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Strontium Supports Capacitation and the Acrosome Reaction in Mouse Sperm and Rapidly Activates Mouse Eggs

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Extracellular Ca^{2+} is required for capacitation and fertilization in the mouse, but very little is known about the ability of other divalent cations to substitute for Ca^{2+}. In this study, Sr^{2+}, Ba^{2+}, and Mg^{2+} were evaluated for their ability to support capacitation, the acrosome reaction, hyperactivated motility, and fertilization. Ba^{2+} proved to be ineffective, but Mg^{2+}-containing medium was able to support capacitation to a greater extent than unsupplemented Ca^{2+}-deficient media; despite this, Ca^{2+} was required for fertilization. In contrast, Sr^{2+} proved capable of substituting for Ca^{2+} in all events . Furthermore, Sr^{2+}-induced responses were indistinguishable from the corresponding Ca^{2+}-induced ones: Sperm capacitated at the same rate and underwent the acrosome reaction to the same extent. However, demonstration of sperm:egg fusion in Sr^{2+} required the use of zona-free eggs. This was due not to the inability of the sperm to penetrate the zona but to the very rapid activation and cortical granule release by eggs in response to Sr^{2+}. When zona-intact eggs were used, the block to polyspermy had been mounted by the time sperm had penetrated the zona. A 15 min exposure to Sr^{2+} was sufficient to block sperm fusion, but a longer exposure was required to ensure the resumption of meiosis in eggs; such a response was surprising in that the eggs were freshly ovulated and not susceptible to activation by many different treatments. Thus Sr^{2+} can profoundly affect both gametes in the mouse: It substitutes completely for Ca^{2+} in sperm responses and rapidly activates eggs, possibly by displacing Ca^{2+} from intracellular stores into the cytoplasm, where the Ca^{2+} can then trigger the various events of activation.

Key words: Sr^{2+}, Ca^{2+}, gamete fusion, egg activation

INTRODUCTION

A requirement for extracellular calcium ions to support successful fertilization has long been recognized in both invertebrates [Loeb, 1915] and mammals [mouse: Iwamatsu and Chang, 1971]. Specifically, a Ca^{2+} influx is required to trigger the acrosome reaction, which leads to release of lytic enzymes and membrane alterations required for interaction with the egg in all species examined [reviewed by Fraser,

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1987]. However, mammalian sperm present a more complicated system; unlike invertebrate sperm, they are not immediately able to fertilize eggs but must undergo a further period of postrelease maturation [Austin, 1951; Chang, 1951]. This has become known as capacitation, and the length of time required to achieve full functional competence appears to be species-specific [Bedford, 1970]. Only upon completion of capacitation can mammalian sperm undergo the acrosome reaction and express the characteristic hyperactivated motility pattern, both of which are required for successful fertilization [Yanagimachi, 1981; Bedford, 1983; Fraser, 1984]. The possibility that extracellular Ca^{2+} is also required to support capacitation has now been examined fairly rigorously in three species. In the guinea pig, there appears to be no such requirement; after incubation of sperm in calcium-deficient medium for a time sufficiently long to support capacitation in complete medium, the introduction of extracellular Ca^{2+} rapidly promotes the acrosome reaction and fertilization [Yanagimachi and Usui, 1974]. In contrast, similar treatments of hamster [Yanagimachi, 1982] and mouse [Fraser, 1982] sperm have shown them not to be fully functional immediately. Mouse sperm, for example, require exposure to extracellular Ca^{2+} for about 1 hr in order to be completely capacitated [Fraser, 1982].

The possibility that other divalent cations are able to substitute for Ca^{2+} during some or all the steps leading up to sperm:egg fusion has been examined to a limited extent in several species. It has been reported that Sr^{2+} was effective in supporting both the acrosome reaction and hyperactivated motility in guinea pig sperm [Yanagimachi and Usui, 1974], hyperactivated motility in mouse sperm [Cooper, 1984], and sperm:egg fusion in the hamster [Yanagimachi, 1978]. There have also been recent reports that human sperm preincubated in Sr^{2+} medium are more successful than their Ca^{2+}-treated counterparts in fertilizing zona-free hamster eggs in the presence of Ca^{2+} [Mortimer, 1986; Mortimer et al, 1986]. However, complete evaluation of a divalent cation such as Sr^{2+} as a substitute for Ca^{2+} has yet to be reported. The present study was undertaken to do this, and, while Sr^{2+}, Ba^{2+}, and Mg^{2+} were initially studied, attention was soon focussed on Sr^{2+} because it presented the most intriguing features.

