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Acceleration of the Acrosome Reaction and Activation of Guinea Pig Spermatozoa by Detergents and Other Reagents

R. YANAGIMACHI

Department of Anatomy and Reproductive Biology,
The John A. Burns School of Medicine,
University of Hawaii
Honolulu, Hawaii 96822

ABSTRACT

In vivo, guinea pig spermatozoa do not begin to undergo the acrosome reaction and to penetrate eggs until 4 to 10 h after they are deposited in the female genital tract. When spermatozoa are incubated in a modified Krebs-Ringer's solution containing 0.003 percent Hyamine, the majority of them exhibit both the acrosome reaction and activation within 10–15 min and become capable of penetrating eggs. The acrosome reaction and activation induced by Hyamine are reversibly blocked when calcium ions are omitted from the medium. Other reagents capable of inducing an accelerated acrosome reaction and/or activation include: Triton, Brig, saponin, nonanol, nystatin, mersalyl acid, dibucane, procaine, neotetrazolium chloride, nitro blue tetrazolium, chloroquine, calcium ionophore, unheated sera of the rabbit and guinea pig, rabbit complement and pronase. The study shows that guinea pig spermatozoa are potentially capable of an immediate acrosome reaction and activation upon leaving the epididymis.

INTRODUCTION

Guinea pig spermatozoa begin to undergo the acrosome reaction and to penetrate eggs in 4 to 10 h in the female genital tract (Yanagimachi and Mahi, 1976). A recent *in vitro* study has shown that the types of energy sources in the environment greatly influence or regulate the timing of the acrosome reaction in guinea pig spermatozoa (Rogers and Yanagimachi, 1975). Thus, the timing of the acrosome reaction is apparently not inherently fixed, but is influenced or regulated by the composition of the medium to which the spermatozoa are exposed. Johnson (cited from Austin et al., 1973) demonstrated that guinea pig spermatozoa can undergo the acrosome reaction *in vitro* within a relatively short time when he observed the acrosome reaction less than 15 min after exposing spermatozoa to unheated rabbit serum. Barros et al. (1973) reported that guinea pig spermatozoa may have an acrosome reaction within a few minutes when placed between a coverslip and slide. Barros et al. claimed that these acrosome-reacted spermatozoa were functionally capacitated, since these spermatozoa, unlike acrosome-intact spermatozoa, were capable of penetrating zona pellucida-free hamster eggs *in vitro*. Although the conditions these investigators employed are unlike the conditions the spermatozoa would encounter in the normal *(in vivo)* environment, these studies nevertheless may contribute to an understanding of the basic mechanisms of the acrosome reaction in the guinea pig. The purpose of this study is to extend these studies and to report various conditions under which guinea pig spermatozoa exhibit the acrosome reaction within a very short period of time.

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MATERIALS AND METHODS

The medium used in this study was a bicarbonate-buffered Krebs-Ringer's solution supplement with pyruvate, lactate, glucose and bovine serum albumin (fraction V, Reheis/Armour) (Biggers et al., 1971, p. 101), which is designated in this paper as BWW medium. The BWW medium from which calcium salt (Ca-lactate) was deleted was called Ca-free BWW medium. Each reagent to be tested was dissolved in BWW medium at various concentrations, and 0.1 ml of each test solution was placed under mineral oil (Squibb) in a watchglass and kept at 38°C. Spermatozoa from the distal portion of a freshly excised cauda epididymis were suspended in fresh BWW medium at approximately 4 to 8 × 10^6 spermatozoa per ml, and 0.1 ml of this suspension was mixed with 0.1 ml of the test solution under mineral oil in the watchglass. These sperm preparations were incubated at 38°C under an air atmosphere and examined under dark-field illumination or with a phase-contrast microscope at various intervals for 1) sperm survival, 2) the pattern of sperm movement and 3) the acrosome reaction. Those

