

## DETACHMENT OF ACROSOMAL CAPS OF BULL SPERMATOZOA BY COCONUT FATTY ACID MONOETHANOLAMIDE ETHOXYLATE

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The detergents at present in use for the separation of mammalian sperm acrosomes have unwanted side effects on the spermatozoa or on the acrosomes themselves. The present study was undertaken in an attempt to obtain a more satisfactory agent. Fresh unfermented coconut milk (CME), obtained from mature coconuts, was boiled and filtered into sterile Erlenmeyer flasks. After cooling, dihydrostreptomycin sulphate (1 mg/ml) was added. Half of the CME was mixed with an equal volume of 0.2 M-phosphate buffer, pH 7.4 (neutral pH CME) and the other half was mixed with an equal volume of 0.2 M-phosphate buffer, pH 5.9 (low pH CME). The buffered solutions were stored in a refrigerator at 2 to 4°C and used as sperm diluents within the week.

A commercial mixed liquid detergent solution (LD), comprising sodium tetrapropylene benzene sulphonate (NaTBS), sodium lauryl ether sulphate (NaLES) and coconut fatty acid monoethanolamide ethoxylate (Empilan) in the ratio of 4:1:1 was purchased as a 10% solution from Thika Wax Works, Nairobi. The pure components were kindly supplied by the management of Thika Wax Works as 10% solutions. Hyamine and Triton X-100 were purchased from Sigma Chemicals, U.S.A.

Good quality semen was obtained from several breeds of European (*Bos taurus*) and indigenous (*Bos indicus*) cattle from the Central Artificial Insemination Service of the Kenya Veterinary Department.

A fresh ejaculate was centrifuged at 1000 *g* for 15 min at 20°C. The pellet was resuspended in low pH CME and recentrifuged as above. This was repeated. The final pellet was resuspended in the low pH CME to a volume equivalent to that of the original ejaculate and the suspension was divided into several 1-ml aliquots.

In the first experiment, the effectiveness of LD and hyamine on the ability to release acrosomal lysozymes was compared. Three samples containing (1) no detergent, (2) 0.2% hyamine and 0.1% Triton X-100 and (3) 0.2% LD and 0.1% Triton X-100, were incubated at 37°C for 90 min. The samples were centrifuged at 500 *g* for 10 min at 20°C. The protein contents of the supernates was determined by the method of Lowry, Rosebrough, Farr & Randall (1951).

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In the second experiment, acrosomal detachment following separate exposure to the three components of LD, namely NaTBS, NaLES and Empilan was investigated. Aliquots (1 ml) of the sperm suspension containing 1% of the above detergents were incubated for 90 min at 37°C as before.

The third experiment was carried out to determine the minimal effective concentration of Empilan and the influence of temperature on acrosomal detachment. Aliquots (1 ml) of sperm suspension containing 0.02, 0.05, 0.1, 0.5 or 1.0% Empilan were incubated at 37°C, room temperature and 2 to 4°C for 2, 3, 4 or 5 hr. Thereafter, the samples were kept at 2 to 4°C and sperm smears were made at intervals of 24, 48 and 72 hr.

In the fourth experiment, the pH of the incubation was changed from 5.9 to 7.4. Aliquots (1 ml) of sperm suspension containing the same concentration of Empilan as above were incubated for the same time intervals.

Sperm smears were air-dried, fixed in absolute alcohol for 5 min and stained for 24 hr in filtered dilute Giemsa stain (2 ml of 0.2 M-phosphate buffer, pH 7.4; 3 ml concentrated Giemsa solution; 25 ml distilled water). The slides were washed in running distilled water to remove excess stain, dried and mounted in DePeX. Smears were photographed under oil-immersion at a magnification of 871 with a Leitz Wetzler orthomat.

