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Acrosomal Status in Fresh and Capacitated Human Ejaculated Sperm

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ABSTRACT

The acrosomal status of human sperm was evaluated by immunofluroescence utilizing a specific monoclonal antibody that recognizes target antigen(s) localized in the acrosomal cap region. Spontaneous acrosomal loss was first examined in sperm preparations used for successful in vitro fertilization of human eggs. In these sperm populations, less than 20% of the sperm underwent degenerative or spontaneous acrosomal loss following 24 h of incubation. The correlation of acrosomal loss with changes in motility and viability suggested that sperm senescence was not necessarily coupled to acrosomal loss. Chemical induction of acrosomal loss by calcium ionophore A23187 and lysophospbatidylcholine (LPC) was characterized. Maximal ionophore induction (10 μM A23187 in media containing calcium) was observed in cells exposed to capacitating conditions in vitro; sperm exposed to noncapacitating conditions did not readily acquire the ability to respond to ionophore. The reaction induced by ionophore was slow (60 min), and at least 30% of the cells were always resistant to induction. In contrast, LPC induced rapid, synchronous acrosomal loss in either freshly ejaculated or capacitated sperm in the presence or absence of extracellular calcium, suggesting that this loss was not a physiologic reaction. These studies may provide a basis for evaluating capacitation and ultimately fertility potential in the human male.

INTRODUCTION

Epididymal or freshly ejaculated mammalian sperm do not normally fertilize mature eggs without first undergoing a period of maturation termed capacitation. Operationally, capacitated sperm have acquired the ability to bind to, penetrate, and fuse with an egg. In the present context, capacitation can more conveniently be thought of as the cohort of biochemical and biophysical changes that occur before the sperm can undergo a physiological acrosome reaction (Bedford, 1970; Yanagimachi, 1981; Bedford, 1983). Evidence is accumulating that this latter reaction occurs principally at the outer margin of the zona pellucida only after sperm-zona interaction. Thus a physiologic acrosome reaction may be induced uniquely in zona-bound sperm by a specific glycoprotein component of the zona (Bleil and Wassarman, 1983; Florman et al., 1984).

Evaluation of human sperm capacitation and fertility potential using human in-vitro fertilization is limited by practical and ethical considerations. However, the study of human sperm function has benefited by the development of two sperm/egg interaction systems: penetration of zona-enclosed nonviable human oocytes and of zona-free hamster eggs (Overstreet and Hembree, 1976; Yanagimachi et al., 1976). Unfortunately, assays involving mammalian eggs are cumbersome, and the performance of only a limited number of sperm is monitored; thus substantial extrapolations are required to assess capacitation or acrosomal status in the entire population. The development of suitable light and immunofluorescence microscopic techniques (Talbot and Chacon, 1981a; Wolf et al., 1985) make it possible to quantify acrosomal status in large populations of sperm rapidly and accurately.

With the ultimate objective of defining the relationships between acrosomal status, capacitation and fertility, we studied human sperm, cultured under capa-

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citating and noncapacitating conditions, from donors of known fertility or from husbands participating in a human, in vitro-fertilization embryo-transfer (IVF-ET) program. An interest in using the evaluation of acrosomal status to quantify the cohort of capacitated sperm led us to examine chemical induction of acrosomal loss by the calcium ionophore A23187 and by the fusogenic agent lysophosphatidylcholine (LPC). Both agents produce fertile, acrosome-reacted sperm populations in other mammalian species (Talbot et al., 1976; Green, 1978; Fleming and Yanagimachi, 1981). Acrosomal loss induced by the calcium ionophore in populations containing capacitated cells is usually slow and incomplete and displays an absolute requirement for extracellular calcium. In contrast, LPC induction of acrosomal loss in the guinea pig (Fleming and Yanagimachi, 1981) and the hamster (Ohzu and Yanagimachi, 1982) is rapid and synchronous. Thus, while the efficacy of both agents in inducing acrosomal loss in mammalian sperm has been described, the possibility that noncapacitated and capacitated cells respond differentially has not been exploited in the human.

MATERIALS AND METHODS

Evaluation of Acrosomal Status

Human sperm were obtained by self-masturbation from healthy adult donors after 24–48 h of sexual abstinence. Ejaculates were allowed to liquify at 37°C for 30–60 min before sperm cells were harvested by centrifugation-resuspension techniques as described previously (Wolf et al., 1985). For indirect immunofluorescence, cells suspended in Dulbecco's phosphate-buffered saline (PBS) were fixed for 15 min at ambient temperature in 1% paraformaldehyde in PBS (pH 7.4) and washed 2 times with 0.2 M glycine to block free-aldehydes (Koehler et al., 1980). As reported previously (Wolf et al., 1985), quantification of acrosomal status is influenced by the time of exposure to paraformaldehyde. Because prolonged exposure can lead to false-negative reactions, we carefully adhered to a 15–20 min fixation time. Cells (5 μl of 1–50 × 10^6 cells/ml) were air-dried in individual wells defined by mylar tape on glass slides precleaned with 3% HCl in methanol. After drying cell lawns were washed 2–3 times with 0.01% Nonidet P-40 (NP-40), 0.1% 2-mercaptoethanol, 1% bovine serum albumin in physiological buffered saline (Solution A). Aliquots (5 μl) of hybridoma supernatant (supplied by Dr. Kathleen Bechtol, Wistar Institute, Philadelphia, PA) were added to each well, and the slides were incubated at ambient temperature for 1 h in a humidified chamber. Cells were washed as above in solution A, and 5 μl of second antibody were added (FITC-goat antimouse immunoglobin G [H+L]; Kirkegaard and Perry, Gaithersburg, MD). Cells were incubated for at least 45 min, washed as above, mounted in glycerol containing 0.2 M n-propylgallate (Giloh and Sedat, 1982), and examined under oil at 500 × with a Leitz Dialux 20 microscope equipped with epifluorescence optics. Cells demonstrating specific fluorescence restricted to the acrosomal cap region of the sperm head were considered positive (acrosome-intact); cells with no detectable specific fluorescence over the acrosomal cap region were scored as negative (acrosome-negative). Previously, we demonstrated by correlation of fluorescence microscopy and transmission electron microscopy that only sperm exhibiting an intact acrosome and overyling plasma membrane display uniform cap fluorescence (Wolf et al., 1985). A minimum of 2 wells were prepared and scored per sample, usually at a two- or threefold difference in sperm lawn density. Initially, acrosomal status was determined for 100 cells in each well; as long as the interwell variation was less than 10%, only 200 cells were scored.