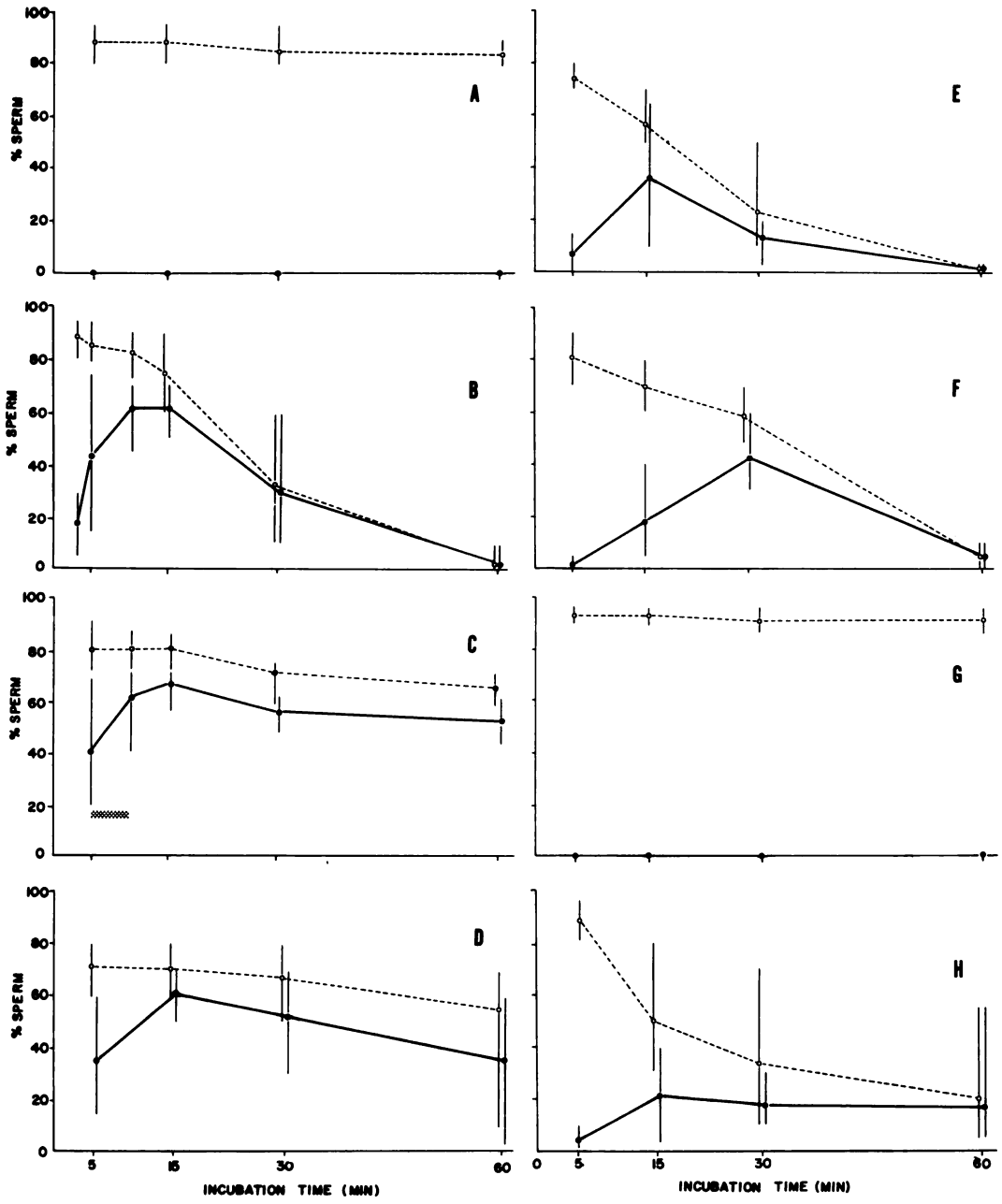

FIG. 1. Survival (○) and acrosome reaction/activation (●) of guinea pig spermatozoa in BWW media containing various reagents. Each vertical line represents the range and mean of 5 to 10 determinations. *(A)* BWW medium without any additional reagent, control; *(B)* BWW medium containing 0.003 percent Hyamine; *(C)* Spermatozoa were treated 5 min with BWW medium containing 0.003 percent Hyamine, then washed and kept in Hyamine-free BWW medium (a dotted rectangle represents the time the spermatozoa were being washed by centrifugation); *(D)* BWW medium containing 0.01 percent Triton; *(E)* BWW medium containing 3 percent unheated rabbit serum; *(F)* BWW medium containing 6 percent unheated guinea pig serum; *(G)* BWW medium containing 50 percent heat-treated rabbit serum; *(H)* BWW medium containing 50 percent heat-treated rabbit serum plus 1.5 mg/ml rabbit complement.

spermatozoa that had lost their acrosomal caps without losing progressive motility were recorded acrosome-reacted. Activation refers to the extremely vigorous movement of spermatozoa characterized by whiplash flexing of the flagella (Yanagimachi and Usui, 1974). Experiments were repeated at least five times using spermatozoa from three or more males. The fertilizing capacity of the spermatozoa was assessed using guinea pig oocytes matured *in vitro* (Yanagimachi, 1974). The spermatozoa to be tested for their fertilizing ability were washed once with BWW medium by centrifugation (× 600 *g* for 4 min), resuspended in fresh BWW medium, and 0.01–0.02 ml of the suspension was introduced into 0.1–0.2 ml of BWW medium containing eggs. Prior to insemination, the cumulus cells surrounding these eggs were removed by treatment with 0.1 percent bovine testicular hyaluronidase (300 USP units/mg, ICN Pharmaceut.) in BWW medium. After incubation for 1 h at 38°C under an air atmosphere, the eggs were examined for evidence of sperm penetration through the zona pellucida and into the egg cytoplasm (Yanagimachi, 1972, 1974).

