases on motility in CE fluid were bell-shaped, with maxima near 50 mM (data not shown).

Time Course of Cyclic AMP Levels in Response to pH Elevation

Sperm cyclic AMP levels and motility were measured at several time intervals following the

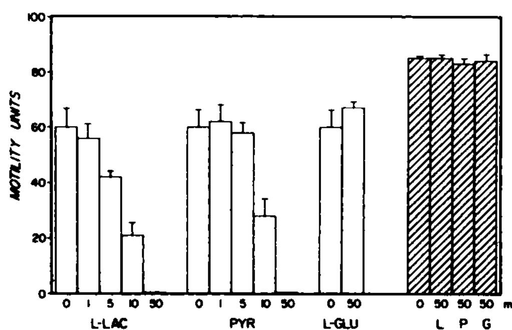

FIG. 4. Effects of weak acids on the motility of CE sperm in assay buffer. Caudal sperm were diluted 1:100 in buffer II at pH 5.5 *(open bars)* containing 0, 1, 5, 10, and 50 mM L-lactic acid; 0, 1, 5, 10 and 50 mM pyruvic acid or 0 and 50 mM L-glutamic acid. Sperm were diluted similarly into buffer II at pH 7.6 *(hatched bars)* containing no additions; 50 mM L-lactic acid (*L*); 50 mM pyruvate (*P*) or 50 mM L-glutamic acid (*G*). Aliquots were removed and assayed after 20 min. Values are means (± SEM) of triplicate determinations from two experiments. The pH of each solution was tested after the experiment and was invariant to 0.1 unit.

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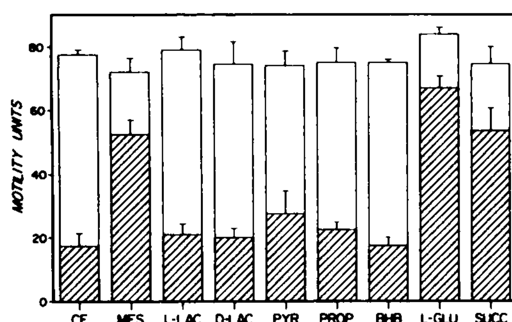

FIG. 5. Effects of weak acids on sperm motility at pH 5.5 and 7.6. Several weak acids were tested to determine their optimum concentration for inhibition of motility at pH 5.5 *(batched bars)* and for reversibility when the pH was elevated to pH 7.5 *(open bars)*. The outcome of these experiments is summarized. CE sperm were diluted into CE fluid *(CF)* or buffer II containing no addition *(MES)*, 10 mM L-lactate, 10 mM D-lactate, 10 mM pyruvate, 5 mM propionate, 10 mM β-hydroxybutyrate, 50 mM L-glutamate or 2.5 mM succinate. Values are means (± SEM) of triplicate determinations in two experiments. The pH was measured before and after addition of NaOH and was within 0.1 unit.

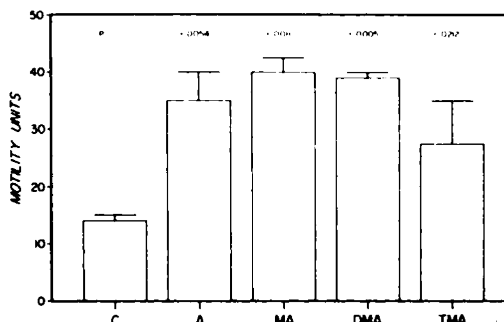

FIG. 6. Effects of addition of weak bases on sperm motility in CE fluid. Sperm were diluted 1:100 in CE fluid containing no addition and serving as a control *(C)*, or 50 mM ammonium chloride *(A)*, methylamine *(MA)*, dimethylamine *(DMA)* or trimethylamine *(TMA)*. Motility was assessed after 20 min at 37°C. Values are means (± SEM) of triplicate determinations from five experiments. The pH was measured before and after the experiment and was 5.8 ± 0.1 in all cases.

elevation of the extracellular pH of CE fluid to 7.6 (Fig. 7). Sodium hydroxide, sufficient to produce a final pH of 7.6, was added to sperm in CE fluid and aliquots removed for assay at the times indicated. An equivalent amount of sodium chloride was added to controls. The increase in the extracellular pH initiated full motility without a significant change in the sperm cyclic AMP levels. Controls showed no change in motility or cyclic AMP.

In this same set of experiments, 3-isobutylmethylxanthine (1 mM) was added to sperm in CE fluid, and the cyclic AMP was measured at the same time intervals and conditions as in the previous experiment. The cyclic AMP concentrations (data not shown), were essentially the same as those shown in Fig. 7. Although motility initiation occurs, (e.g., see Fig. 6, Carr and Acott, 1984, *companion paper*), the sperm cyclic AMP concentration did not change significantly. The extracellular pH, measured before and after the addition of the phosphodiesterase inhibitor was invariant to 0.1 pH units.