MATERIALS AND METHODS

Media

The basic medium was a modified Tyrode's medium lacking pyruvate and lactate and containing glucose and 1.8 mM $CaCl_2$ [Fraser, 1983b]. Where noted, the composition was altered so that $CaCl_2$ was replaced by 1.8 mM $SrCl_2$, $MgCl_2$, or $BaCl_2$. Calcium-deficient medium was prepared by omitting $CaCl_2$; this medium supports neither full capacitation nor fertilization in vitro in the mouse [Fraser, 1982]. All media contained crystalline BSA (Sigma, Poole, Dorset, United Kingdom) at 4 mg/ml.

In Vitro Fertilization

Sperm suspensions were prepared by releasing the equivalent of two cauda epididymides from mature male TO mice into 1 ml medium. When several media were evaluated simultaneously, half the contents of one epididymis from each of two males were released into 0.5 ml of each medium. Suspensions were preincubated for a total of 120 min, with samples being removed at 30 and/or 120 min for assessment

of fertilizing ability. These aliquots were diluted approximately tenfold to yield a final concentration of 1–2 $\times$ 10^6 sperm/ml; droplets of 300 μl were prepared and eggs were added.

Mature female TO mice were induced to superovulate with 7.5 IU PMSG (Intervet Laboratories, Cambridge, United Kingdom) followed 54 hr later by 5 IU hCG (Pregnyl; Organon, Morden, Surrey, United Kingdom). Fourteen hours after hCG, eggs were released from oviducts directly into sperm suspensions. When appropriate, hCG was injected asynchronously to ensure that only freshly ovulated eggs would be used; in TO mice, ovulation has just been completed by 14 hr following the hCG injection [Fraser, 1979].

All incubations were carried out at 37°C in 30 mm plastic culture dishes (Sterilin; Teddington, Middlesex, United Kingdom); droplets of medium were overlaid with autoclaved liquid paraffin (Boots, Nottingham, United Kingdom). The gas mixture used for equilibration of media and incubation of gametes was 5% CO_2: 5% O_2:90% N_2.

Assessments

The majority of eggs were transferred from the sperm suspensions to droplets of fresh medium at 65 min after gamete mixing and fixed at 75 min by adding excess neutral buffered formalin. After staining and mounting, eggs were assessed for fertilization [Fraser, 1983a]. Eggs were considered to be fertilized if they had resumed the second meiotic division and contained a fertilizing sperm head. The proportion of eggs at telophase II with a fully decondensed sperm head, indicating rapid sperm penetration, was calculated. Eggs were considered to be activated if they had resumed meiosis but lacked a fertilizing sperm head.

In some experiments using sperm suspensions preincubated in Sr^{2+} medium, eggs were also removed at 15 min after mixing. These were transferred immediately to Ca^{2+} medium with hyaluronidase to remove cumulus cells and excess sperm; they were washed, placed in a droplet of Ca^{2+} medium, and incubated until 75 min after mixing, when they were fixed as above.

Acrosome loss was assessed in sperm suspensions that were filtered through short columns of Sephadex G-25 in Pasteur pipettes [Fraser, 1985] to select motile cells. The eluted sperm were fixed in formalin; slides were prepared [Fraser, 1983b], and at least 100 sperm in each sample were assessed for the presence or absence of the acrosome.

To evaluate cortical granule loss in response to Sr^{2+}, cumulus-intact eggs were added to a droplet of Sr^{2+} medium and incubated for 35 min. Eggs were transferred to a Ca^{2+}-containing hyaluronidase solution for cumulus cell removal, washed briefly, and fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4. After postfixation in 1% osmium tetroxide and dehydration in graded alcohols, eggs were embedded in Epon resin. Thin sections were stained with uranyl acetate/lead citrate and examined in a JEOL electron microscope. Hyperactivated motility was assessed in diluted suspensions. When appropriate, data were analyzed using Cochran's test for the combination of 2 $\times$ 2 contingency tables [Snedecor and Cochran, 1967].

RESULTS

Series Ia: Effect of Sr^{2+} vs Ca^{2+} on Capacitation and Fertilization

Zona-intact eggs. Sperm suspensions were prepared from the same males in medium containing either Ca^{2+} or Sr^{2+} and also in Ca^{2+}-deficient medium. At both

30 and 120 min after release of sperm, aliquots were removed and diluted as detailed below and eggs were added. Each suspension was diluted into Ca^{2+}-containing medium and a second aliquot of the Sr^{2+} suspension was diluted into Sr^{2+} medium (N = 3).