Some samples of spermatozoa treated with rabbit sera or some other reagents were fixed with 3 percent glutaraldehyde in 0.1M phosphate buffer (pH 7.4), postfixed with 1 percent osmium tetroxide in 0.1M phosphate buffer (pH 7.4), dehydrated with ethanol and embedded in Epon. Thin sections stained with uranyl acetate and lead citrate were examined with a Philips 300 or an RCA EMU-4 electron microscope.

RESULTS AND DISCUSSION

When spermatozoa were incubated in BWW medium without any additional reagents, none or only a very few (0.01–0.2 percent) showed the acrosome reaction and activation within 1 h (Fig. 1A; also cf. Yanagimachi, 1972; Yanagimachi and Usui, 1974). When spermatozoa were incubated in BWW medium containing 0.003 percent (v/v) Hyamine 2389 (Rhom/Harris), the acrosome reaction and activation could first be seen in 2 to 3 min. By 10 min 45–70 percent of the spermatozoa showed the acrosome reaction and activation (Fig. 1B). The concentration of Hyamine in the medium was critical. Low concentrations (<0.0003 percent) were ineffective and high concentrations (>0.01 percent) were lethal, killing 100 percent of the spermatozoa within 5 min. Spermatozoa that had undergone activation and the acrosome reaction as a result of continuous Hyamine treatment did not survive beyond 1 h (Fig. 1B), but when spermatozoa were treated for only 5 min then washed and kept in Hyamine-free BWW medium, they survived well beyond 1 h (Fig. 1C). When spermatozoa were incubated in Ca-free BWW medium containing 0.003 percent Hyamine, over 90 percent of the spermatozoa remained highly motile for over 2 h, but none of them showed the acrosome reaction or typical activation. However, when spermatozoa were first incubated in the Hyamine-containing medium for 15 to 60 min then 2–5 mM $CaCl_2$ was added to the medium, the acrosome reaction and activation could be seen in the majority of the spermatozoa within 10 min. The acrosome reaction and activation of spermatozoa were also observed in BWW medium containing 0.01 percent (v/v) Triton X-100 (Sigma Chem.) (Fig. 1D).

