

## The appearance of new electrical properties in the plasma membrane of ram spermatids

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**Summary.** The « en bloc » staining of ram spermatids with electronegative colloidal iron at pH 9 caused particle precipitation only on the portions of plasma membrane covering both the postacrosomal lamina and the perinuclear ring. This area of the plasma membrane, implicated in fusion with the oocyte, was characterized by unique electrical properties appearing in the testis.

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### Introduction.

Morphological evidence (Fawcett, 1975), as well as differences in the biochemical composition of the plasma membrane, indicate that there are specialized zones on the spermatozoon cell surface (Koehler, 1978). Some of the components of this cell surface are specific to the male germ cell line (Gachelin *et al.*, 1976); they may be found all over the spermatozoon or only on the flagellum, around the mid-piece and on the subacrosomal zone of the plasma membrane (Millette and Bellve, 1977). This paper reporting ram spermiogenesis, describes the appearance and maintenance of special electrical properties of that portion of the plasma membrane covering the postacrosomal region of the sperm head and implicated in gamete fusion during fertilization.

### Material and methods.

Pieces of testis were fixed for 10 to 24 hrs with 1 to 5 p. 100 freshly depolymerized formaldehyde (Millonig and Bosco, 1967) in 0.07 M phosphate buffer at pH 7.4. The pieces were then washed for 24 hrs in the buffer and for 1 min. in distilled water before being incubated for 24 hrs at 4 °C in electronegative colloidal iron at pH 9, 10 or 11, according to Puvion and Banquet (1971). After washing for 1 hr at 4 °C with buffered solutions of the same pH and composition (iron excepted) as those of the incubation medium, the pieces were dehydrated and embedded in epon.

*Control blocks* were either acetylated for 4 hrs according to Lison (1953), deaminated for 1 hr according to Lillie (1954), or fixed with 4 p. 100 glutaraldehyde in 0.1 M phosphate buffer at pH 7.4 before being incubated in the colloidal iron solution.

Ultrathin sections were observed without post-staining using a Philips EM 300 electron microscope. The stages of spermiogenesis were evaluated according to Clermont and Leblond (1955).

## Results.

With the technique used, the only cellular component stained at pH 9 was the plasma membrane covering the postacrosomal lamina of old spermatids and testicular spermatozoa (fig. 3). The contrast progressively developed backwards during stage 12 (fig. 1) owing to the forming postacrosomal lamina (fig. 2). In addition, the membrane covering the perinuclear ring was stained from stage 10 to the end stage 12 (figs. 1, 2), and was unstained when the perinuclear ring disappeared in stage 13; the portion of membrane covering the postnuclear band was not stained (fig. 2). Staining intensity was lower in tissues incubated at pH 10 and was zero after incubation at pH 11.

Controls: « en bloc » acetylation (fig. 4) or nitration, as well as glutaraldehyde fixation, abolished membrane colloidal iron binding.

## Discussion.

According to Puvion and Banquet (1971), the mean diameter of electronegatively charged colloidal iron particles does not permit their penetration into intact cells. Thus, electronegative colloidal iron may be used as an extra-cellular tracer that binds the electropositively charged basic residues according to the pH of the incubation medium.

