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The spermatid manchette of mammals : formation and relations with the nuclear envelope and the chromatin

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Summary. This paper presents evidence that the microtubules of the manchette in ram, goat, boar, stallion and bull spermatids are linked to both the nuclear envelope and the chromatin by fibers transpiercing the nuclear envelope. It is suggested that this organization, allowing redistribution of chromatin prior to spermiation, is similar to half a mitotic apparatus.

Introduction.

Up to now, microtubules have been described in the spermatids of all the species studied. In annelids (Webster and Richards, 1977), insects (Friedlander, 1976 ; Godula, 1979), whip scorpions (Phillips, 1976), fishes (Zirkin, 1975), amphibians (Picheral, 1972 ; Sandoz, 1974), sauropsidae (Nagano, 1962 ; Nicander, 1967 ; McIntosh and Porter, 1967 ; Marchand, 1977 ; Maretta, 1977) and such mammals as protheria (Sapsford *et al.*, 1969 ; Phillips, 1970 ; Rattner, 1972 ; Harding *et al.*, 1976), rodents (Gardner, 1966 ; MacKinnon and Abraham, 1972 ; Rattner and Brinkley, 1972 ; Woloswick and Bryan, 1973), lagomorphs (Pedersen, 1969 ; Ploen, 1971), cats (Burgos and Fawcett, 1955), ongulates (Courot and Fléchon, 1966 ; Zirkin, 1971) and primates including man (de Kretser, 1969 ; Holstein, 1976), the microtubules are arranged to form a well differentiated caudal sheet or manchette around the nucleus. They remain less organized in hydrae (West, 1978), chaetognaths (Van Deurs, 1975) and scorpions (Phillips, 1973) but, as seen in other species, these spermatid microtubules are located near or in contact with the nuclear envelope (NE), and are only present during the nuclear elongation phase.

This situation leads numerous authors to postulate that the microtubules exert a mechanical role in shaping the spermatid nuclei. However, an alternative hypothesis — the establishment of a macromolecular lattice between the DNA and the nucleoproteins — has been proposed by Fawcett *et al.* (1971) and Loir and Courtens (1979).

This controversy indicates that the role(s) of the manchette remains to be established. The present paper reports new data on the relations of the manchette and the nucleus in the spermatids of five mammalian species.

Material and methods.

Small pieces of ram, boar, stallion, goat and bull testes, obtained after the castration of anaesthetized animals, and the nuclei of ram spermatids, prepared by sonication as described by Loir and Courtens (1979), were fixed with 4 p. 100 glutaraldehyde in 0.1 M phosphate buffer, pH 7.4 for 24 h (1 h at 20 °C and 23 h at 4 °C) before being osmicated for 2 h with 2 p. 100 osmium tetroxide in 0.2 M phosphate buffer and embedded in Epon 812. Ultrathin sections, mounted on bare copper grids, were stained with uranyl acetate and lead citrate. The steps of spermiogenesis are those described by Courtens and Loir (1981) for rams, goats, boars and stallions, and by Courtens *et al.* (1980) for bulls.

Results.

1. *The nuclear envelope in early and full step* 8 *spermatids.* — In the five studied species, the NE displayed 3 zones before any microtubules appeared in the cytoplasm.

1) In the most anterior zone, located under the acrosome, the NE was closely apposed to the chromatin. The two NE membranes were contiguous, and no pores were visible (fig. 1).

2) The equatorial zone of the NE, located just beneath the posterior part of the acrosome, was apposed to the caudally-developing peripheral layer of dense chromatin. The two nuclear membranes were separated by a wider space, as under the acrosome. No pores were visible (fig. 1).

3) The posterior part of the NE was apposed to the chromatin not already condensed into a layer of peripheral chromatin. The two membranes were separated by a large space, and numerous pores were present (fig. 1).