Results from this series are presented in Table 1. After 30 min preincubation in either Sr^{2+} or Ca^{2+} and assessment in Ca^{2+}, a mean of 60–70% of eggs were fertilized, and no significant differences in fertilizing ability could be detected between the groups. The large standard errors indicate the variability in fertilizing ability after a short preincubation; in essence, sperm are only partially capacitated. There was no indication that Sr^{2+} either inhibits or accelerates capacitation relative to Ca^{2+}. That it can substitute for Ca^{2+}, however, is emphasized by the much lower fertilization rate (2%) obtained with suspensions preincubated in Ca^{2+}-deficient medium and assessed in the presence of Ca^{2+}. A surprising observation was made in the suspensions maintained throughout in Sr^{2+}, namely, the spontaneous activation of approximately half the eggs, with very few of the remaining eggs possessing fertilizating sperm.

Only half or fewer of fertilized eggs in all groups had reached telophase II with fully decondensed sperm heads (data not shown), indicating that sperm penetration did not begin immediately upon gamete mixing. This is consistent with the profiles observed when partially capacitated sperm are used [eg, Fraser, 1983b]. In contrast, most of the activated eggs had reached telophase II, suggesting that the response to Sr^{2+} was a rapid one. In all experimental series this activation produced haploid eggs.

After 120 min preincubation in Sr^{2+} or Ca^{2+}, again no significant differences in fertilizing ability could be detected when sperm were assessed in the presence of Ca^{2+}. Over 90% of eggs were fertilized (Table 1), with 98–100% of these having reached telophase II and possessing fully decondensed sperm heads (data not shown). Thus Sr^{2+} was able to substitute for Ca^{2+} during capacitation. In contrast, relatively few eggs (17%) were fertilized by suspensions preincubated in Ca^{2+}-deficient medium and assessed in the presence of Ca^{2+}; none of these had advanced stages of nuclear development.

Hyperactivated motility was observed in all suspensions, whether incubated in the presence of Ca^{2+} or Sr^{2+}. These observations are consistent with those of Cooper [1984].

Again, a high proportion of eggs incubated with Sr^{2+} sperm suspensions had undergone rapid, spontaneous activation (Table 1) as evidenced by the prevalence of the telophase II stage (data not shown). Although only 12% of eggs were fertilized, careful examination prior to fixation revealed that almost all eggs had several to many sperm actively swimming in the perivitelline space. This was equally true for eggs possessing an obvious second polar body (hence either activated or fertilized) and for those lacking it (eventually assessed as being unfertilized). Thus Sr^{2+} appeared able to support both capacitation and zona penetration, but the support of sperm:egg fusion was still unproved.

Zona-free eggs. To discriminate between the possibilities either that Sr^{2+} failed to substitute for Ca^{2+} during gamete fusion or that egg activation was simply so rapid that, by the time sperm had penetrated the zona, the cortical reaction had occurred zona-free eggs were used. Sperm suspensions were preincubated for 120 min in Sr^{2+} and diluted in Sr^{2+}; then zona-free eggs (prepared by sequential treatment with

TABLE 1. Fertilizing Ability of Mouse Sperm Preincubated in Ca^{2+}-Deficient, Ca^{2+}-Containing, or Sr^{2+}-Containing Medium and Assessed in the Presence of Ca^{2+} or Sr^{2+}

Length of pre-incubation (min)	Medium		Eggs		
	Preincubation	Fertilization	Total No.	Fertilized[a]	Activated[a]
30	Ca^{2+}	Ca^{2+}	109	60.3 ± 27.5	0
	Sr^{2+}	Sr^{2+}	92	2.0 ± 1.4	57.3 ± 17.8
	Sr^{2+}	Ca^{2+}	112	70.7 ± 20.3	0
	0	Ca^{2+}	62	2.0 ± 2.8	0
120	Ca^{2+}	Ca^{2+}	99	96.3 ± 2.9	0
	Sr^{2+}	Sr^{2+}	73	12.3 ± 0.3	55.3 ± 18.1
	Sr^{2+}	Ca^{2+}	74	91.0 ± 6.7	0
	0	Ca^{2+}	60	16.6 ± 14.0	0
Zona-free eggs	Sr^{2+}	Sr^{2+}	37	97.0 ± 4.2	0

[a]Mean % ± SEM.

hyaluronidase and pronase as described in Fraser [1983a] using Ca^{2+}-deficient medium) were added (N = 2). Evaluation at 75 min indicated that essentially all were fertilized (97%; Table 1) with fully decondensed fertilizing sperm heads, and a mean of 44% were polyspermic (data not shown). Therefore, Sr^{2+} can support sperm:egg fusion as well as the preceding events in gamete interaction.