DISCUSSION

Bovine sperm are maintained in a quiescent state in the caudal epididymis. The pH of bovine CE fluid is 5.8, while the pH of seminal plasma is 6.8; elevation of the pH or dilution of CE fluid allows the initiation of motility. The apparent pK of serum motility, as a function of the pH of the medium is 6.6 in CE fluid and

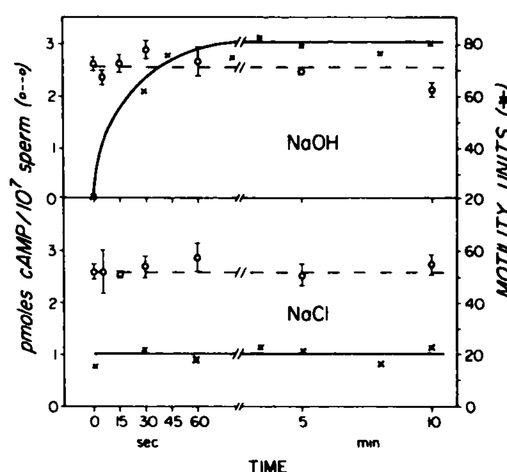

FIG. 7. Effects of elevation in pH on the motility and cyclic AMP levels of sperm in CE fluid. CE sperm were diluted 1:100 into parallel tubes containing CE fluid and the motility was assessed. Sodium hydroxide or sodium chloride was added and the reaction terminated with TCA at the times indicated for cyclic AMP assay as described in *Materials and Methods* and previously (Carr and Acott, 1984, companion paper). Motilities were assessed in other parallel tubes. Cyclic AMP values are means (± SEM) of triplicate cyclic AMP determinations of triplicate tubes from two experiments. The pH was measured before and after each addition and was within 0.1 unit.

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5.3 in assay buffer. These data, and those from the preceding paper (Carr and Acott, 1984), are best explained by the presence, in bovine CE fluid, of a pH-dependent inhibitor of sperm motility. This inhibitor is active at the physiological pH of CE fluid, i.e., 5.8, and inactive at the pH of seminal plasma. The effect of pH is not directly on the sperm, but is mediated by this quiescence factor, or activity, in CE fluid. Dilution of the CE fluid at either pH allows motility initiation. Therefore, in the bull there are two ways of initiating motility: *1)* by dilution, and *2)* by elevation of the pH of CE fluid. In the normal course of events both would occur, simultaneously, upon ejaculation.

The pH of bovine CE fluid was reported to be 6.8 ± 0.4 (n=3) by Wales et al. (1966). These authors did not mention any details of the pH measurement and the emphasis of the paper was the measurement of ionic and organic components of the fluid. Our pH measurements were made at time intervals from 5 min to several hours postmortem by two independent methods. Our electrode, meter and calibration followed NBS recommendations and we used NBS standard buffers. We measured large numbers of samples under a variety of conditions without cooling the sperm, centrifugation or freezing the fluid and with attention to gas uptake by the fluid. Our value for CE fluid is approximately one pH unit lower than theirs.

The time required for the initiation of motility after elevation of the pH of CE fluid is short, compared with the time required for the initiation of motility in response to dilution at pH 5.5 or 7.5 (Fig. 2A and B). This time difference implies that CE fluid contains a factor(s) that mediates both the quiescence and the initiation of motility in response to changes in CE fluid pH. Dilution of CE fluid may allow a rapid dissociation of the factor from the sperm, therefore it could not mediate the response to changes in the pH of the media. This rapid response is also observed when motile sperm are resuspended in CE fluid (Fig. 3, Carr and Acott, 1984, companion paper). We have insufficient data, at this time, to fully interpret these time courses. It is clear that the mechanism is not simple. We speculate that some factor in CE fluid interacts with the sperm membrane to modulate a transport event, e.g., proton efflux. Other mechanisms are also possible.

The inhibition of sperm motility in buffer, containing a variety of permeant weak acids (Figs. 4 and 5), appears to be due to a reduction of the intrasperm pH. This possibility is supported by previous measurements of the intracellular pH of bovine CE sperm that were exposed to weak acids and bases (Babcock, 1983; Babcock et al., 1983). Our experiments were designed to match, as closely as possible, the conditions that Babcock used in this study. The inability of impermeant weak acids to inhibit motility and the lack of stereospecificity seen with lactate, i.e., the metabolically inactive form is as effective as the metabolically active form, corroborate this conclusion. The loss of inhibition at pH 7.6 could be due to the reduction in the concentration of the associated form of the acids. The reduction of intracellular pH by weak acids is based on the ability of the uncharged, associated form of the acid to enter the cells and then dissociate, releasing a proton. The extracellular pH change from 5.5 to 7.5 would be expected to reduce the concentration of the associated form of the acid by approximately 99%. This low level of the associated form of the acid would not be inhibitory (Fig. 4).