2. *The microtubules of the manchette in late step* 8 *spermatids.* — The microtubules were observed as isolated, straigth, short organelles always located near zones 2 and 3 of the NE (fig. 2). When present in zone 2, they were oriented antero-posteriorly and

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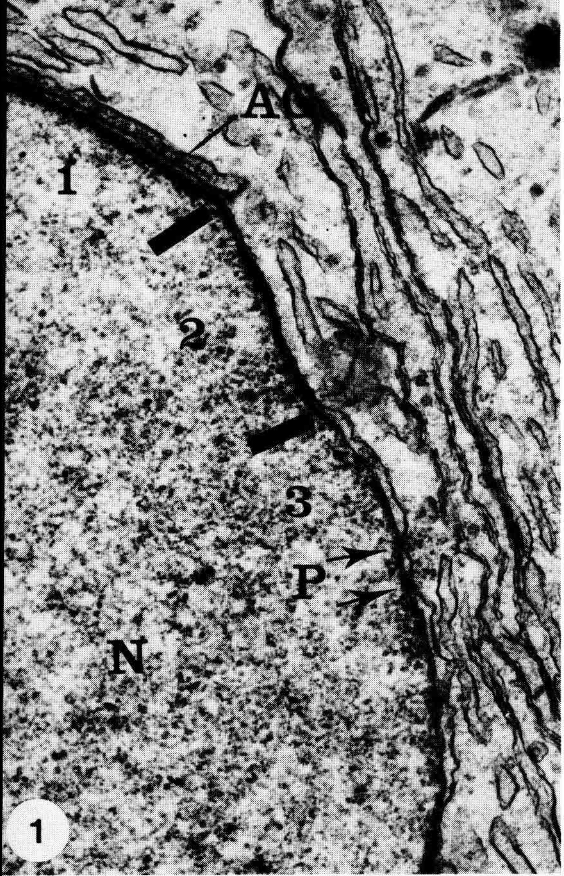
FIG. 1. — *Early step* 8 *goat spermatid* : the two NE membranes are very close together under the acrosome (AC) in zone 1 ; they are separated by larger spaces in zones 2 and 3. Nuclear pores (P) only occur in zone 3. Condensation of the peripheral chromatin layer is not complete in zone 3, as in the other zones. N = nucleus. × 26 000.

FIG. 2. — *Middle step* 8 *stallion spermatid* : numerous brush-like projections are present along the NE ; some of these are linked to microtubules (M) in zone 2. Nuclear pores are only present in zone 3. F = flagellum ; N = nucleus. × 10 000.

FIG. 3. — *Early step* 9 *boar spermatid* : microtubules (M), not in contact with the plasma membrane (PM), form a conical-shaped manchette. AC = acrosome ; F = flagellum ; N = nucleus. × 15 000.

FIG. 4. — *Step* 9 *ram spermatid* : microtubules (M), linked to the NE (arrows), are now longer. The perinuclear ring is not present (compare with fig. 5). N = nucleus ; PM = plasma membrane. × 42 000.



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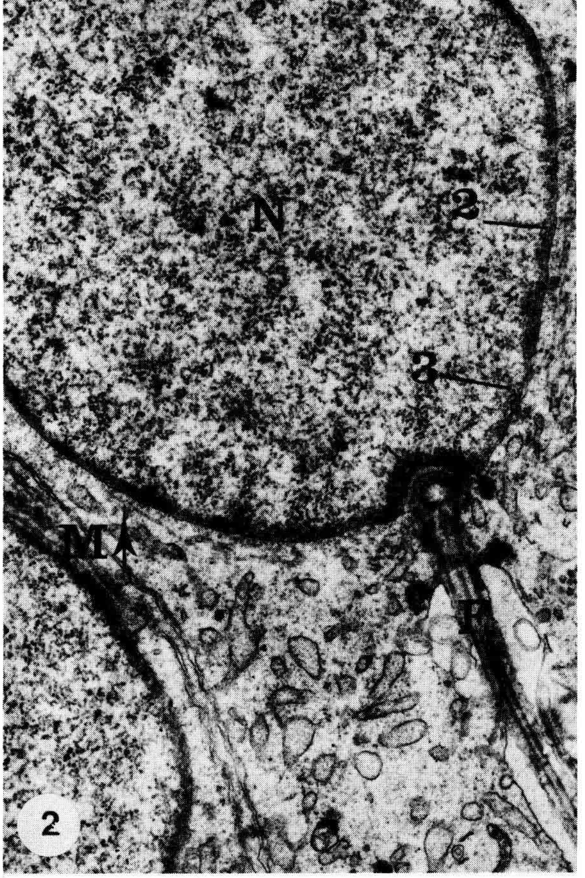