Series Ib: Effect of Ba^{2+} and Mg^{2+} on Capacitation and Fertilization

As part of Series I, sperm suspensions were also prepared in medium containing Ba^{2+} or Mg^{2+} to allow comparisons with Ca^{2+} and Sr^{2+}. Suspensions were preincubated for 120 min and then assessed in Ba^{2+} or Mg^{2+} (N = 1) or in Ca^{2+} (N = 3).

Results obtained with these divalent cations differed from those obtained with Sr^{2+}. When evaluated in Ca^{2+}, the Ba^{2+}-preincubated suspensions proved to be poorly fertile, with 28.0% ± 19.2% of eggs fertilized. These results did not differ significantly from those obtained with sperm preincubated in Ca^{2+}-deficient medium. In contrast, suspensions preincubated in Mg^{2+} were consistently more fertile than those exposed to Ba^{2+}, with a mean of 52.3% ± 19.0% of eggs fertilized. When compared with the 16.6% ± 14.0% obtained when sperm from the same males were preincubated in Ca^{2+}-deficient medium, these significantly different ($P < 0.05$) results suggest that the presence of Mg^{2+} promotes more advanced stages of capacitation than does the virtual absence of divalent cations. In the one replicate when sperm were evaluated in the presence of Ba^{2+} or Mg^{2+}, no eggs were fertilized but four of 22 (18%) eggs in the Ba^{2+} group were activated.

Motility was somewhat reduced (about 10% fewer motile cells) in suspensions preincubated in Ba^{2+} or Mg^{2+} compared with those in Ca^{2+} or Sr^{2+}. Even after dilution into Ca^{2+}, sperm exhibited erratic movements rather than the flowing pattern observed in controls. Hyperactivated motility was observed in some sperm in the Mg^{2+}-treated suspensions (fewer than Ca^{2+} or Sr^{2+}), but it was rarely observed in the Ba^{2+}-treated sperm.

Series II: Acrosome Loss in the Presence of Ca^{2+}, Sr^{2+}, Mg^{2+}, or Ba^{2+}

Sperm suspensions were prepared from the same males in media containing either Ca^{2+}, Sr^{2+}, Mg^{2+}, or Ba^{2+} and incubated for 120 min. They were then filtered as described above and assessed (N = 4). In two of the replicates, the effect

of diluting the Mg^{2+} suspensions into Ca^{2+} and assessing after 10 min was also examined.

Results were consistent with the fertilization data obtained in Series I. A high proportion of sperm incubated in either Ca^{2+} or Sr^{2+} had undergone the acrosome reaction (means of 35.5% and 34.8%, respectively; Fig. 1), whereas very few had done so in Mg^{2+} or Ba^{2+} (6.0% and 4.3%, respectively). Adding Ca^{2+} to the Mg^{2+} -preincubated sperm did not trigger an increased response within 10 min (7.5%; data not shown), which suggests that these sperm were not yet fully capacitated.

Series III: Rapidity of the Activation Response in Eggs Exposed to Sr^{2+}

It was evident from results in Series I that the freshly ovulated eggs underwent activation in less 75 min. Sperm were clearly visible in the perivitelline space, and this would be expected to occur from about 25–30 min onward [Fraser, 1983a]. To examine this more carefully, eggs were added to 120 min-preincubated Sr^{2+} sperm suspensions. Approximately half were removed at 15 min, manipulated in Ca^{2+} medium to remove cumulus cells and excess sperm as described above, and incubated in Ca^{2+} medium; the other half were allowed to remain in the Sr^{2+} sperm suspension. At 75 min, both were fixed and assessed for activation/fertilization. At the same time, cumulus-intact eggs were incubated for 75 min in sperm-free Sr^{2+} medium, fixed, and assessed. For comparison, some were also incubated in Ca^{2+} -deficient and Ca^{2+} plus Sr^{2+} media.

Results are presented in Figure 2. Data indicate that most eggs incubated in Sr^{2+} for 15 min were either unfertilized (mean of 60%) or activated (36%). The effect of lengthening exposure to Sr^{2+} resulted in more eggs being activated, ie, reaching telophase II (54%), than remaining unactivated, ie, still at metaphase II with no sign of sperm incorporation (31%). Again, almost all these eggs, whether activated