The initiation of sperm motility in CE fluid, induced by the addition of weak bases, appears to involve an elevation of the intracellular pH. Permeant weak bases are able to cross the plasma membrane and to elevate the intracellular pH by absorbing a proton. The weak bases that we and others have used have pH above 9.0. Since CE fluid is only inhibitory at low pHs, the concentration of the active form of the bases was very low; it is not surprising that they were unable to initiate full motility. The percent of motility approaches that seen in other experiments, although the vigor is relatively low, e.g., 50% motile with vigors from 2–3. This is interesting, but we can only speculate on its meaning. Possibly the intracellular pH is elevated in most of the sperm, but not enough to produce full motility.

Taken together, our evidence and the measurements of the intracellular pH changes in bull sperm in response to the addition of weak acids and bases (Babcock, 1983; Babcock et al., 1983), indicate that the intracellular pH can regulate bovine sperm motility in buffer and in CE fluid. The physiological maintenance of sperm quiescence in CE fluid by the quiescence factor is likely to involve regulation of the intracellular pH. The utilization of weak bases and acids to manipulate intracellular pH is not without complications (Babcock, 1983; Babcock et al., 1983; Boron, 1983; Roos and Boron,

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1981). We have not measured the intracellular pH of bovine sperm in CE fluid in response to elevations of the extracellular pH or dilution. This will be necessary before we can make stronger conclusions about the role of intracellular pH in the physiological initiation of motility.

We have not attempted to unravel the involvement of bicarbonate and carbon dioxide in this process, because of the complexity of their interactions with the intracellular pH. Instead, we have attempted to control for this variable by saturation of the CE fluid and buffers with oxygen and nitrogen and by degassing them. Bovine CE fluid contains no measurable bicarbonate (Zaneveld and Chatterton, 1982). Incubation of sperm in CE fluid with gentle shaking does not result in the initiation of motility, although some carbon dioxide is undoubtedly absorbed; the pH is also unchanged after 1 h. The same results are obtained for CE semen diluted under oil with degassed CE fluid or buffers as are obtained for air-equilibrated solutions. The addition of bicarbonate or carbon dioxide to the various incubation solutions that we have used produces variable, biphasic responses in motility and pH with time and bicarbonate concentration (data not shown). Simultaneous and continuous measurement of the intracellular pH and motility will be required to interpret these effects. This modulation is of potential physiological importance and deserves study in the future.

Another acknowledged regulator of sperm motility is cyclic AMP. The data in Fig. 7 show an uncoupling of sperm cyclic AMP levels and the initiation of sperm motility by the elevation of CE fluid pH. We have also reported that the initiation of motility by dilution of CE fluid does not involve an elevation of sperm cyclic AMP levels (Carr and Acott, 1984, *companion paper*). Several reports of the uncoupling of motility and cyclic AMP levels have been published (Amann et al., 1982; Babcock et al., 1983; Hammerstedt and Hay, 1980). The simplest explanation of our data that is consistent with other observations in the literature (for review see Garbers and Kopf, 1980), is that cyclic AMP and the quiescence factor both regulate sperm motility. During maturation of sperm in the epididymis, cyclic AMP is elevated to a level that is permissively high for motility. Subsequent removal or inactivation of the quiescence factor initiates motility without a further increase in sperm cyclic AMP levels.

The ability of the cyclic AMP phosphodiesterase inhibitor, 3-isobutyl-methylxanthine, to initiate motility without producing an elevation in cyclic AMP concentrations, may be explained by the fact that it is also a weak base. Vijayaraghavan et al. (1983) have postulated that these inhibitors can induce motility by acting as weak bases and elevating the intracellular pH of bovine caput sperm. Our data clearly corroborate this possibility, although we cannot eliminate other explanations. They also reported that 8-bromo-cyclic AMP is unable to stimulate motility in bovine caput serum in the absence of bicarbonate. We reported that dibutyryl cyclic AMP is not able to initiate CE sperm motility in CE fluid (Carr and Acott, 1984). This is consistent with our postulate that cyclic AMP is already permissively high in CE sperm. They concluded that an elevation in cyclic AMP, which occurs during epididymal maturation (Hoskins and Casillas, 1975), and a subsequent increase in intracellular pH are required for the induction of caput sperm motility. Our observations are best explained by this dual regulation of CE sperm motility.

We are currently characterizing the pH-dependent quiescence factor that is present in CE fluid. The CE fluid factor behaves analogously to weak acids in buffer, and it is possible that CE sperm regulate themselves with their own waste products, i.e., lactate, thereby conserving energy for future need. Lactate concentrations are relatively high in bovine CE fluid. i.e., 4–5 mM (Wales et al., 1966). However, the time courses that we observed suggest a more complex mechanism, involving more than simply a build-up of lactate.

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