Sperm Capacitation

Ejaculated, washed sperm from proven fertile donors or from husbands participating in an IVF-ET program were incubated in either Ham's F-10 containing 7.5% heat-inactivated maternal serum or Biggers, Whitten and Whittingham medium (BWW, Biggers et al., 1971) containing 0.3–3.5% human serum albumin (HSA), pH 7.6, in 15-ml conical tubes. Incubation in Ham's F-10 medium was conducted in 5% CO_2, 5% O_2, and 90% N_2, while those in BWW were in 5% CO_2, 95% air. In some experiments motile supernatant sperm were recovered after incubation by allowing nonmotile cells and debris to settle and aspirating off the supernatant fraction. The remaining cells constituted the pellet. Dulbecco's PBS was used in some experiments as a substitute medium that does not support capacitation but does support motility and viability for brief incubations. Unless indicated, sperm incubated in BWW with 3.5% HSA were washed twice with BWW containing 0.3% HSA, resuspended in BWW with 0.3% HSA, and exposed to specific conditions as itemized below.

Calcium concentrations were varied in some experiments. In the standard medium, the final calcium concentration was 1.7 mM, pH 7.6. Calcium-free medium was prepared by deleting calcium, adjusting osmolarity with sodium chloride, and adding 1 mM ethylene glycol-bis (β-amino ethyl ether) tetra-acetic acid (EGTA).

Preparation of Ionophore A23187 and Lysophosphatidylcholine

Calcium ionophore A23187 (free acid; Calbiochem Corp., La Jolla, CA) was dissolved in dimethylsulfoxide (DMSO) to a final concentration of 5 mM. Aliquots of stock were frozen and maintained at a constant −20°C, and were thawed and diluted immediately before use. Lysophosphatidylcholine (LPC, Sigma Chemical Corp., St. Louis, MO) was prepared immediately before use (1 mg in 200 μl of physiological saline) and then diluted to the desired concentration.

Chemical Induction of Acrosomal Loss

Sperm were washed into BWW with 0.3–3.5% HSA as indicated and incubated with 1–50 μM ionophore A23187 or 25–200 μg/ml of LPC. In the former case, sperm were also incubated either in BWW containing DMSO or in BWW medium alone. Incubations were carried out for the indicated times at 37°C before fixation and processing for acrosomal status evaluation. Standard incubation conditions (as defined in these experiments) consisted of 10 μM ionophore for 60 min or 100 μg/ml LPC for 15 min.

Determination of Sperm Motility and Viability

Sperm were analyzed for percentage of motility (at least 100 sperm scored) and forward progression (scale of 1–4; Amelar and Dubin, 1982) by phase contrast microscopy at 200 ×. Sperm viability was determined by incubating equal volumes of sperm solutions and Trypan Blue (3.0% in physiological saline) for 30 min at 37°C. Viable sperm excluded the dye; dead sperm stained a dark blue. At least 100 sperm were scored in each sample.

Statistical Analysis

Comparisons between different groups were made using the Student's *t*-Test; analysis of variance or linear regression was performed using the statistical package on a DECVAX-11/780 computer, University of Texas Health Science Center at Dallas, Medical Computing Resources Center.

RESULTS

Incidence of Spontaneous Acrosomal Loss

A high percentage of freshly ejaculated sperm from proven fertile donors were viable, motile and acrosome-intact. In 10 different donors, the mean percentage of intact cells was 88.0 ± 6.1% (SD). Moreover, these sperm did not undergo marked spontaneous or degenerative acrosomal loss in whole semen (i.e., under noncapacitating conditions): a corresponding mean value of 80.8 ± 12.9% (SD) was obtained when the sperm from 10 different donors were subjected to a 6- h incubation in semen before acrosomal quantification.

During incubation under capacitating conditions sperm acquire the ability to fertilize mature oocytes in vitro. Undoubtedly several sperm processes occur concurrently over this time: capacitation, motility, and viability changes, as well as physiologic and/or degenerative acrosomal loss. Of interest was whether any of these events were coupled and if so, which ones. These events were first examined using sperm from fertile husbands participating in a human IVF program. Spontaneous acrosomal loss and viability decline were limited in the relatively motile sperm populations recovered in the supernatant fractions of up to 24-h incubations in Hams F-10 medium with 7.5% heat-inactivated serum (Table 1). Significant declines in the percentage of motile cells were noted by 24 h of incubation. Thus, motility loss was not necessarily coupled to acrosomal loss. By 24 h the motility loss seen in the relatively nonmotile pellet population was accompanied by a significant decrease in acrosome-intact cells. Viability levels also decreased; however, since the percentage of acrosome-intact sperm exceeded the percentage of viable cells, it was clear that at least some nonviable cells retained acrosomes. In order to examine these relationships over broader ranges, data obtained for all cell populations incubated under capacitating conditions for up to 24 h were plotted together (Fig. 1). As anticipated, considerable variability was observed in motility scores, and motility was less strongly correlated with acrosomal status (Fig. 1b; $r = 0.62$) than was viability (Fig. 1a; $r = 0.76$). Extrapolation of results to a point at which all cells would be immotile or nonviable indicated that 72% and 58% respectively of such sperm retain intact acrosomes.