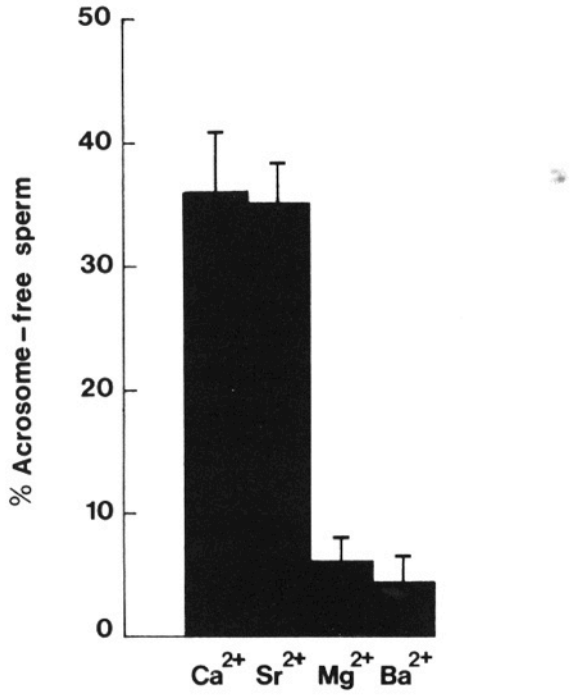

Fig. 1. Acrosome loss in mouse sperm suspensions incubated for 120 min in media containing 1.8 mM Ca^{2+}, Sr^{2+}, Mg^{2+}, or Ba^{2+}. Data are presented as mean % ± SEM.

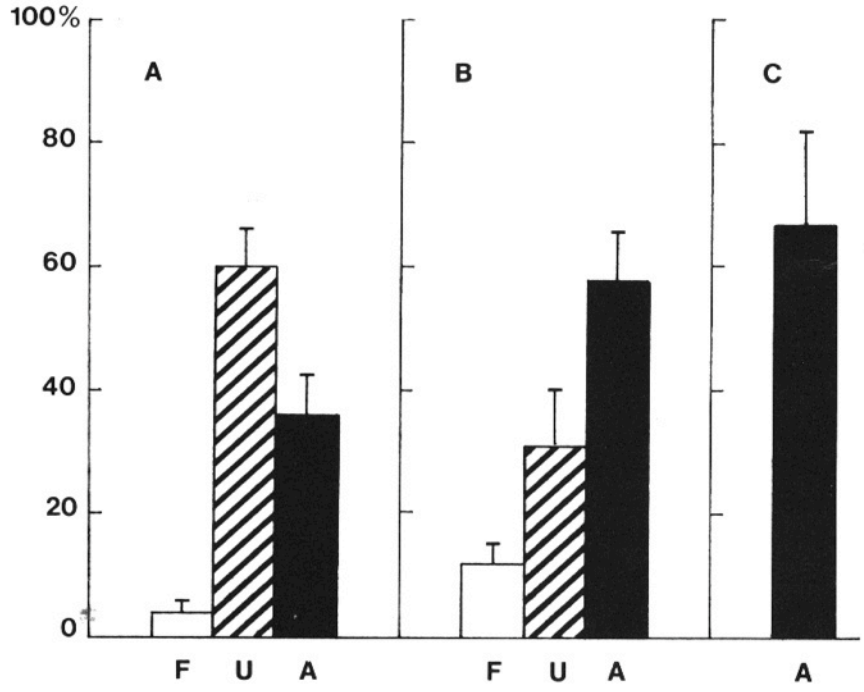

Fig. 2. The proportion of eggs that were fertilized (F), unfertilized (U), or activated (A) after the following treatments: **A:** incubation for 15 min in Sr^{2+} sperm suspensions followed by cumulus cell removal and incubation for 60 min in Ca^{2+} medium; **B:** incubation for 75 min in Sr^{2+} sperm suspension; **C:** incubation for 75 min in sperm-free Sr^{2+} medium.