Table 1 lists the reagents which were capable of inducing accelerated acrosome reaction and/or activation of the spermatozoa. The reagents belonging to group I (including Hyamine and Triton) induced both the acrosome reaction and activation. Those belonging to group II induced activation of spermatozoa within 10 min, but the incidence of the acrosome reaction was low within the 1 h test period. Saponin (group III) was lethal at high concentrations (>0.01 percent), killing 100 percent of spermatozoa within 5 min. At lower concentrations (0.001–0.003 percent) it induced the acrosome reaction, but the vast majority of spermatozoa failed to display activated movement.

Spermatozoa which had undergone accelerated acrosome reaction and activation as a result of treatment with Hyamine, Triton, nystatin and neotetrazolium chloride were capable of penetrating eggs within 1 h (Table 2).

Freshly collected unheated sera of the rabbit and guinea pig could induce the acrosome reaction and activation at certain concentrations (Figs. 1E and F, Figs. 2–5). High concentrations (e.g., 50 percent) of unheated sera were lethal, killing 100 percent of the spermatozoa within 5 min by randomly disrupting sperm membranes (Fig. 6: also cf. Bedford, 1970; Russo and Metz, 1974). Heat-treated (56°C, 30 min) sera were not at all detrimental to spermatozoa even at high concentrations (e.g., 50 percent), but were virtually ineffective in inducing the acrosome reaction and activation within 1 h (Fig. 1G). However, when rabbit complement (1.5 mg/ml; Gibco) were added to these heat-treated sera, an appreciable proportion of the spermatozoa underwent the acrosome reaction and activation within 15 min (Fig. 1H). Rabbit complement alone (1.5 mg/ml in BWW medium) induced the acrosome reaction in about 30 percent of the spermatozoa within 15 to 30 min. Guinea pig complement (1.5–6.0 mg/ml; Gibco) could also induce

TABLE 1. Reagents capable of inducing accelerated acrosome reaction and/or activation of guinea pig spermatozoa.

Reagent (source)	Conc. in incubation medium	Duration of exposure (min)	% sperm that underwent acrosome reaction[a]
Group I: induces both activation and acrosome reaction			
Hyamine 2389 (Rhom/Harris)	0.003% (v/v)	5	5-75
		15	50-70
Triton X-100 (Sigma)	0.01% (v/v)	5	15-60
		15	50-70
Brig 96 (Sigma)	0.003% (v/v)	10	2-10
		30	30-40
1-Nonanol (Sigma)	0.1% (v/v)	10	5-20
		30	5-10
Nystatin (Sigma)	0.1% (v/v)	30	15-60
		60	30-80
Mersalyl acid, Na salt[b]	0.003% (w/v)	15	5-10
		30	40-50
Dibucaine[c]	0.01% (w/v)	10	0.5-15
		30	10-20
		60	5-40
Neotetrazolium chloride (Sigma)	0.03% (w/v)	10	5-10
		30	10-30
		60	30-60
Nitro blue tetrazolium (Sigma)	0.03% (w/v)	10	0-10
		30	35-40
Group II: induces activation, but incidence of acrosome reaction is very low			
Chloroquine (Sigma)	0.01% (w/v)	30	0
		60	0-0.5
Procaine HCl (Sigma)	0.1% (w/v)	30	0-3
		60	0-3
Ionophore A-23187[c]	0.1% (w/v)	30	0-5
		60	0.1-5
Ionophore X-537A[c]	0.1% (w/v)	30	0-5
		60	0.5-5
Group III: induces acrosome reaction without obvious activation			
Saponin (Sigma)	0.001% (w/v)	10-15	10-50
		30	10-50

[a] % of acrosome-reacted sperm in the total population; range of 4-10 determinations, using 3 to 5 different males.

[b] Gift from Dr. Per Eric Lindhal.

[c] Gift from Dr. George Poste and Dr. Garth L. Nicolson.