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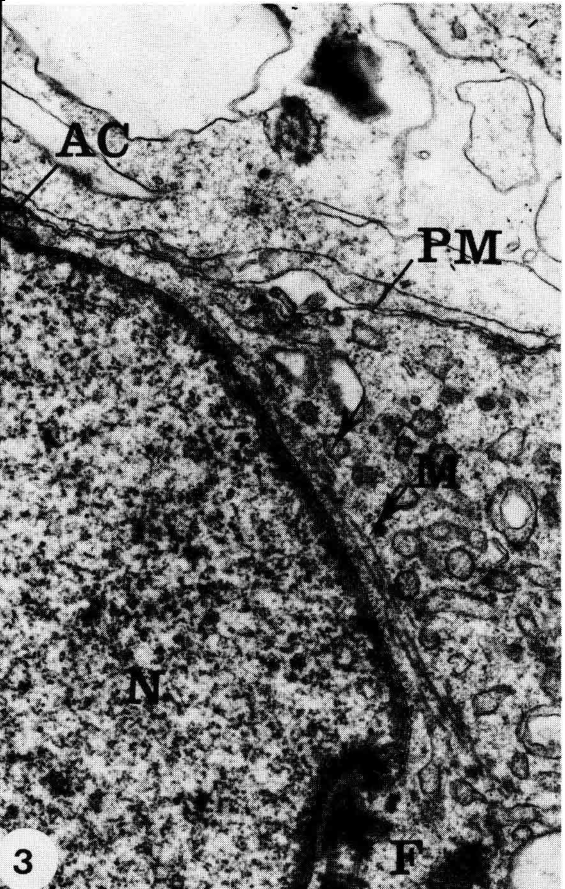

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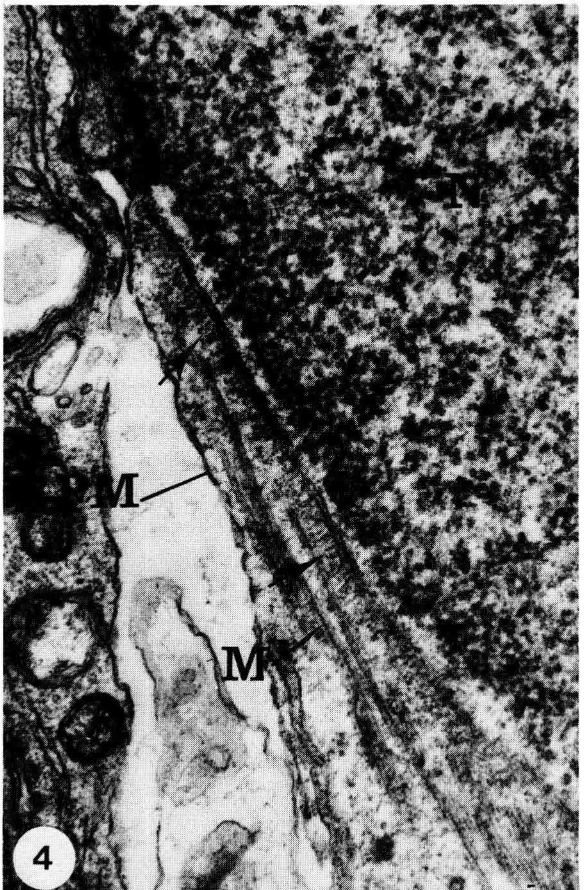

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were linked to the envelope by numerous short, brush-like projections perpendicular to the nuclear surface.

These projections were present before the microtubules were joined to the envelope (fig. 2). In conveniently oriented sections, the anterior end of each microtubule, free in the cytoplasm, was surrounded by small patches of electron-dense amorphous material (fig. 3).