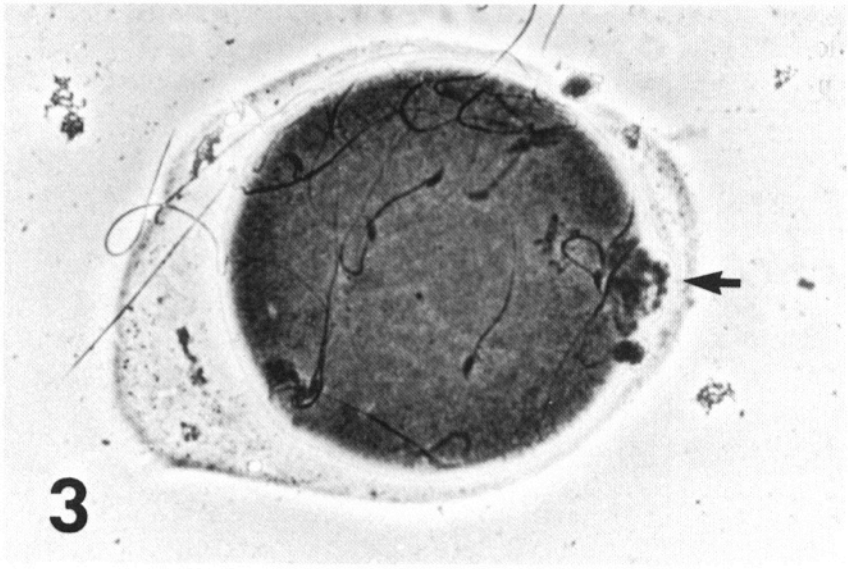

Fig. 3. Sr^{2+}-activated egg with numerous sperm inside the zona pellucida; no sperm have been incorporated into the egg cytoplasm. The second polar body is indicated by an arrow. ×500.

or unactivated, had numerous sperm vigorously moving in the perivitelline space; a light microscopic preparation of a typical egg is shown in Figure 3. Because sperm can fuse with eggs in the presence of Sr^{2+} (Series I), these results suggest that the failure to achieve fertilization was due to a very rapid Sr^{2+}-induced activation response by the eggs. A 15 min exposure is sufficient to prevent sperm fusion, although a longer exposure is required to promote the full nuclear response, leading

to extrusion of the second polar body. Since cumulus cells and unattached sperm were removed after this short incubation, the sperm observed in the perivitelline space at 75 min must have associated with the zona within 15 min and maintained the interaction during the various washing stages.

A similar proportion of eggs (65%; Fig. 1) underwent rapid, spontaneous activation when incubated for 75 min in sperm-free Sr^{2+} medium. Again, most had reached telophase II by the time of fixation. In contrast, none of the eggs incubated in either Ca^{2+} plus Sr^{2+} or Ca^{2+}-deficient medium showed signs of activation (data not shown).

Series IV: Electron Microscopic Evaluation of Eggs Incubated in Sr^{2+} Medium

Results indicated that the majority of eggs had lost cortical granules by the time of fixing. A few granules were seen in isolated regions of the cortex, but these can be seen in fertilized eggs as well. A typical section is shown in Figure 4. Occasionally, structures resembling intact cortical granules were observed in the perivitelline space and/or the zona pellucida.

DISCUSSION

The data from the present study provide unequivocal evidence that in mouse sperm Sr^{2+} can completely substitute for Ca^{2+} in all of the steps leading up to and including fusion with the egg: capacitation, hyperactivated motility, acrosome reaction, sperm:zona interaction, zona penetration, and gamete fusion. It is true that the exclusion of $CaCl_2$ from the medium does not totally eliminate calcium ions, because of trace amounts of Ca^{2+} in other salts, but these low levels of Ca^{2+} are insufficient to promote complete capacitation and fertilization [Fraser, 1982] and therefore cannot account for the results obtained in the presence of Sr^{2+}. The data in Table 1 clearly demonstrate marked differences between sperm suspensions preincubated in Sr^{2+}

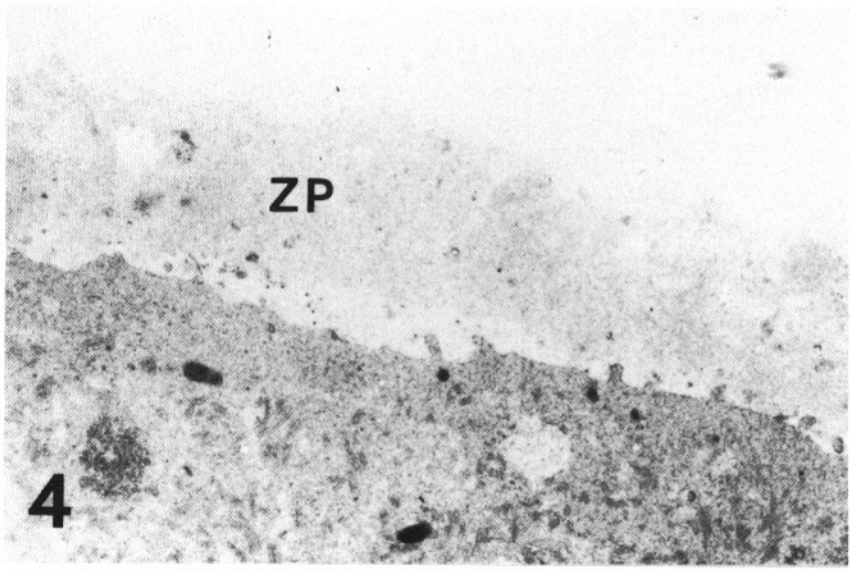

Fig. 4. Cortical region of a mouse egg incubated for 35 min in Sr^{2+} medium; most cortical granules have disappeared. ZP, zona pellucida. ×5,000.