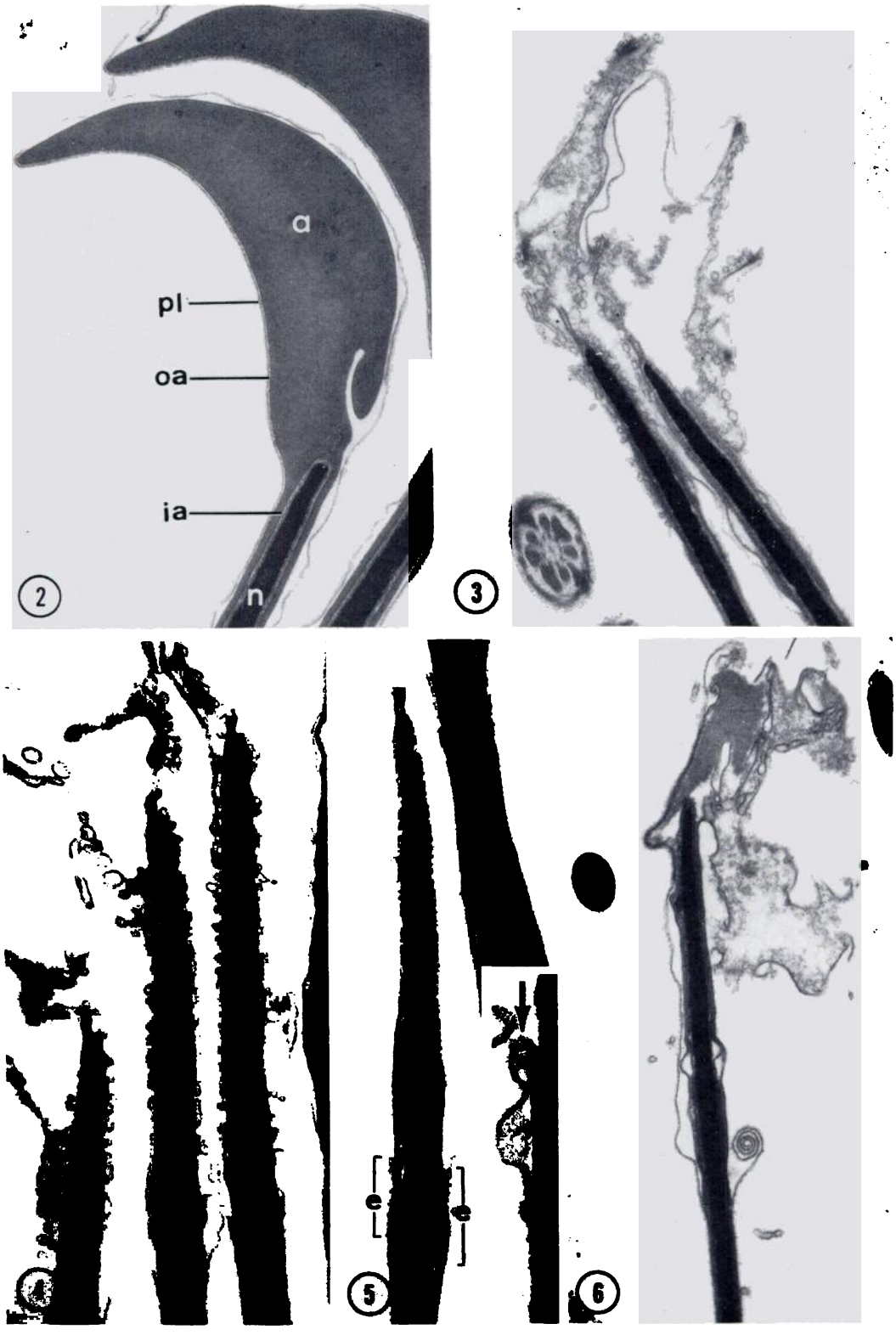

TABLE 2. Fertilizing ability of spermatozoa treated with hyamine, triton, nystatin and neotetrazolium chloride.