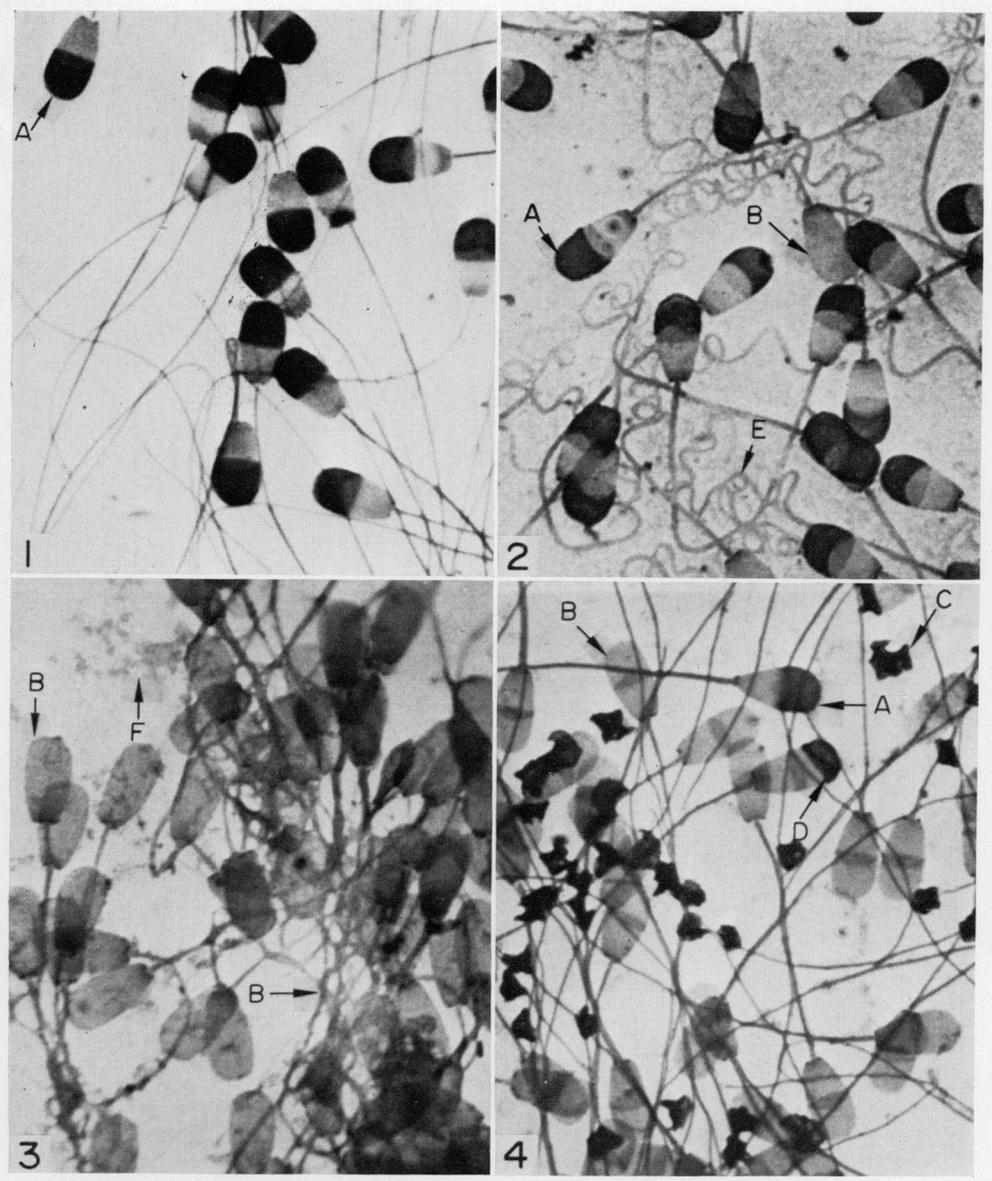
It was found that proteins released by hyamine and by the LD were similar in all the trials. The actual quantities varied with the population of spermatozoa in the original ejaculate. With both agents, separation of the acrosomes was complete. Most of the acrosomes in the hyamine-treated samples, however, were dissolved leaving very few intact acrosomal caps. The reverse was true in LD-treated samples in which intact caps were fairly numerous. There was a tendency for LD-treated spermatozoa to flocculate on incubation.