TABLE 1. Acrosomal loss in sperm incubated under capacitating conditions.[a]

	Acrosome-intact cells (%)[b]	Motile cells (%)[b]	Viable cells (%)[b]
Fresh ejaculate	92.3 ± 8.1	71.3 ± 2.2	93.3 ± 8.9
Supernatant (6-h)	86.5 ± 7.1	74.6 ± 9.3	87.7 ± 7.2
Supernatant (24-h)	83.1 ± 7.1	46.3 ± 31.8	67.8 ± 22.4
Pellet (6-h)	81.8 ± 7.5	34.7 ± 18.1	69.8 ± 16.8
Pellet (24-h)	74.1 ± 10.3	18.4 ± 13.9	55.3 ± 20.9

[a]Sperm were incubated in Hams F-10 with 7.5% heat-inactivated maternal serum for 6–24 h for use in human in vitro fertilization. Results are expressed as the mean ± SD. All males (N = 11) were considered fertile since all fertilized at least 50% of their wives' eggs with an overall fertilization rate of 82.2%.

[b]The individual means of these groups are not significantly different. Percentage of cells that were acrosome-intact by analysis of variance: 1=2=3, 2=3=4 (therefore groups 1 and 4 are significantly different); Percentage of motility: 1=2; 3=4; 4=5. Percentage of viability: 1=2=3=4; 3=4=5.

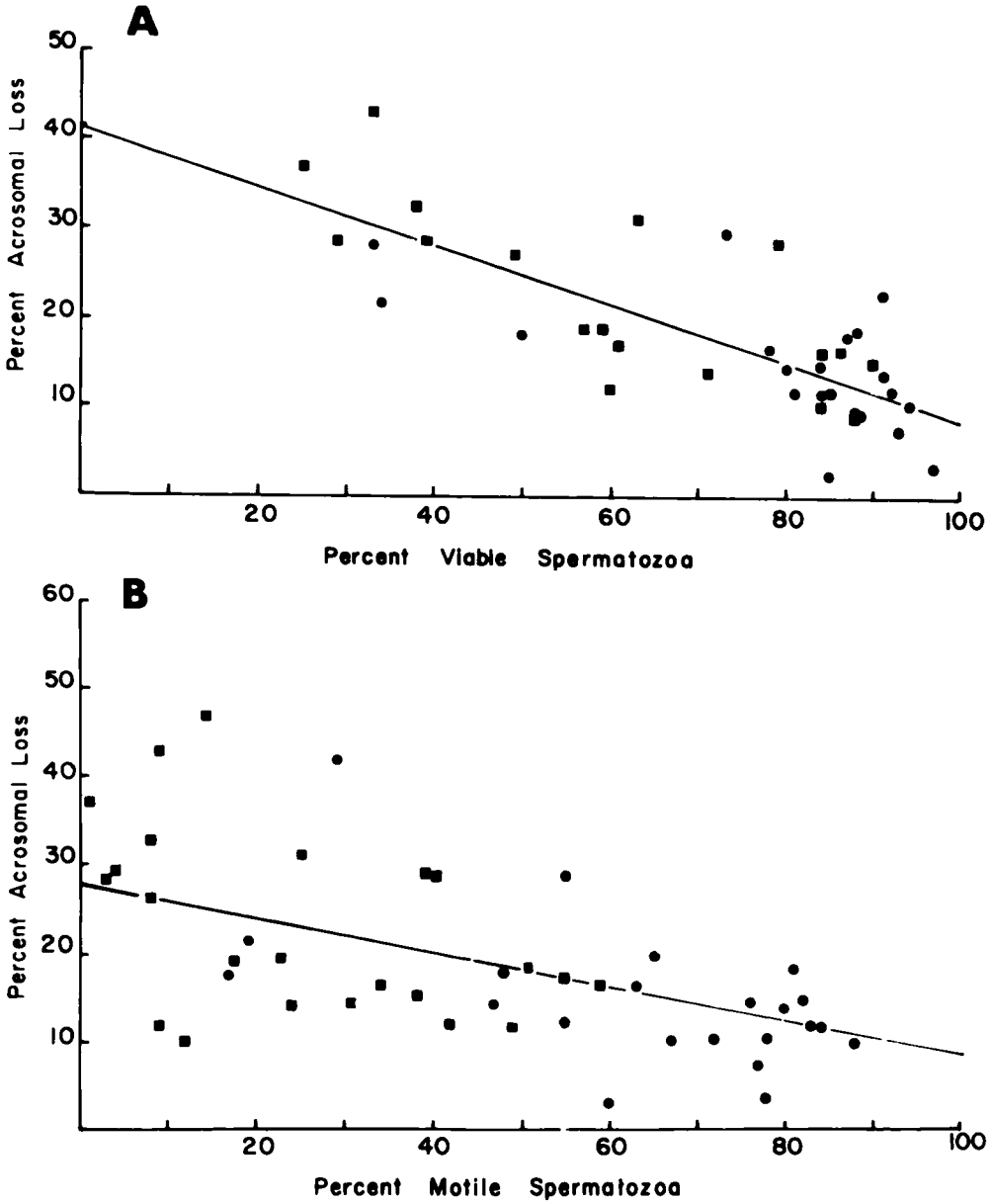

FIG. 1. The incidence of spontaneous loss of acrosomes was measured in sperm incubated under capacitating conditions. Sperm were washed and incubated in Hams F-10 medium supplemented with 7.5% maternal serum. Aliquots of sperm from either the supernatant or the pellet were assayed at 6 and 24 h for viability (*A*) or motility (*B*) and compared to acrosomal status. (■): Sperm taken from the pellet (●): Sperm taken from the supernatant.