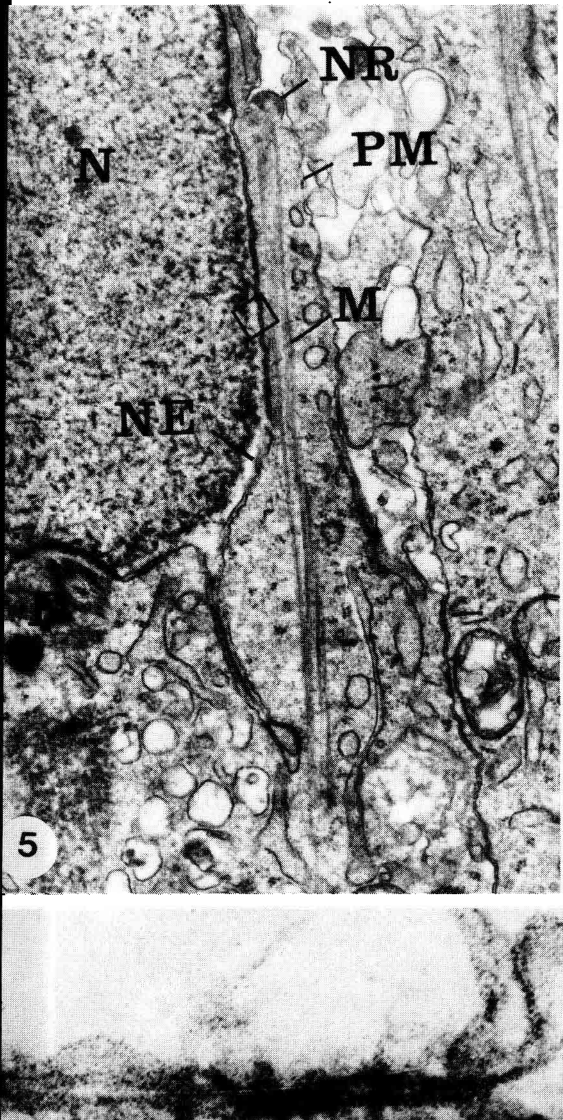
3. *The nuclear envelope and manchette in step* 9-10 *spermatids.* — When the compaction of the peripheral chromatin layer was complete, the NE was progressively lifted from the chromatin. The separation started at the posterior part of the nucleus and progressed anteriorly until all portions of the NE, not covered by the acrosome or the implantation fossa of the flagellum, were separated from the condensed chromatin (fig. 4). Numerous fibers were present between the condensed chromatin and the detached NE (fig. 5) ; some transpierced the two NE membranes, joining both the chromatin and the manchette (fig. 8). The manchette microtubules were longer than those observed in step 8 and formed almost parallel arrays arranged in several layers surrounding the nucleus. They were then attached together by many bridges, and those located nearest the nucleus were linked to the raised NE in the zone devoid of nuclear pores (figs. 5, 6, 8). The manchette was conical-shaped. Patches of electron-dense material, present at the anterior part of the manchette, were common to 3 or 4 tubules which did not make contact with the plasma membrane in very early step 9 spermatids (fig. 4), but later joined a thickened part of that membrane (the nuclear ring) in full step 9 goat, ram, boar and bull spermatids and in step 10 stallion spermatids (fig. 5).

4. *The nuclear envelope and the manchette in step* 10 *and* 11 *spermatids.* — In step 10 spermatids, the manchette formed a flattened cylinder composed of parallel microtubules. Its shape in cross-sections corresponded to the shape of the nucleus but was larger (figs. 5, 6, 8). Links between the microtubules and between the manchette and the NE were numerous. The NE was lifted from the chromatin, forming the nuclear pocket (figs. 5, 6, 8) which was filled with a reticular array of fibers joining both the chromatin and the NE or both the chromatin and the manchette through the nuclear membranes (fig. 8). The microtubules were longer in step 11 than in the preceding steps.

FIG. 5. — *Step* 10 *bull spermatid* : the manchette (M) is linked to the plasma membrane (PM) by the nuclear ring (NR). The NE is detached (square) from the chromatin (N). × 13 000.

FIG. 6. — *Step* 11 *stallion spermatid* : cross-section of the nucleus (N). The microtubules of the manchette (M) are linked together (single arrow) and to the nuclear envelope (double arrow). × 26 000.