Exposure to NaLES caused little separation of the acrosomal caps but its effect on the sperm tails was quite marked. With short incubations, the tails tended to spiral but the spermatozoa remained separate; with longer incubation, the spermatozoa clumped together by their tails. The tails themselves showed evidence of swelling, breakage and dissolution of the plasma membranes (Pl. 1, Figs 1 and 2).

The extent of clumping was most marked with NaTBS even within minutes of addition. After incubation for 2 hr in a 1% concentration, all the acrosomal membranes had been dissolved away from the spermatozoa and the spermatozoa appeared in tight clumps (Pl. 1, Fig. 3).

The most effective separation of the acrosomal caps with minimal fragmentation or dissolution of the membranes was obtained with Empilan (Pl. 1, Fig. 4); the spermatozoa were intact and unfragmented, and the tails were straight, resembling those of the controls (Pl. 1, Fig. 1). Most spermatozoa in the other treatments had shed their acrosomes.

At pH 5.9, 1% Empilan was as effective at room temperature as it was at 37°C in causing acrosomal detachment. At 2 to 4°C, however, this concentration of Empilan caused the same degree of separation only after 3 days. When the pH was raised to 7.4, almost 100% acrosomal detachment was effected by incubation in 1% Empilan at 2 to 4°C for 2 hr. Even 0.1% Empilan at pH 7.4 was adequate to cause almost 100% detachment of acrosomal caps after incuba-



Appearance of bull spermatozoa after exposure to various detergents during incubation at 37°C for 2 hr followed by incubation at 2 to 4° for a further 70 hr. A = Spermatozoon with intact acrosome; B = spermatozoon with completely detached acrosome; C = detached acrosomal membrane; D = spermatozoon with partly detached acrosome; E = spiralled and swollen tails; F = acrosomal debris.

FIG. 1. Control spermatozoa.

FIG. 2. Spermatozoa after incubation in 1% sodium lauryl ether sulphate.

FIG. 3. Spermatozoa after incubation in 1% sodium tetrapropylenebenzene sulphate.

FIG. 4. Spermatozoa after incubation in 1% coconut fatty acid monoethanolamide ethoxylate (Empilan).

tion for 2 hr at room temperature or 37°C. This was regarded as the optimal operating condition.

Hathaway & Hartree (1963) found that cetyltrimethylammonium bromide (CTAB) caused dissolution of acrosomal membranes and clumping of the denuded spermatozoa. It is evident that CTAB, NaTBS and hyamine do not attack the acrosomal membranes specifically, but NaLES appeared to attack the sperm tails and the action of Empilan was mainly on the acrosomes. This demonstrates not only a difference in the acrosomal membranes from the rest of the sperm plasma membrane but also that specificity of action could be achieved by different detergents.

The staining characteristics of intact control acrosomes and those of acrosomes separated with Empilan were similar. The denuded spermatozoa that had been treated with Empilan also had the same pale-staining sub-acrosomal regions and red tails as the control spermatozoa. This suggests that Empilan did not effect acrosomal detachment through disruption or dissolution of the membrane but by loosening the cementing material between the acrosomal caps and the sperm heads.

The small quantities of Empilan needed to effect total separation and the relative unimportance of operating temperature favour its substitution in place of the detergents at present in use. It remains to be shown, however, that Empilan does not inactivate the released acrosomal enzymes. The demonstration by Topps & Elliot (1965) that the more powerful detergent, CTAB, did not inactivate acrosomal enzymes may indicate that Empilan would be safe to use in this respect.

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#### REFERENCES

- HATHAWAY, R. R. & HARTREE, E. F. (1963) Observations on the mammalian acrosome: experimental removal of acrosomes from ram and bull spermatozoa. *J. Reprod. Fert.* 5, 225-232.
- LOWRY, O. H., ROSEBROUGH, N. J., FARR, A. L. & RANDALL, R. J. (1951) Protein measurement with the Folin phenol reagent. *J. biol. Chem.* 193, 265-275.
- TOPPS, J. H. & ELLIOT, R. C. (1965) Enzymatic action of lipoglycoprotein preparation from sperm acrosomes on rabbit ova. *Nature, Lond.* 205, 498-499.