Chemically Induced Acrosomal Loss

The results presented above indicate that human sperm immediately recovered from whole semen or washed motile sperm incubated in capacitating medium for up to 24 h do not readily undergo spontaneous or degenerative loss of acrosomes. Since it is possible to induce acrosomal loss chemically, we were interested in determining if incubated cells might be uniquely responsive to chemical induction. Two agents, calcium ionophore A23187 and LPC, were selected for use in inducing acrosomal loss.

Conditions for induction by calcium ionophore A23187. Since our initial objective was to induce maximal loss of acrosomes, we defined parameters that contributed to the efficiency of induced response without regard to the recovery of motile, fertile sperm. First, to determine dependency on ionophore concentration, washed sperm were preincubated for 6 h in BWW medium containing 3.5% HSA. Approximately 40% of the population lost acrosomes after a 60-min exposure to 5 μM A23187 (Fig. 2); this percentage was not increased further by exposure to concentrations as high as 50 μM. Ionophore concentrations of 5 μM and above were associated with a marked and rapid reduction in sperm motility to less than 5%.

Penetration rates in the zona-free hamster egg bioassay are influenced by the concentration of albumin, presumably through an effect on sperm capacitation (Wolf and Sokoloski, 1982). To evaluate the influence of albumin on capacitation and ionophore-induced loss of acrosomes, sperm were incubated in BWW with either 0.3 or 3.5% HSA prior to and during ionophore exposure. Sperm were washed and then resuspended in BWW containing either 3.5 or 0.3% HSA and exposed to 10 μM ionophore before aliquots were removed for evaluation of acrosomal status. In all samples, a maximal response to ionophore was seen within 60 min (Fig. 3). Ionophore exposure times longer than 2 h did not further increase the total number of reacted sperm. Acrosomal loss was greatest in sperm preincubated in 3.5% HSA and subsequently exposed to ionophore in BWW with only 0.3% HSA present. Exposure to ionophore in 3.5% HSA was relatively ineffective, suggesting that albumin may have been chelating the ionophore.

Another variable potentially influencing the stoichiometry of ionophore to sperm and hence the efficiency of induced loss of acrosomes was sperm concentration. This relationship is shown in Figure 4 for three ejaculates from each of two different donors. A maximal response in both donors was observed in the range of $1-10 \times 10^6$ cells/ml with a marked decrease in response apparent at higher concentrations of sperm. The variability of donor response to ionophore at a standard sperm concentration was investigated in a separate group of experiments (Table 2). It was apparent that a donor dependency existed in chemical induction even when differences in sperm concentrations were eliminated.

On the basis of these results, standard conditions for inducing acrosomal loss in capacitated sperm were defined as 10 μM ionophore for 60 min at 37°C in BWW containing 0.3% HSA at a sperm concentration of less than 10×10^6 cells/ml.

Ionophore induction of fresh and incubated cells. Once standard conditions for ionophore induction had been established, the relationship between ionophore inducibility and the physiological state of sperm (i.e., capacitated or noncapacitated) could be examined. Sperm from eight different donors were incubated either under noncapacitating (whole

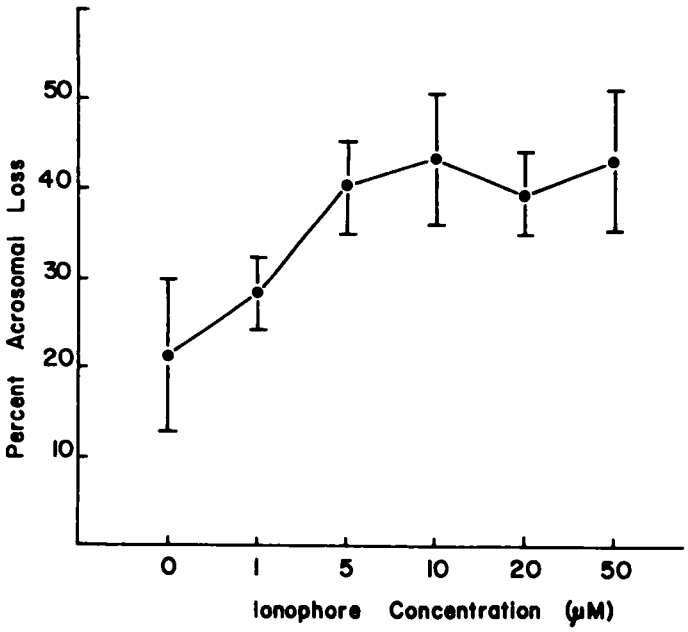

FIG. 2. Calcium ionophore A23187 concentration and loss of acrosomes in sperm incubated under capacitating conditions. Sperm were washed and incubated in BWW medium with 3.5% HSA for 6 h. Sperm were then washed and resuspended in BWW containing 0.3% HSA. Ionophore (1–50 μM, final concentration) was added and sperm were incubated an additional 60 min before acrosomal status was quantified by indirect immunofluorescence. Mean ± SD of five different ejaculates. Ionophore concentrations of 5–50 μM were not significantly different from each other but were different from the 1 μM-treated group ($p<.01$).