-containing and Ca^{2+}-deficient media, with only the former attaining full functional competence during the preincubation phase. The possibility than Sr^{2+} can substitute in a similar manner for Ca^{2+} during human sperm capacitation has been suggested recently [Mortimer, 1986; Mortimer et al, 1986]. The data also indicate that the temporal facet of capacitation was similar in both Sr^{2+} and Ca^{2+}: After 30 min preincubation, sperm were only partially capacitated; after 120 min, they were fully capacitated. Thus Sr^{2+} neither inhibited nor accelerated capacitation relative to Ca^{2+}. Of the other two cations assessed, preincubation in Ba^{2+} had no positive effect on fertilizing ability compared with Ca^{2+}-deficient medium, which suggests that Ba^{2+} cannot substitute for Ca^{2+} during capacitation. In contrast, Mg^{2+} can apparently partially substitute for Ca^{2+} during capacitation, since sperm preincubated in Mg^{2+} were significantly more fertile when Ca^{2+} was added than were sperm preincubated in Ca^{2+}-deficient medium.

Previous studies have reported Sr^{2+} effects on motility in mouse sperm. Cooper [1984] found that Sr^{2+} supported hyperactivated motility, as confirmed here, but did not link this to an evaluation of egg penetration. Heffner et al [1980] reported that Sr^{2+} supported motility (not specifically hyperactivated) but not sperm-zona binding. The present results are at variance with the latter. Motile sperm were seen to be firmly adhering to zonae when eggs were removed from Sr^{2+} sperm suspensions at 15 min; furthermore, many sperm were observed subsequently to be moving in the perivitelline space and these would have had to initiate interaction with zonae prior to penetration. Certainly the media used in the two studies differed; that used by Heffner et al [1980] was a minimal one containing only Tris-HCl and NaCl in addition to Sr^{2+}, whereas a complete medium was used in the present study. One possible explanation for the failure to observe sperm-zona binding in the earlier study is even more rapid Sr^{2+}-induced egg activation, with attendant changes in the zona properties that could block sperm binding, in the minimal medium than in the complete one.

Sr^{2+} again proved able to substitute for Ca^{2+} in supporting the acrosome reaction, unlike both Ba^{2+} and Mg^{2+}. Sr^{2+} has also been reported to support the acrosome reaction in guinea pig sperm when added to suspensions preincubated in Ca^{2+}-deficient medium [Yanagimachi and Usui, 1974]. Despite the enhanced fertilizing ability of mouse sperm suspensions preincubated in Mg^{2+}, compared with Ca^{2+}-deficient medium, when Ca^{2+} was introduced, acrosome loss was not increased within 10 min of adding Ca^{2+}. This suggests that sperm had not yet completed capacitation in the presence of Mg^{2+}.

Initially it proved difficult to demonstrate that Sr^{2+} can support sperm:egg fusion because of the rapid activation of eggs, but by using zona-free eggs, which permitted sperm direct access to the plasma membrane, normal fusion was possible. Subsequent early stages of development appeared indistinguishable from those observed in eggs fertilized in Ca^{2+} medium. Similarly, Yanagimachi [1978] reported that Sr^{2+} was able to replace Ca^{2+} during hamster sperm:zona-free egg fusion. However, it should be pointed out that those sperm had been preincubated in Ca^{2+} medium to permit capacitation and the acrosome reaction prior to the fusion assessments, whereas mouse sperm were fully functional in the continuous presence of Sr^{2+}.

The activation of eggs in response to Sr^{2+} was unexpected. In general, activation of mouse eggs is considerably more effective in aged eggs than in freshly ovulated eggs, and there are strain-related differences in the responses [Whittingham, 1980;