FIG. 7. — *Step* 13-14 *ram spermatid nucleus after sonication* : after the disappearance of the manchette, the nuclear envelope (NE) is transpierced by numerous fibers originating in the chromatin. × 190 000.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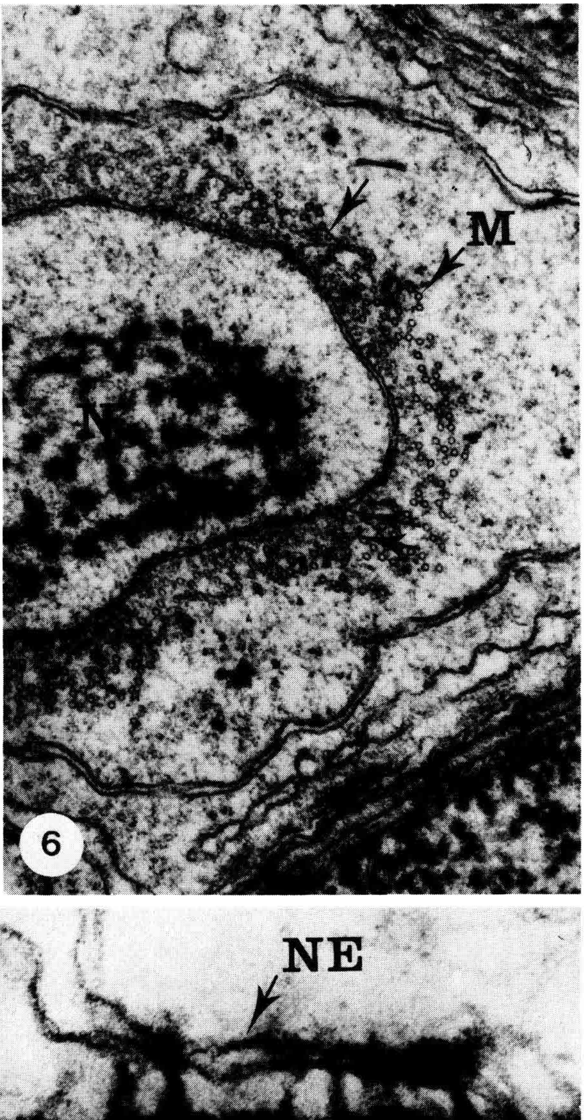
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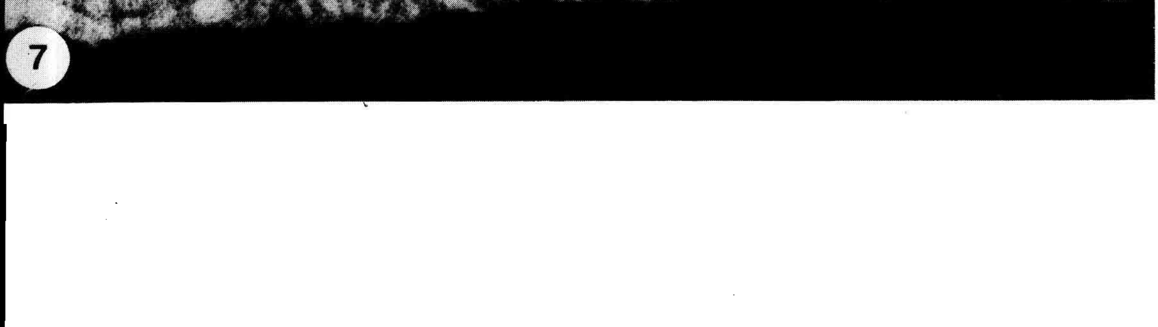
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5. *The nuclear envelope and the manchette in step* 12 *spermatids.* — The manchette slipped backwards along the NE. During this process, the microtubules remained parallel. The manchette, wider in front of the redundant NE, remained attached to the lateral part of the nuclear pocket by means of connecting fibers (fig. 9). These or other links, also present on the most posterior part of the redundant NE, were not fixed to the manchette (fig. 9). In many zones, the fibers transpierced the NE and joined the chromatin through the nuclear pocket (fig. 9). The perinuclear ring also slipped backwards along the plasma membrane (fig. 9). When the manchette reached the posterior part of the nucleus, the microtubules dissociated, leaving the nuclear ring in place along the plasma membrane before it, in turn, disappeared (fig. 10).

6. *The nuclear envelope in sonicated step* 12-14 *spermatids.* — The sonication of spermatids destroyed the cytoplasm and most of the nuclei. Only step 12-15 spermatid nuclei resisted destruction. The NE, better resolved in material treated that way, was lifted from the nucleus and transpierced by fibers originating in the chromatin and ending in the cytoplasm (possibly the perinuclear substance) (fig. 7). Fiber number or concentration was greater than that when the manchette was present in the earlier steps of spermiogenesis in intact cells.

Discussion.