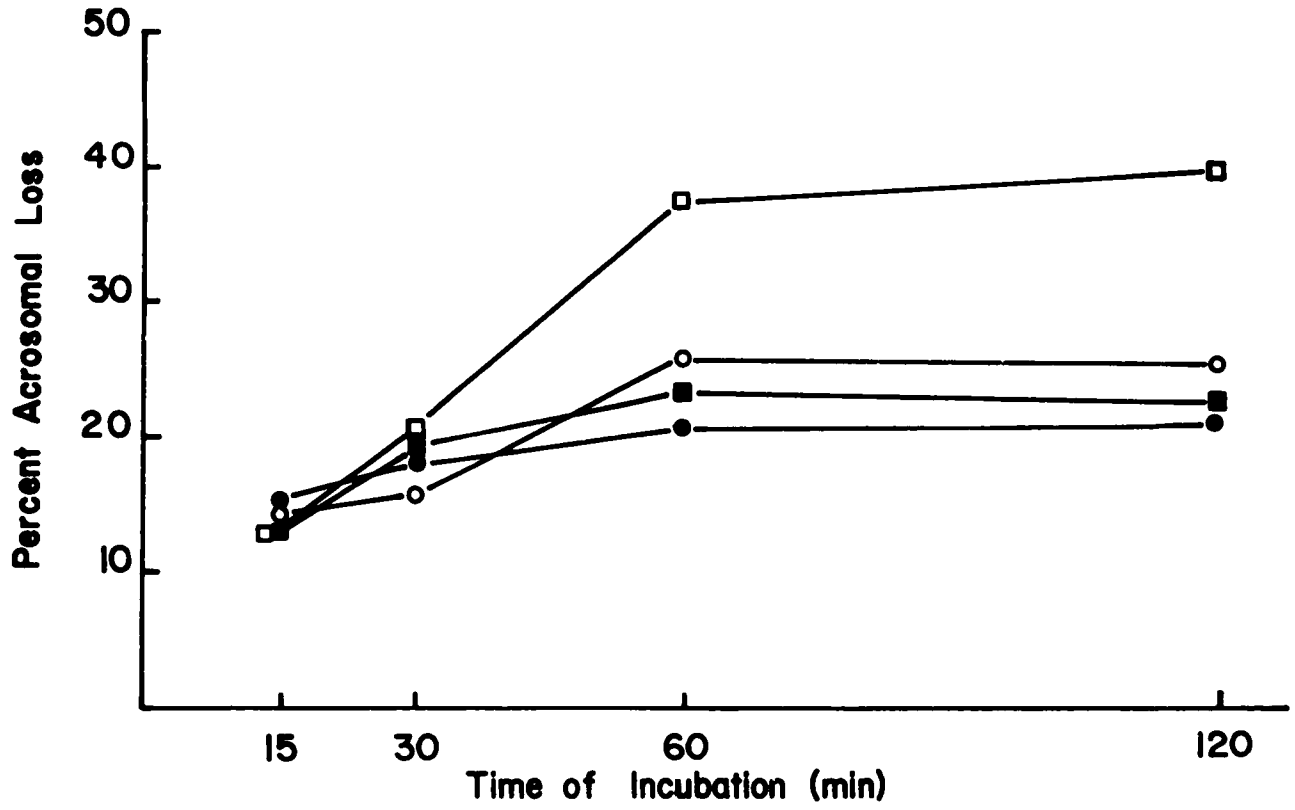

FIG. 3. The influence of albumin concentration on capacitation and ionophore-dependent loss of acrosomes. Sperm were washed and incubated for 6 h in BWW medium containing either 0.3 or 3.5% HSA. Following incubation, sperm were washed and resuspended in BWW containing either 3.5% HSA or 0.3% HSA. Ionophore (10 μM) was added and determinations of acrosomal status were made at the indicated times. (●): incubate in 0.3% HSA, followed by 3.5% HSA; (○): incubated in 0.3% HSA, followed by 0.3% HSA; (■): incubate in 3.5% HSA, followed by 3.5% HSA; (□): incubate in 3.5% HSA, followed by 0.3% HSA.

semen) or capacitating conditions (BWW with 3.5% HSA) for 6 h (Fig. 5). Sperm incubated in whole semen demonstrated very low levels of reacted cells. Indeed, no differences appeared between cells aged in whole semen, cells aged and exposed to ionophore, and cells harvested directly from fresh semen as discussed earlier. In contrast to the situation with noncapacitated sperm, a statistically significant increase in induced acrosomal loss followed ionophore exposure in sperm capacitated in BWW with 3.5% HSA ($p<.001$) at sperm concentrations less than 10×10^6.

Acrosomal loss induced by LPC. Initial conditions for LPC conditions of acrosomal loss were based on our experience with ionophore and a report by Fleming and Yanagimachi (1981). The optimal albumin concentration for LPC induction in sperm preincubated for 6 h was 0.3%; the response was rapid, synchronous, and dependent on LPC concentration (Fig. 6). At a LPC concentration of 100 μg/ml, the reaction was complete within 15 min. Induction of acrosomal loss by LPC was also dependent upon sperm concentration. In a series of experiments with six different donors, acrosomal loss following LPC treatment (100 μg/ml) was noted in

TABLE 2. Variability in response of donor sperm to ionophore.

Donor number	Acrosome-intact sperm (%)[a]	
	Control[b]	Ionophore[b]
1	88.3 ± 2.2	73.5 ± 9.3
2	87.9 ± 4.2	44.2 ± 10.5
3	89.3 ± 4.1	47.0 ± 13.9

[a]Sperm ($1-10 \times 10^6$ sperm/ml) were incubated for 6 h in BWW with 3.5% HSA, washed, incubated in BWW with 0.3% HSA ± 10 μM ionophore for 60 min and processed for acrosomal status. Means ± SD represent averages of four or more different ejaculates.

[b]Analysis of variance indicates no significant difference in controls (donor groups 1–3); ionophore-treated sperm, donor group 1 is significantly different from donor groups 2 and 3 ($p<0.001$). A significant difference between control and ionophore-treated sperm is seen in donor groups 2 and 3 ($p<0.001$).

