Ultrastructure of Rabbit Spermatozoa After Treatment with Lysolecithin and in the Presence of Hamster Somatic Cells[1] (37482)

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Description of the processes which occur during fusion between spermatozoa and somatic cells is available from a limited number of studies (1, 2). Moreover, these investigations were largely concerned with the technicological aspects of cell fusion and the initiation of DNA synthesis by spermatozoa within somatic cells. The latter phenomenon was observed only when spermatozoa were treated with lysolecithin (LL) but not when the fusion process took place in the presence of Sendai virus (1). The present studies were concerned with the changes that occurred in the ultrastructure of spermatozoa treated with LL and mixed with somatic cells.

Materials and Methods. Spermatozoa. Semen was obtained with an artificial vagina from adult Standard New Zealand rabbits. Cauda epididymidal contents were collected immediately after killing other rabbits. All spermatozoa were centrifuged (45*g*) 3 times in calcium-free Krebs–Ringer phosphate solution (37°) fortified with potassium penicillin (100 IU/ml) and streptomycin (50 μg/ml).

Somatic cells. For mixing experiments, primary cultures of F5-1 Syrian hamster cells transformed by Simian Virus 40 (SV40) were trypsinized, suspended in Hanks' solution and divided into pellets containing 5×10^6 cells/tube.

Mixing procedure in the presence of LL. Pellets containing 5×10^7 spermatozoa were pretreated for 1–3 min with LL (Supelco, Inc., Bellafonte, PA) that had been freshly prepared to a final concentration of 600 or 1200 μg/ml (3). The pretreated spermatozoa were mixed with 5×10^6 F5-1 cells in Hanks' solution. The cell mixture was centrifuged at 180*g* and the supernatant was discarded. The pellet was once more treated with LL (600 μg/ml) for 1–3 min, and 0.4 ml of inactivated (56° for 30 min) fetal bovine serum was added to stop the action of LL. Following recentrifugation at 180*g*, Eagle's basal medium was added to the cells and the pellets were held at 37° for 20 min.

Sampling; electron microscopy. Samples of the washed spermatozoa and the pretreated spermatozoa were removed for electron microscopy. Some of the cells were also removed from the mixture; those remaining were seeded in plastic petri dishes and placed in an incubator at 37°. The culture medium was changed every 2 days and samples of cells were fixed for electron microscopy after 20 min, 3, 18, 48, and 96 hr of incubation.