Sperm were preincubated In medium with:	For:	No. of exp.	Total no. of eggs insem.	No. (%) of eggs penetrated at 1 h after insem.
0.003% Hyamine	5-10 min	3	15	15 (100)[a]
0.01% Triton	5-10 min	2	10	10 (100)[a]
0.1% Nystatin	20-25 min	2	8	8 (100)[a]
0.03% Neotetrazolium chloride	20-25 min	2	11	11 (100)[a]
No additional reagent (control)	20-25 min	3	20	0 (0)

[a]Spermatozoa penetrated both zona pellucida and egg cytoplasm.

the acrosome reaction, but much less effectively than the rabbit complement. The spermatozoa activated and acrosome-reacted by treatment for 15 min with 3 percent unheated rabbit serum in BWW medium penetrated the zona pellucida of 70 percent of 20 eggs (3 experiments) within 1 h.

Of various enzymes tested pronase (84–120 UPK units/mg; Calbiochem.) had the most interesting effects on spermatozoa. At concentrations of 100–200 units/ml in BWW, it induced activation of virtually all the spermatozoa by 30 min. Activation of the spermatozoa lasted for 1 to 2 h. Occasionally some (1 to 15 percent) of the activated spermatozoa showed an acrosome reaction when examined between 30 and 60 min after the start of incubation. When activated spermatozoa were mounted between a slide and coverslip, many of the spermatozoa became firmly attached by their heads to the glass surfaces and soon lost their acrosomal caps as well as their mobility. Activation of spermatozoa by pronase was reversibly blocked when calcium ions were omitted from the medium. Treating pronase with heat (95°C for 1 h, in distilled water) completely abolished its sperm-activating capacity. BWW medium containing 1 to 4 × 10^4 units of trypsin per ml (crystalline, Types I and III; Sigma), the rouleaux or head-to-head stacks of the spermatozoa were dissociated in 2 to 3 h, but the spermatozoa showed neither activation nor the acrosome reaction within the 3 h test period. Other enzymes listed below were all ineffective in inducing the acrosome reaction and activation of spermatozoa above control levels at least within the 2 h test period: α-chymotrypsin (cryst., Sigma, Type II; 40–200 units/ml), β-chymotrypsin (cryst., Sigma; 40–150 units/ml), γ-chymotrypsin (cryst., Sigma, Type II, 40–150 units/ml), bromelain (Sigma, Grade II; 2000–4000 units/ml), papain (cryst., Sigma; 45 units/ml), hyaluronidase (Sigma, Types I and II; 300 units/ml), neuraminidase (Schwartz/Mann, 50 units/ml), β-glucuronidase (Miles/Servac; 2000 units/ml: Sigma, Types L-1 and B-1; 200–500 units/ml), lipase (Sigma, Type VI; 1–2 × 10^4 units/ml), phospholipase

FIG. 2. A parasagittal section through the anterior portion of spermatozoa prior to treatment (control). A, acrosome; ia, inner acrosomal membrane; n, nucleus; oa, outer acrosomal membrane; pl, plasma membrane. ×20,000.

FIGS. 3 and 4. Parasagittal sections through anterior portions of spermatozoa exposed to 3 percent unheated rabbit serum for 10 min, showing the acrosome reaction–multiple fusions ("vesiculation") between the outer acrosomal membrane and the overlying plasma membrane. The reaction is more advanced in the spermatozoa shown in Fig. 4 than in those in Fig. 3. The bulk of the acrosomal contents has been lost in both cases. Similar membrane vesiculation is seen in spermatozoa treated with reagents of group I listed in Table 1. ×20,000.

FIG. 5. A sagittal section through the anterior portion of a spermatozoon exposed to 3 percent unheated rabbit serum for 15 min, showing the completion of the acrosome reaction. ×25,000. Inset, the equatorial segment (e) of the acrosome at a higher magnification (×73,000); note that the plasma membrane and the outer acrosomal membrane are fused and continuous (arrow). Treatment of spermatozoa with reagents of group 1 listed in Table 1 produces the effects shown in this figure.

FIG. 6. Random disruption of sperm membranes following treatment of spermatozoa with 50 percent unheated rabbit serum for 5 min. ×14,000. Similar or harsher disruption of membranes occurs when spermatozoa are treated with excessively high concentrations of the reagents listed in Table 1.