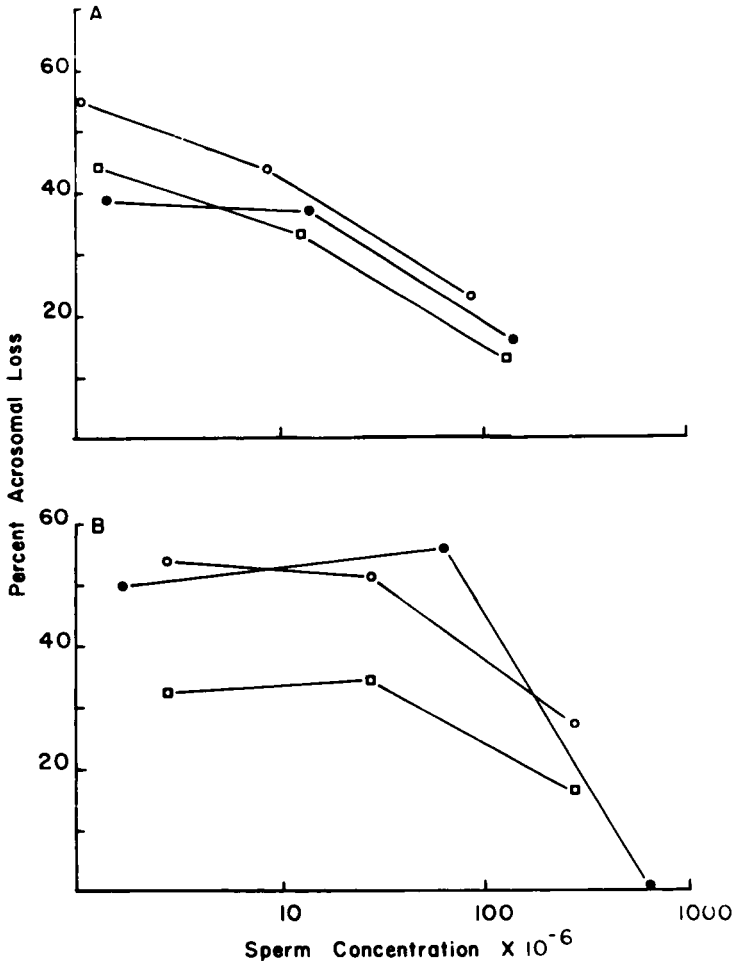

FIG. 4. Intra- and interdonor variability in response to a standard ionophore concentration at different sperm concentrations. Sperm were washed and incubated for 6 h in BWW medium with 3.5% HSA. Following incubation, sperm were washed into BWW with 0.3% HSA and diluted to the indicated sperm concentrations. Ten μM ionophore was added to the dilution. After one hour, sperm were assayed for acrosomal status. (*A*) and (*B*) represent two different donors. Three different ejaculates were analyzed for each donor (○, ●, □).

94.9% ± 4.3 (SD) of the cells at concentrations of 1–10 × 10^6 sperm/ml but in only 58.3% ± 26.7 (SD) at sperm concentrations of 25–100 × 10^6 sperm/ml.

Fresh and incubated cells induced with LPC. We compared the responsiveness to LPC of capacitated sperm (6 h of preincubation in BWW with 3.5% HSA) with that of noncapacitated sperm (from fresh, whole semen). Noncapacitated sperm from eight different donors were acrosome-negative (92.3 ± 5.4% SD) following exposure to LPC; a corresponding value of 89.4% ± 11.8 (SD) was observed after exposure to capacitating conditions. These findings suggested that induction by LPC was independent of capacitation. To test this directly, freshly ejaculated sperm were washed in either BWW containing 0.3% HSA or PBS and exposed to different concentrations of LPC for 15 min before processing (Table 3). Phosphate-buffered saline was chosen since we found previously that sperm incubated in PBS do not capacitate under our standard conditions and albumin is not present to interfere with LPC induction. In PBS, a near maximal response was seen at a LPC concentration of 50 μg/ml; in BWW a concentration of 100 μg/ml of LPC was required to elicit the same response. Hence, sperm responsiveness to LPC was dependent on albumin but independent of capacitation.

Given the apparent nonphysiologic response of sperm to the concentration of LPC tested, a role for extracellular calcium in the reaction appeared unlikely. To determine whether extracellular calcium was required for LPC induction, noncapacitated sperm were washed in PBS or BWW containing 0.3% HSA with or without added calcium and incubated for 15 min in LPC (100 μg/ml) before acrosomal

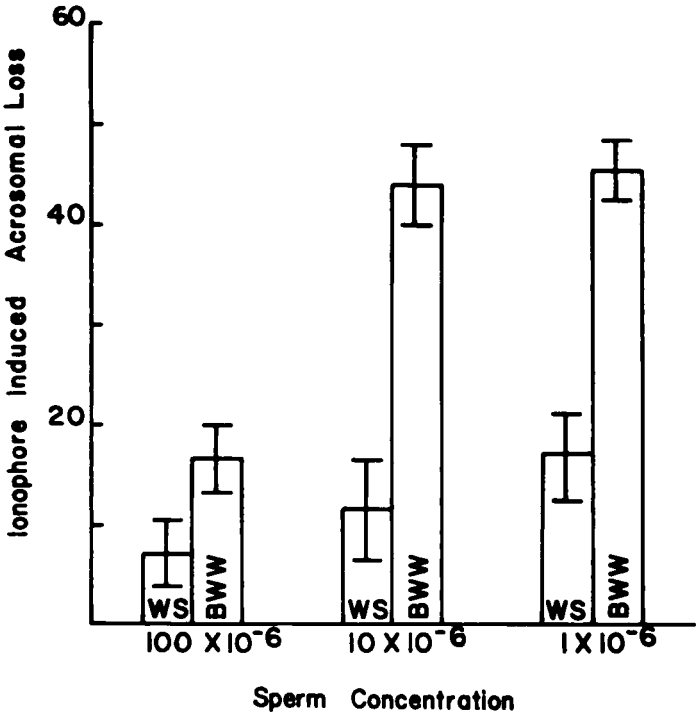

FIG. 5. Acrosomal loss induced by calcium ionophore A23187 in sperm incubated under capacitating and noncapacitating conditions. Ejaculates from different donors were either left in whole semen or washed into BWW with 3.5% HSA. Sperm were incubated for 6 h before all samples were washed and resuspended at the indicated concentrations in BWW with 0.3% HSA and 10 μm ionophore. After 1 h of incubation, sperm were assayed for acrosomal status. Each *bar* represents the mean ± SD of 8 different donors. The differences between the two groups were statistically significant ($p<0.01$) at sperm concentrations of 10 × 10^6 or less.