The present morphological data on the appearance or assembly of manchette microtubules in ram, goat, stallion, boar and bull spermatids have been previously described in other species (Bray, 1979 ; Raff, 1979 ; see Introduction) or are evident in many photomicrographs already published on this subject. However, the relations of the microtubules with the NE have not received much attention in mammals, except for the rat in which relations with the redundant NE are fully documented (MacKinnon and Abraham, 1972).

Our results show that before any manchette microtubules are present inside the cytoplasm, zones of the external NE membrane are predifferentiated, for further microtubular binding, by short perpendicular projections facing the cytoplasm. Observation

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FIG. 8. — *Step* 11 *stallion spermatid* : fibers transpierce (arrows) the NE. Some are linked to the manchette (M), others to both the manchette and the condensed chromatin (double arrow). × 45 000.

FIG. 9. — *End of step* 12 *stallion spermatid* : the nuclear ring (NR) and the manchette (M) slip backwards (large arrows), being attached to the redundant NE by fibers (arrows), some of which pass through both membranes. Fibers, not linked to the manchette, are seen in zone 2. Nuclear pores (NP) face the flagellum (F). × 42 000.

FIG. 10. — *Early step* 13 *bull spermatid* : the manchette has disappeared. The nuclear ring (NR) is still present. The nuclear pocket (NPK) contains 2 basal knobs (BK), one of which is seen in the section. × 40 000.

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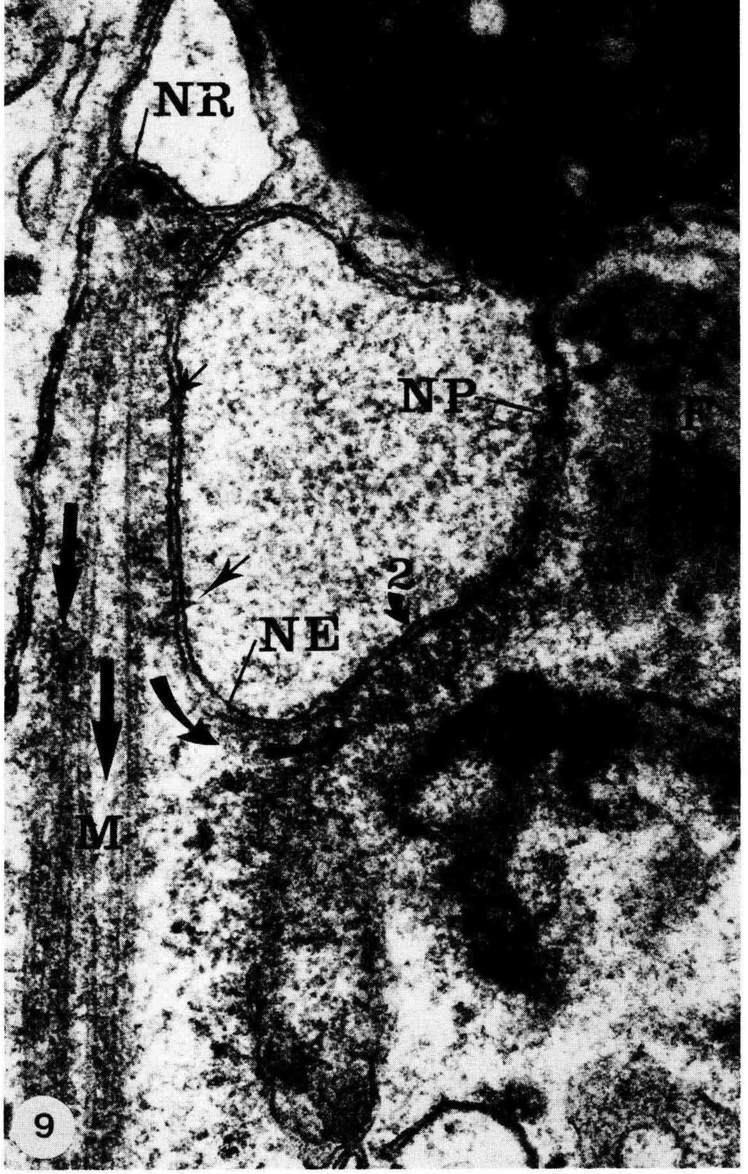

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of these projections reveals that they are only present in front of the zone of condensing chromatin, and that they face other fibers between the inner membrane of the NE and the condensing chromatin in such a way that the NE is the anchorage zone of both the intra and extra-nuclear fibers.