C (Sigma, Type I; 0.25–5 units/ml), α-amylase (cryst., Sigma, Type II-A; 500–1000 units/ml), β-amylase (Sigma, Type I-B; 200–600 units/ml), cellulase (Sigma, Type II; 0.7–1.0 units/ml), α-glucosidase (Sigma, Type I; 3 units/ml), β-glucosidase (Sigma, 1 mg/ml), amyloglucosidase (Sigma, Grade II; 2000–4000 units/ml), β-galactosidase (Sigma, Grade III; 0.2 units/ml), and galactose oxidase (Sigma, Type I-B; 200–600 units/ml). Other enzyme preparations that were capable of inducing the acrosome reaction and activation of the spermatozoa were chitinase (Schwartz/Mann, 0.1–0.3 mg/ml) and α-glucosidase (Seikagaku Kogyo, 0.03–1 mg/ml); however, these enzymes were prepared from the fungus, *Streptomyces*, and it is likely that these preparations are contaminated with pronase.

Other reagents or conditions which proved to be ineffective in inducing activation and the acrosome reaction above the control levels within 2 h test period include: high and low pH values (8.5–9.5 and 6.0–7.0, BWW medium), hypo- and hypertonicity (160–230 m osmol and 320–410 m osmol, BWW medium), valinomycin (Sigma, 0.003–0.1 percent), oligomycin (Sigma, 0.001–0.03 percent), cytochrome C (Sigma, 0.003–0.1 percent), polymyxin B sulfate (Sigma, 60–6,000 USP units/ml), strophanthin-G (Sigma, 0.001–0.1 percent), strophanthidin (Sigma, 0.001–0.1 percent), sodium tetraphenylborne (K & K Lab., 0.0001–0.01 percent), acetylcholine chloride (Sigma, 0.1–10 mM), caffeine (Sigma, 0.001–0.01 percent), ethanol (0.1–3 percent), acetone (0.1–3 percent) and plant lectins (e.g., Concanavalin A, wheat germ lectin and *Ricinus communis* lectin; 5–250 mg/ml). Nicotin (Sigma, 0.1 percent) and phenylbutazone (Sigma, 0.03–0.1 percent) in BWW medium enhanced the motility of spermatozoa, but induced neither the acrosome reaction nor typical activation.

The mechanism(s) by which detergents (e.g., Hyamine and Triton), and several other reagents induced an accelerated acrosome reaction and/or activation of guinea pig spermatozoa is unknown at the present time. It is possible that these reagents rapidly modify surface components and/or structural components of the sperm plasma membrane itself and make the membrane permeable (or leaky) to external calcium ions which, according to Yanagimachi and Usui (1974), triggers the acrosome reaction and activation of guinea pig spermatozoa. How these reagents alter the membrane molecules and what kinds of molecular changes are induced by these reagents must be the subjects of further investigation. One thing which is evident from the present study is that guinea pig spermatozoa are potentially capable of an immediate acrosome reaction and activation upon leaving the epididymis. Thus all the components necessary for the acrosome reaction and activation appear to be within the spermatozoa upon the completion of their epididymal maturation.

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ADDENDUM

After the manuscript was submitted, Wooding published a paper (J. Reprod. Fert. 44, 185–192, 1975) in which he reported that treatment of bull spermatozoa with 0.01 percent Hyamine for 15 sec to 75 min induced vesiculation between the outer acrosomal membrane and the plasma membrane in 18–26 percent of the spermatozoa. Treatment of spermatozoa with bovine uterine fluid for 3 to 4 h also produced a similar reaction in 35–40 percent of spermatozoa. According to him, the reaction was blocked by azide rather than by the absence of calcium in the medium. Wooding studied the effects of Hyamine and uterine fluid with electron microscopy, and was not very sure if the acrosome-treated spermatozoa were motile. It would be interesting to learn if all the reagents (including Hyamine) reported in the present paper to be effective in inducing the accelerated acrosome reaction of guinea pig spermatozoa are equally effective for bovine spermatozoa as well as the spermatozoa of other mammalian species.

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RECOMMENDED REVIEWS

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