All samples were fixed in a cold (4°) 3%

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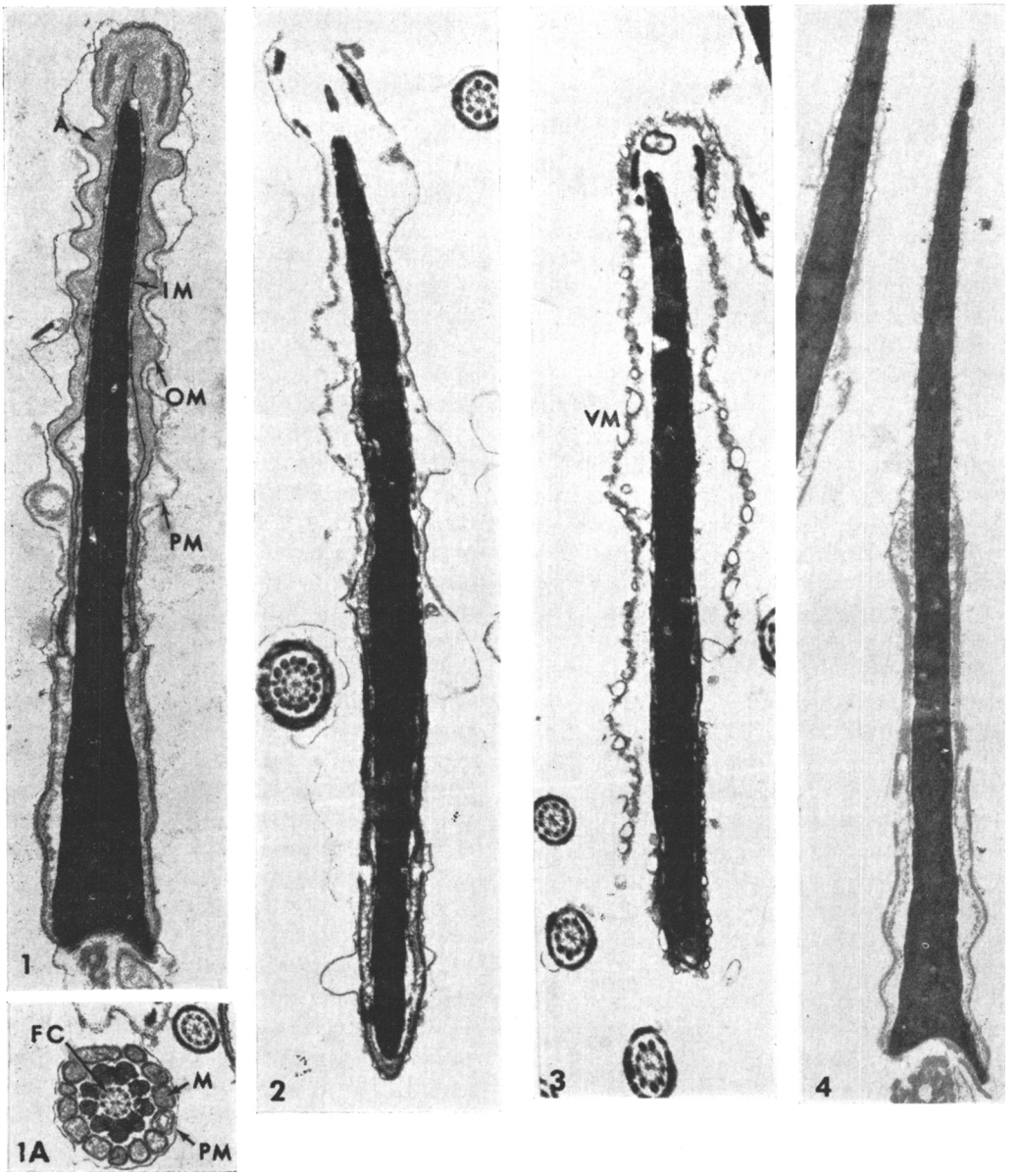

FIG. 1. Ejaculated rabbit sperm after washing. Note presence of the acrosome (A), and outer (OM) and inner (IM) acrosomal membranes. Wrinkling of the plasma membrane (PM) and OM is typical for sperm fixed after washing. ×20,300. (1A) As in Fig. 1. Cross section of the midpiece of the flagellum. Mitochondrial structure (M), axial filament complex (FC), and plasma membrane (PM) are visible. ×20,300. Figs. 1–4 were obtained from material fixed in glutaraldehyde–osmium in 0.05 *M* cacodylate buffer.

FIG. 2. Rabbit sperm after exposure to 600 μg/ml of lysolecithin. Loss of the acrosomal contents with partial disruption of the plasma membrane and outer acrosomal membrane is visible. ×20,300.

FIG. 3. Vesicles (VM) formed from fusion of plasma membrane and outer acrosomal membrane surround head of rabbit sperm after pretreatment with 600 μg/ml of lysolecithin. ×23,100.

FIG. 4. Rabbit spermatozoan after treatment with 600 μg/ml of LL. Complete disappearance of the plasma and the outer acrosomal membrane is sometimes seen after exposure of rabbit sperm to 600 μg/ml of lysolecithin.

glutaraldehyde solution for 2 hr, washed for 12 hr, then postfixed in 2% osmium tetroxide solution for 3 hr. Solutions used for fixing or washing were 0.05 *M* cacodylate buffer, 0.1 *M* cacodylate buffer, or Eagle's basal medium. After dehydration in ethyl alcohol solutions of increasing concentrations, the specimens were embedded in Epon 812. Thin sections were cut with a diamond knife, stained with uranyl acetate followed by lead citrate and then examined in either a Hitachi HS-8 (at 50 kV) or a Philips 300 (at 80 kV) electron microscope.

Results. Untreated spermatozoa. After washing, there was no visible difference in morphology between cauda epididymal and ejaculated spermatozoa. As has been noted by others (4), the plasma membrane and both the outer and inner acrosomal membranes were wrinkled and separated with 0.05 or 0.1 *M* cacodylate buffer (Fig. 1).