The short microtubules attached to the NE first form a coat apposed to the hemispherical-shaped posterior part of the nucleus. Progressive microtubular elongation, together with the formation of bridges between the microtubules, has been observed in many species (Courot and Fléchon, 1966 ; MacKinnon *et al.*, 1973). Considering that these inter-microtubular links have almost constant dimensions in a given step of spermiogenesis (MacKinnon *et al.*, 1973) and that the number of microtubules is constant for a given species (Rattner and Brinkley, 1972), the only possible shape the manchette could take is that made by parallel walls and/or bundles, provided that the bridges occur all along the neighboring microtubules and that all the microtubules are of equal length. This is the shape of the manchette reported in most mammalian species, and we believe that its change in shape from hemispherical to conical and finally to a flat cylinder, is the result of linkage between adjacent microtubules and of microtubular elongation. The subsequent lifting of the NE from the chromatin suggests that the manchette plays a mechanical role in this phenomenon. The resulting nuclear pocket would facilitate chromosomic movements during spermiogenesis. That these movements occur is suggested by the work of von Hofgartner *et al.* (1978) in vertebrates ; they are observed when the chromosomic centromeres are stained by appropriate techniques, as in urodeles (MacGregor and Walker, 1973 ; Schmid and Krone, 1976) and birds (Dressler and Schmid, 1976). The chromosomic centromere regions in these species are progressively separated into two sets, the first one corresponding to a chromosome under the acrosome, and the second set to the other chromosomes which migrate backwards during nuclear elongation, finally stopping at the posterior part of the nucleus. In birds, the second set is divided into two small symmetrical patches near the implantation plate of the flagellum (Dressler and Schmid, 1976). In mammals, we suggest that such movements exist and that the basal knobs would result from centromere migration.

The arguments in favor of this hypothesis are the following :

1) The basal knobs are formed only in late elongating spermatids, just before the manchette disappears (Courtens and Loir, 1981).

2) The basal knobs display specific staining properties and would thus be composed of heterochromatin (Loir and Courtens, 1979).

3) The present results give further data on the way the knobs may be formed, considering that the links observed between the manchette, the NE and the condensing chromatin slip backwards along and through the NE when the manchette moves backwards. The anatomical arrangement we describe, providing a stable support for slipping nuclear material, may, in some way, be similar to half a mitotic apparatus. Slippage through the persisting NE is common during protozoal mitosis (Mazia, 1961). However, the chromatin structure in mitosis and spermiogenesis is not the same. The movement of individual chromosomes in the mass of condensing chromatin, specific of elongation spermatids, may be difficult to imagine. However, these movements may be facilitated when nucleoproteins, responsible for early chromatin condensation, are lost from

the nuclei. In the presently studied species, this takes place when the manchette slips backwards, the basal knobs are formed (Courtens and Loir, 1981) and the DNA is somewhat unmasked (Darzynkiewicz *et al.*, 1969 ; Loir and Hochereau-de Reviers, 1972). Other evidence of chromosomic movement is found in salamander spermatids whose chromatin is almost fully condensed (MacGregor and Walker, 1973) when the centromeres migrate backwards (Schmid and Krone, 1976).

Because intra-nuclear movements and manchette slippage would occur at the same time in the same direction, it seems reasonable to postulate that the bridges between the manchette and the chromatin are not motile with respect to the manchette, and that the manchette's movement is related to the intra-nuclear movement of the chromatin. The resulting advantage of such a movement would appear at the time of male pronucleus formation, the centromeres being near the future sperm aster. Spermiogenetic nuclear events would thus be equivalent to (i) a metaphase (chromatin condensation phase) of the haploid genome, and (ii) an anaphase taking place in the direction opposite to the acrosome.

The numerous fibers transpiercing the NE of old spermatids, when the manchette has disappeared, differ both in number and length from those present earlier. Their significance will be discussed elsewhere (Courtens and Loir, in preparation).

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Résumé. Les microtubules de la manchette des spermatides de Bélier, Taureau, Etalon, Verrat, et Bouc sont reliés à la fois à l'enveloppe nucléaire et à la chromatine par des fibres traversant l'enveloppe nucléaire. Cette relation suggère que la manchette est équivalente à un demi-fuseau achromatique à partir duquel s'organise la redistribution de la chromatine avant la spermiation.

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