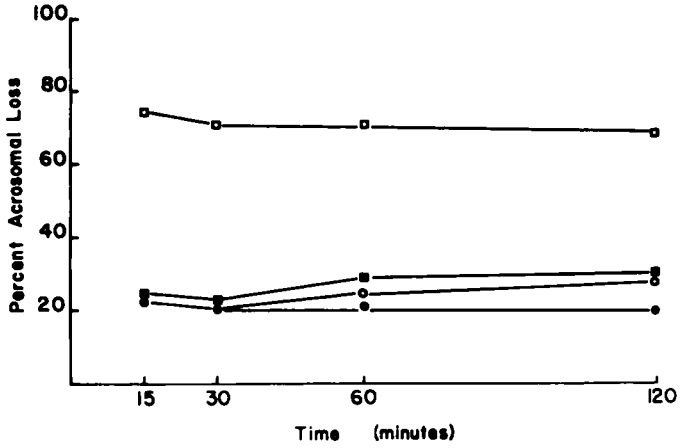

FIG. 6. Acrosomal loss induced by LPC in sperm incubated under capacitating conditions. Sperm were prepared and incubated in BWW with 3.5% HSA for 6 h. Following incubation, sperm were washed into BWW with 0.3% HSA at a final concentration of 10×10^6 sperm/ml. LPC was added at the indicated concentrations and the incubation was continued. Aliquots of sperm were assayed for acrosomal status at the indicated times. (●): control; (○): 25 μg/ml LPC; (■): 50 μg/ml LPC; (□): 100 μg/ml LPC.

status was evaluated (Table 4). All samples displayed similar acrosomal loss. Therefore, extracellular calcium is not a requirement for LPC induction of acrosomal loss under these conditions.

Exposure of sperm to LPC (100 μg/ml) resulted in a rapid loss of motility (in less than 2 min) in greater than 90% of the cells, concomitant with a variable loss of viability. The dramatic decrease in motility and the possible nonspecific nature of acrosomal loss induced with LPC suggested a very rapid and extensive disruption of acrosomal and plasma membrane integrity. This observation was confirmed by examination of LPC-treated sperm by transmission electron microscopy. Two notable features were apparent. First, there was a complete absence of the outer acrosomal and overlying plasma membrane, and second, the acrosomal contents appeared completely dispersed. There was no ultrastructural evidence of membrane vesiculation as seen in sperm treated with calcium ionophore (Wolf et al., 1985). Limited disruption of the plasma membrane was also noted over the remainder of the sperm surface.

DISCUSSION

In this study, we have confirmed that sperm incubated under capacitating conditions do not readily undergo spontaneous loss of acrosomes in vitro, and that sperm senesence is not necessarily linked to loss of acrosomes. The ability of capacitated or noncapacitated cells to respond to the fusogenic agents calcium ionophore A23187 and LPC was examined after conditions for incubation were optimized. Induction of acrosomal loss by the calcium ionophore A23187 was greatest in preincubated, capacitated sperm, whereas LPC caused a nonphysiologic disruption of the acrosome and overlying plasma membrane, independent of sperm preincubation or extracellular calcium (Table 5).

The low incidence of spontaneous loss of acrosomes in human sperm following short incubation periods (Talbot and Chacon, 1981a,b; Gould et al., 1983; Plachot et al., 1984; this study) contrasts with that found in other species (Talbot et al., 1976; Liu et al., 1979; Rogers, 1981; Bedford, 1983). After 6 h of incubation, the level of motile sperm in the supernatant fraction that reacted spontaneously remained low (13.5%), increasing to only 16.9% after 24 h of incubation. Similary, Plachot et al. (1984) observed that only a small portion of live sperm (16%) underwent an acrosome reaction by 17 h of incubation.

Increased intracellular calcium appears to be the primary signal for inducing the acrosome reaction in capacitated sperm (Singh et al., 1978; Triana et al.,

TABLE 3. Loss of acrosomes in noncapicated sperm induced by LPC.[a]

LPC concentration μg/ml	Acrosome-negative cells (%)[b]					
	0	25	50	75	100	200
BWW (N=5)	17.0 ± 0.7	22.0 ± 9.1	25.4 ± 5.4	44.8 ± 33.5	84.6 ± 4.6	89.8 ± 6.9
PBS (N=5)	12.2 ± 3.8	61.8 ± 28.1	80.0 ± 16.1	88.2 ± 4.9	87.6 ± 5.9	90.8 ± 1.9

[a]Ejaculated sperm were washed in the indicated medium and incubated with different concentrations of LPC for 15 min before they were processed for acrosomal evaluation.

[b]Mean ± SD of at least 100 sperm in each experiment.