Spermatozoa after treatment with LL. In contrast to untreated spermatozoa, pronounced morphological changes were observed after treatment with LL (Figs. 2–4). In some spermatozoa, disruption of the plasma and the outer acrosomal membranes, with the disappearance of the acrosomal contents, was seen (Fig. 2). The inner acrosomal membrane, the subacrosomal cleft, and the equatorial segment remained intact. In the majority of pretreated spermatozoa, the inner acrosomal membrane was retained but, instead of the plasma membrane and outer membrane, a row of electron transparent vesicles was present (Fig. 3). This change appeared to be similar to that described (5) in capacitated rabbit spermatozoa undergoing the acrosome reaction at the zona pellucida. A few spermatozoa had lost most of their acrosomal contents and all of the vesiculated remnants (Fig. 4). Treatment with LL had no apparent effect on the ultrastructure of the nucleus, the midpiece and the remainder of the flagellum (Fig. 1a).

Effects of mixing. After being mixed for 20 min with F5-1 cells, pretreated spermatozoa were surrounded by cytoplasmic pseudopodia of the somatic cells. Usually between 1 and 6 spermatozoa were close to the surface of one somatic cell. When the mixed culture was maintained for 3 hr, spermatozoa adhered to the somatic cell membranes primarily at the midregion of the spermatozoal head where the subacrosomal substance remained.

At 18 hr after mixing, spermatozoa, found in the cytoplasm of somatic cells, had lost their plasma membrane, acrosomal contents, and most of their perinuclear material. Spermatozoa were seen frequently in cytoplasmic vacuoles in the somatic cells. In these spermatozoa, the subacrosomal cleft and the inner acrosomal membrane were still present. Moreover, the post acrosomal dense lamina were generally visible.

After 48 hr of culture, uniform disaggregation of spermatozoal chromatin was visible in one instance within the cytoplasm of a somatic cell (Fig. 5). The disaggregated chromatin was in the form of an ovoid body composed of loosely packed threads. The spatial relationship of the ovoid body to the midpiece of the flagellum suggests that it represents chromatin of the spermatozoal nucleus.

In most instances, the changes seen in spermatozoa ingested by somatic cells were degenerative in nature and were not dependent upon the age of the culture. Many spermatozoa persisted unchanged in cytoplasmic vacuoles of the somatic cells, for up to 96 hr of culture.

Discussion. In describing the effect of LL on somatic cells, Lucy (6) postulated that LL acts primarily by rearranging lipids of the cell membrane into a micellar arrangement. We have not determined whether the same mechanism is operative in spermatozoa treated with LL. However, exposure of spermatozoa to LL seems to potentiate their incorporation into F5-1 cells.

The loss of plasma and outer acrosomal membranes and acrosomal contents was accompanied by the appearance of small vesicles surrounding the heads of LL-treated spermatozoa. When LL-pretreated spermatozoa were placed in contact with somatic cells, in the presence of LL, encirclement of the spermatozoa by pseudopodia of the somatic cells occurred within 20 min. This was followed by the adherence of the spermatozoa at their midregion to the somatic cell cytoplasmic membrane.