Kaufman, 1983]. Although it has been reported that the inclusion of Sr^{2+} will increase the activation rate in Ca^{2+}-deficient medium of mouse eggs recovered approximately 3 hr or more postovulation [Whittingham and Siracusa, 1978], the eggs used in the present study were recovered just at the completion of ovulation in this strain [Fraser, 1979]. There was no indication that these eggs incubated in Ca^{2+}-deficient medium activated, and, indeed, activation has essentially never been observed in this in vitro fertilization system whatever the medium composition (unpublished observations). The response to Sr^{2+} was very rapid; a 15 min exposure resulted in only 4% of eggs being fertilized (Fig. 2) compared with 91% when similarly preincubated sperm were assessed in Ca^{2+} (Table 1). The fact that only a minority of unfertilized eggs showed nuclear evidence of activation after 15 min in Sr^{2+} followed by 60 min in Ca^{2+}, whereas the majority had responded after 75 min in Sr^{2+} (Fig. 1), suggests that a short time in Sr^{2+} will effectively mount a block to sperm fusion but that a longer exposure is required to ensure resumption of meiosis. Ultrastructural examination of eggs incubated for 35 min in Sr^{2+} revealed that the majority of cortical granules had been discharged. This presumably accounts for the inability of sperm to fuse with the eggs, since gamete fusion in this system is initiated at around 35 min [Fraser, 1983a] when zona-intact eggs are used in Ca^{2+} medium. It should be pointed out that these results are in direct contrast to those reported by Wolf et al [1979], which suggested that the triggering of cortical granule release by pretreatment of eggs with ionophore A23187 in the presence of Ca^{2+} did not prevent their fertilization by capacitated sperm. However, the inophore did not promote cortical granule loss to the same extent as normal fertilization; this may indicate that the experimental treatment was either too brief or used a concentration too low to initiate a true blocking response.

In almost all activated eggs, the response to Sr^{2+} resulted in a rate of nuclear development indistinguishable from that observed in eggs fertilized by capacitated sperm: Within 75 min, telophase II had been reached, and frequently the second polar body was visible; thus all activated eggs were haploid. These direct observations reinforce the rapidity of the activation response initially deduced from the low rate of fertilization despite the presence of sperm in the perivitelline space. No attempt was made to culture the eggs in order to observe possible further development; fertilized eggs from this strain do not develop readily beyond the two-cell stage [Fraser, 1977].

Egg activation appears to be elicited by an increase in intracellular free Ca^{2+} resulting in large part from release of intracellular Ca^{2+} stores [Whittingham, 1980]. Some support for this has been provided by experiments demonstrating that 1) invertebrate and mammalian eggs underwent activation in response to ionophore A23187 in Ca^{2+}-deficient medium [Steinhardt et al, 1974] and 2) mouse eggs activated in response to injected Ca^{2+} [Fulton and Whittingham, 1978]. Sr^{2+} was also effective in the latter system. More direct evidence comes from recent experiments that have measured a significant rise in intracellular Ca^{2+} during early stages of both artificial activation (alcohol-induced) and fertilization of mouse eggs [Cuthbertson et al, 1981]. The mechanism(s) whereby Sr^{2+} induces activation is unclear at present. It has been suggested that Sr^{2+}, rather than being the primary effector, displaces bound Ca^{2+} within the cell and that it is this free Ca^{2+} that then initiates the response [Whittingham and Siracusa, 1978]. These authors noted that the presence of extracellular Ca^{2+} in addition to Sr^{2+} or other divalent cations inhibited activation, as was also observed in the present study. This may indicate an effect of extracellular Ca^{2+} on either the divalent cation transport systems regulating movement across the plasma

membrane or the intracellular buffering systems that normally maintain a low concentration of intracellular free Ca^{2+}; in the absence of extracellular Ca^{2+}, these systems may be altered. Current evidence suggests that, at fertilization, the production of inositol 1,4,5-trisphosphate (Ins P_3) in the cortical region of the egg stimulates an autocatalytic cycle of intracellular Ca^{2+} release and further Ins P_3 production, and it has been demonstrated that injection of Ins P_3 into unfertilized sea urchin eggs will initiate activation [Whitaker and Irvine, 1984]. It seems possible that treatments that cause activation initiate intracellular Ca^{2+} release and trigger such an intracellular cycle, thereby mimicking the sperm. In addition, evidence obtained in frog eggs indicates that increased free Ca^{2+} in the cytoplasm inactivates factors that block resumption of the second meiotic division [Masui et al, 1977; Lonka and Masui, 1984]. The relatively longer exposure to Sr^{2+} that was required for ensuring resumption of meiosis, compared with cortical granule extrusion, may reflect a similar inactivation process in mouse eggs.

In conclusion, Sr^{2+} is able to substitute for Ca^{2+} in all the processes mouse sperm undergo from capacitation to fusion with the egg. All the responses measured, physiological, morphological, and temporal, are essentially indistinguishable irrespective of which cation is present. Whether this indicates that Sr^{2+} transport across the sperm membranes utilizes the same systems or different ones is not yet known. Sr^{2+} also promoted egg activation despite the fact that the eggs were freshly ovulated and not prone to spontaneous activation under many other experimental conditions. A short (15 min) exposure to Sr^{2+} was sufficient to trigger cortical granule extrusion, but a longer time was required to ensure resumption of meiosis. The time course of the nuclear response was also rapid; the telophase II/second polar body stage was evident within 75 min.

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