TABLE 4. Induction of acrosomal loss in noncapacitated sperm by LPC in the presence and absence of calcium.[a]

	Acrosome-negative cells (%)[b]		
	Control	LPC with 1.7 mM calcium	LPC without calcium (1mM EDTA)
BWW (N=5)	17.0 ± 8.1	81.7 ± 10.4	77.8 ± 9.7
PBS (N=5)	12.5 ± 4.4	94.6 ± 1.2	87.0 ± 9.0

[a]Sperm were washed in the indicated medium and exposed to 100 μg/ml of LPC before they were processed for acrosomal status.

[b]Mean ± SD of at least 100 sperm in each experiment.

1980; Yanagimachi, 1981). Control of calcium permeability and the nature of the stimulus required to induce an acrosome reaction in vivo are not well understood. Therefore, in the absence of a known biological inducer, studying the susceptibility of sperm to ionophore would appear to be a logical approach to evaluating the capacitated state of those sperm. Indeed, we were able to establish that an enhanced population of cells incubated under capacitating conditions responded to ionophore induction, at least when compared with sperm incubated under noncapacitating conditions (i.e., whole semen). This suggests that exposure to seminal plasma blocks or prevents capacitation, perhaps directly or indirectly through some alteration in membrane permeability to calcium. The persistent presence of even dilute seminal plasma apparently prevents attachment and penetration of zona-free hamster and salt-stored human eggs (Kanwar et al., 1979). In the bovine, calcium uptake in epididymal sperm can be inhibited by adding a protein of small molecular weight isolated from seminal plasma (Rufo et al., 1982).

Under the present treatment conditions, a significant population of viable sperm never responded to ionophore induction. This is consistent with the expectation that capacitation is asynchronous. Variation in the kinetics of capacitation has been described for human sperm used in the zona-free hamster egg bioassay (Perreault and Rogers, 1982; Wolf and Sokoloski, 1982; Saito et al., 1984). Alternate approaches to maximize loss of acrosomes might include synchronization in low extracellular calcium (Wolf and Sokoloski, 1984), a more rigorous selection of motile sperm using Percoll gradients (Suarez et al., 1985), or utilization of a physiological trigger for induction, such as solubilized zona (Bleil and Wassarman, 1983). The variability seen in human sperm morphology (Amelar and Dubin, 1982) suggests that substantial numbers of sperm may be unresponsive to ionophore induction because of defective or absent acrosomes.

A number of agents in addition to calcium ionophore A23187 induce an accelerated acrosome reaction in mammalian sperm: detergents (Wooding, 1975), methoxyverapamil (Singh et al., 1978), and lipids (Fleming and Yanagimachi, 1981). We chose to examine the effect of one lipid, LPC, which stimulates the acrosome reaction when added to guinea pig and hamster sperm (Fleming and Yanagimachi, 1981; Ohzu and Yanagimachi, 1982; Llanos and Meizel, 1983) while retaining fertility and motility for at least 2 h (Fleming and Yanagimachi, 1981). We found that sperm treated with LPC underwent rapid acrosomal (85–90%) and motility loss in the absence of extracellular calcium, and that preincubation under capacitating conditions was not a prerequisite for induction. The rapid loss in sperm motility was probably due in part to disruption of the integrity of the sperm plasma membrane by LPC and an influx of calcium into the sperm. If lower concentrations of LPC were used, sperm retained partial motility but did not show acrosomal loss. Further work on LPC induction might demonstrate a concentration of LPC effective in inducing acrosomal loss without the resulting loss in motility. Human ejaculated sperm treated with the concentrations of LPC used here do not undergo a physiological acrosome reaction; this was corroborated by ultrastructural analysis of LPC-treated sperm, which demonstrated that the integrity of acrosomal and plasma membranes was highly perturbed after LPC treatment.

It has not been established whether acrosome-reacted sperm can fertilize human eggs in vitro or in

TABLE 5. Summary of calcium ionophore A23187 and LPC results.

	A23187	LPC
Is capacitation required?	Yes	No
Is calcium required?	Yes	No
Optimal HSA concentration for reaction	0.3%	0.3%
Optimal HSA concentration for reaction	1–10 × 10^6	1–10 × 10^6
Time required for maximum reaction	60 min	2–15 min
Characteristics of reaction	Asynchronous-slow	Synchronous-rapid

vivo and, if so, how long they retain their fertilizing capability. Plachot et al. (1984) found no correlation between acrosomal status and fertility in vitro when wives' eggs were inseminated approximately 17 h before acrosomal status was evaluated. Aitken et al. (1984) addressed this question by treating human sperm with high concentrations of ionophore (50–100 μM) and then measuring the penetrating capability of the sperm by using the zona-free hamster egg bioassay. Aitken et al. (1984) reported significantly increased penetrability after treatment, but did not confirm increased loss of acrosomes. The possibility that motile, acrosome-reacted human sperm retain their fertilizing capability for at least a limited time cannot be eliminated. Certainly sperm of other species retain their fertilizing capability following induction of the acrosome reaction (Fleming and Yanagimachi, 1981).

Idiopathic infertility exists in a large number of couples. In some, failure of sperm to capacitate or undergo an acrosome reaction may be responsible. We can now examine relationships between spontaneous or chemically induced acrosome reactions in populations of fertile and subfertile males to extend our understanding of this phenomenon. Are subfertile males with normal semen parameters capable of undergoing induced loss of acrosomes at the same rate as known fertile males? Research on the oligospermic male suggests that factors other than reduced concentration may influence fertility in vitro (Byrd and Wolf, 1984; Wolf et al., 1984). In contrast to sperm from normal males, sperm treated with calcium ionophore from oligospermic males do not exhibit enhanced penetratibility of zona-free hamster eggs (Aitken et al., 1984). Perhaps some of these subfertile males can attribute their infertility to a defect in capacitation, an inability to undergo an acrosome reaction or normal sperm-egg membrane fusion. The present studies may provide a basis for evaluating some of the more obvious sperm dysfunctions that contribute to male infertility.

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