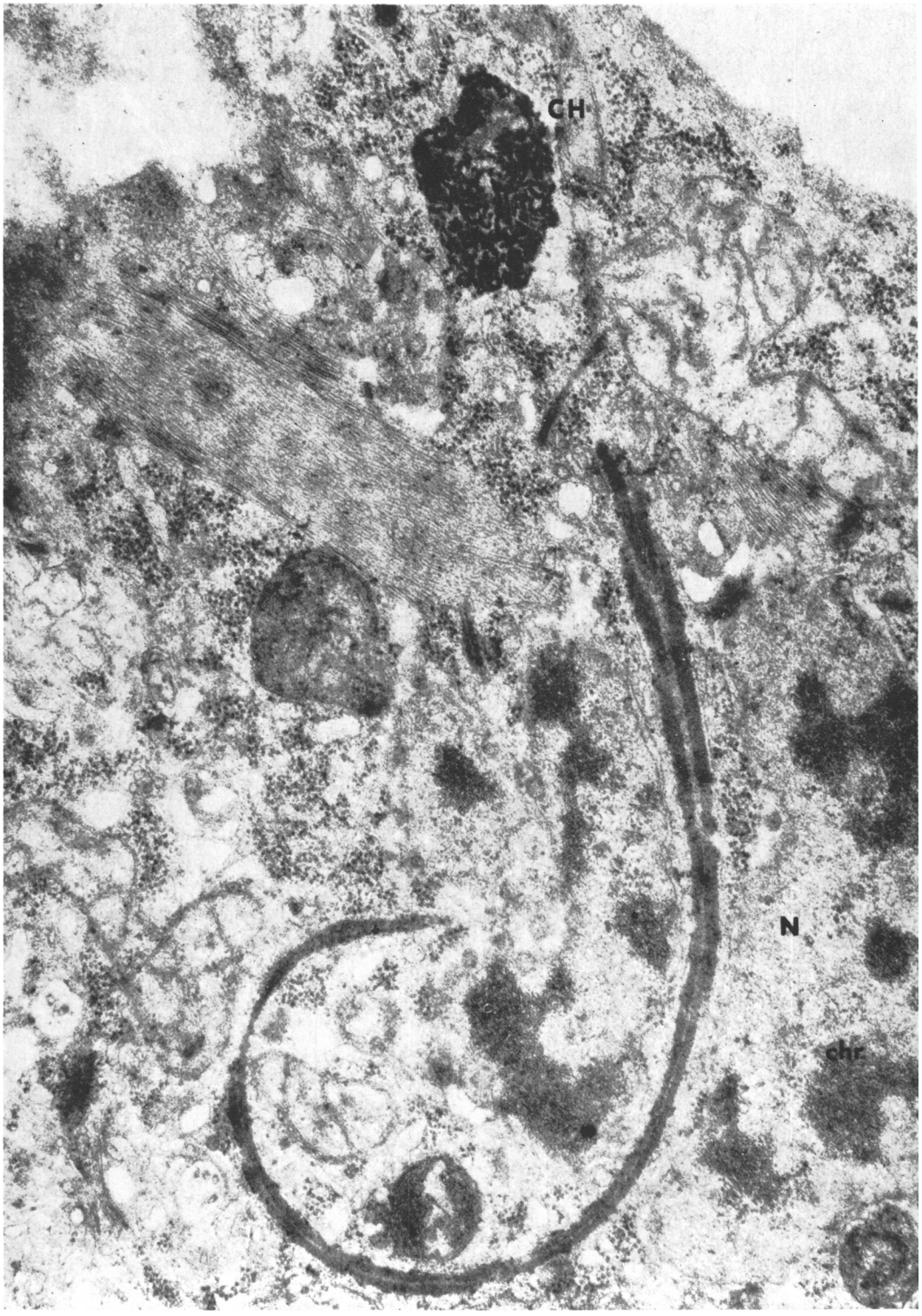

FIG. 5. Lysolecithin pretreated rabbit sperm 48 hr after mixing with F5-1 somatic cells in the presence of LL. Note the marked disaggregation of spermatozoal chromatin (CH). The spermatozoal flagellum is seen in the somatic cell cytoplasm. The F5-1 nucleus (N) displays a particularly clumped chromatin (chr). ×47,000.

The presence of vacuoles surrounding spermatozoa in F5-1 cells may indicate that spermatozoa had entered the somatic cell through phagocytosis (7). Alternatively, spermatozoa might have been attached to the wrinkled, invaginated, cytoplasmic membrane of the somatic cell and only appeared to be in a cytoplasmic vacuole. However, since degeneration of spermatozoal chromatin generally does not occur in an orderly sequence (5), the changes observed would suggest that spermatozoa are actually localized inside the cytoplasm of the somatic cell.

From the description presented here, it can be concluded that spermatozoa are affected by LL treatment in that the cell membrane is removed. Incorporation of spermatozoa, after treatment with LL, into somatic cells seems to be potentiated in a still unknown manner.

Summary. Rabbit spermatozoa pretreated with lysolecithin (LL) were mixed with SV40 transformed hamster somatic cells (F5-1 line) in the presence of LL and studied with the electron microscope. After LL pretreatment, small vesicles surround the spermatozoal head and appear to replace the plasma and outer acrosomal membranes. When mixed with F5-1 cells, pretreated spermatozoa are first encircled by somatic cell pseudopodia. Adhesion of the midregion of the spermatozoal head to membranes of the somatic cells, and incorporation of spermatozoa into somatic cells is potentiated in an undetermined manner.

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