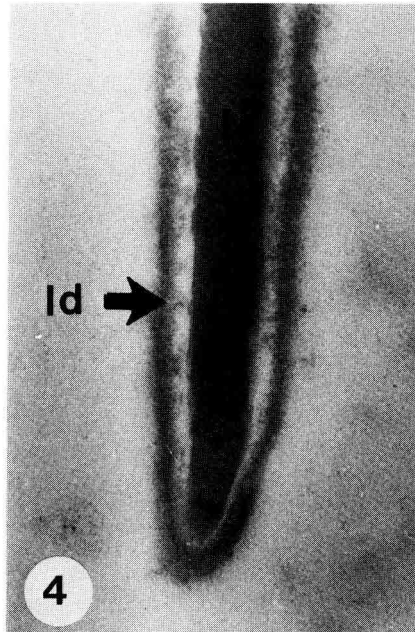
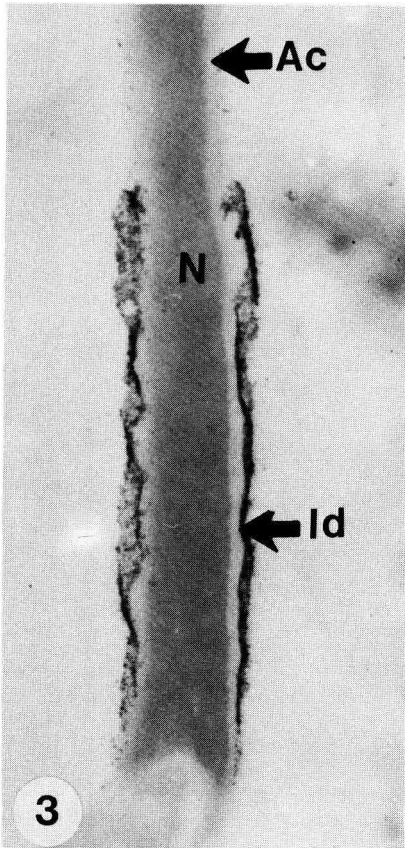
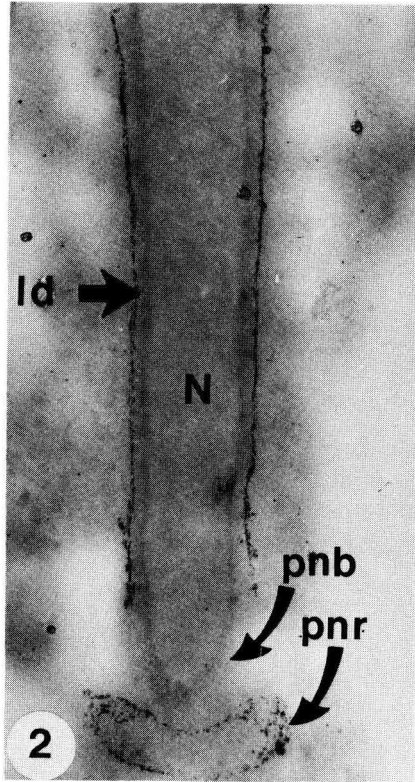
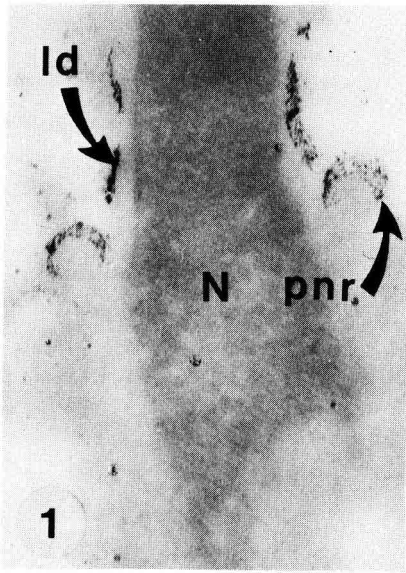
The absence of staining observed in the present study after glutaraldehyde fixation, nitration, or acetylation indicated that proteins were involved in colloidal iron binding. In this experiment using paraformaldehyde as a reversible fixation (Scott *et al.*, 1968), the fact that good binding could only be obtained with a pH lower than or equal to 10 indicated that there may be many more basic residues than acidic ones in the stained regions of the membrane. The most basic residues, arginine and lysine (Puvion and Banquet, 1971; Ganter and Jolles, 1969), have not been observed in spermatid plasma membranes after staining for lysine (Courtens and Loir, 1975) or for arginine (Loir and Courtens, 1979; Courtens, 1978a).

With these techniques, lysine residues were only found in some spermatid organelles. Of these, the previously described postnuclear band (Courtens *et al.*, 1976),

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FIG. 1, 2, 3. — Cells fixed with formaldehyde and stained « en bloc » with negatively charged colloidal iron at pH 9. Portions of the plasma membrane covering both the perinuclear ring (pnr) and the postacrosomal lamina (ld) are stained. The membrane covering the acrosome (Ac) and the postnuclear band (pnb) is not labelled. Fig. 1:  $\times 67\ 000$  mid-stage 12 spermatid. Fig. 2:  $\times 48\ 000$  end of stage 12. Fig. 3:  $\times 50\ 000$  testicular spermatozoon.

FIG. 4. —  $\times 42\ 000$ ; testicular spermatozoon fixed with formaldehyde and acetylated prior to staining with colloidal iron. The plasma membrane covering the postacrosomal lamina (ld) is not labelled.



perinuclear ring and postacrosomal lamina were nearest to the stained portions of the plasma membrane. This observation suggested at least three hypotheses to explain the binding of electronegatively charged colloidal iron to the plasma membrane :

- 1) the plasma membrane covering the perinuclear ring and postacrosomal lamina may be rich in basic residues such as asparagine, glutamine or histidine (pK = 9.8, 9.5 and 9.2, respectively) and/or very poor in acidic residues. This hypothesis does not explain why the plasma membrane covering the perinuclear ring lost its binding affinity when the ring disappeared ;
- 2) the electropositive charge of underlying organelles, i. e. the postacrosomal lamina and the perinuclear ring, might be sufficient to attract negatively charged colloidal iron through the plasma membrane ;
- 3) components of the postacrosomal lamina and perinuclear ring might closely penetrate the plasma membrane in some places.

The last explanation is plausible since regularly-spaced knobs have been observed on the surface of the postacrosomal lamina and the perinuclear ring in both freeze-etched specimens and ultrathin sections (Rattner and Brinkley, 1972 ; Koehler, 1966, 1970) ; they are very evident when the organelles are occasionally detached from the plasma membrane (Courtens, 1978b). The present observations do not allow a more definite explanation ; nevertheless, it appears that that portion of the spermatid which, in the spermatozoon will be involved in gamete fusion (Yanagimachi and Noda, 1970), has original electrical properties. These properties may be correlated with a special chemical composition or with the appearance of fertilization sites in the plasma membrane. Recent investigations indicate that they are unmodified in ram spermatozoa taken from both the epididymis and the female genital tract (Courtens and Delpech, 1979).

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**Résumé.** La coloration en bloc des spermatides de bélier par le fer colloïdal électronégatif à pH 9 révèle exclusivement les portions de membrane plasmique qui recouvrent la lame postacrosomique et l'anneau périnucléaire. Cette zone membranaire impliquée dans la fusion des gamètes présente des propriétés électriques particulières.

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