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The Manchette in Stage 14 Rat Spermatids: A Possible Structural Relationship with the Redundant Nuclear Envelope

E. A. MacKinnon and J. P. Abraham
Department of Anatomy, Queen's University, Kingston, Ontario

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Summary. The manchette or caudal tube has been examined in Stage 14 rat spermatids. The microtubules of the caudal tube have been found to be partially sheathed by smooth endoplasmic reticulum which appears to be continuous with the outer nuclear membrane of the redundant nuclear envelope. The microtubules in caudal regions of the manchette have been noted to be interconnected by links of unusual size and morphology. It is suggested that the caudal tube consists at this stage of development of two structures, membrane and microtubules and that the links between the microtubules appear to play a role in the structural order noted in the position of the tubules of the manchette. The possible significance of these links in relation to motility is discussed.

Key words: Spermiogenesis — Rat — Spermatids — Manchette — Microtubules.

Introduction

The microtubules of the manchette in rooster spermatids have been exactingly described and considered partially functional in the development of sperm head anisometry (McIntosch and Porter, 1967). Systems of microtubules possibly analogous to the vertebrate manchette have also been described in various invertebrates (see review in Clark, 1967). However, since the pioneering work of Burgos and Fawcett (1955) only occasionally has attention been directed towards manchette ultrastructure in mammalian species (Sotelo and Trujillo-Cenóz, 1958; Porter, 1965).

We are undertaking a study of changes that occur in manchette morphology during rat spermiogenesis. The present article concerns Stage 14 spermatids in which the microtubules of the manchette, or caudal tube, and membranes originating in close proximity to or from the redundant nuclear envelope (Franklin, 1968) appear to be structurally related. At this particular stage of spermiogenesis unusual links exist between microtubules in the caudal regions of the manchette which to our knowledge have not been previously described.

Materials and Methods

Adult male Wistar (Woodland) albino rats were killed and their testes were immediately removed. The tissue was flooded with 3.5% glutaraldehyde (Sabatini *et al.*, 1963) buffered to pH 7.2–7.5 with 0.1 M phosphate buffer. Fixation was carried out at room temperature for two hours, during which the seminiferous tubules were sliced into short pieces. The tubules were rinsed in 6.5% sucrose washing buffer for two hours, postfixed in cold 1% OsO_4, buffered with 0.1 M sodium phosphate pH 7.2–7.4 for $1^1/_2$ hours and then embedded in Epon

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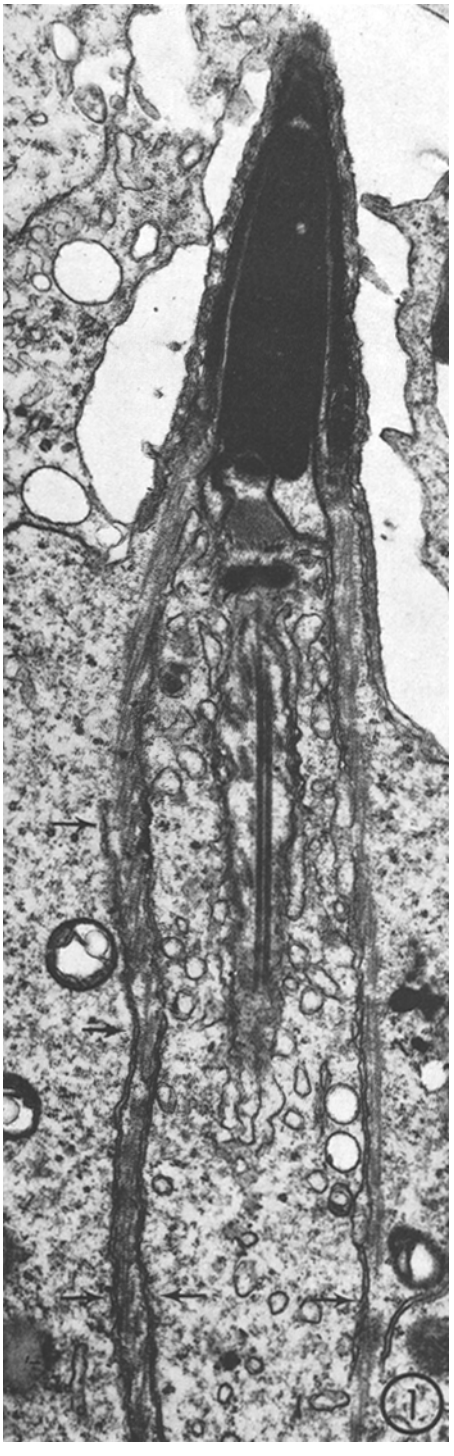


Fig. 1. A Stage 14 spermatid in median section. Arrows point to membranes on either side of the caudal tube. × 8200

(Luft, 1961). Sections were cut on a Reichert OMU 2 ultramicrotome and stained with uranyl acetate and lead citrate (Venable and Coggeshall, 1965). Electron micrographs were taken with a Hitachi HU-11 E electron microscope operated with an accelerating voltage of 75 kV on $3^1/_4 \times 4$ high contrast Kodak Projecter Slide plates.

Stage 14 spermatids and their particular cell associations were recognized according to the criteria of Clermont and Perry (1957) on thick sections of epon embedded tubules stained with toluidine blue (Bencosme *et al.*, 1959). Our analysis in this report is limited to the caudal portions of the spermatid heads and the cytoplasm adjacent to this region. Fig. 13 represents a diagram of a Stage 14 spermatid. As much, it is meant to contain information pertinent to this study, some of which is not illustrated by electron micrographs for reasons of illustrative economy.

Observations

The axial filament in Stage 14 spermatids is partially surrounded by a cylindrical structure consisting of two unit membranes and numerous smooth surfaced vesicles (Figs. 1, 2, 9, 12). This structure appears to increase in diameter in a caudal direction from the nucleus (Figs. 9, 12). Both the axial filament and its membranous sheath are partially enclosed by additional double unit membranes which appear on either side of anterior regions of the caudal tube (Figs. 1–3) in 14 and earlier stage spermatids. Sections in various planes reveal that the inner membranes of this second sheath often appear to completely underlie the manchette microtubules whereas the outer membranes more frequently appear to be incomplete (Figs. 1, 2).

The inner membranes of the sheath surrounding the microtubules of the caudal tube, or manchette, are closely

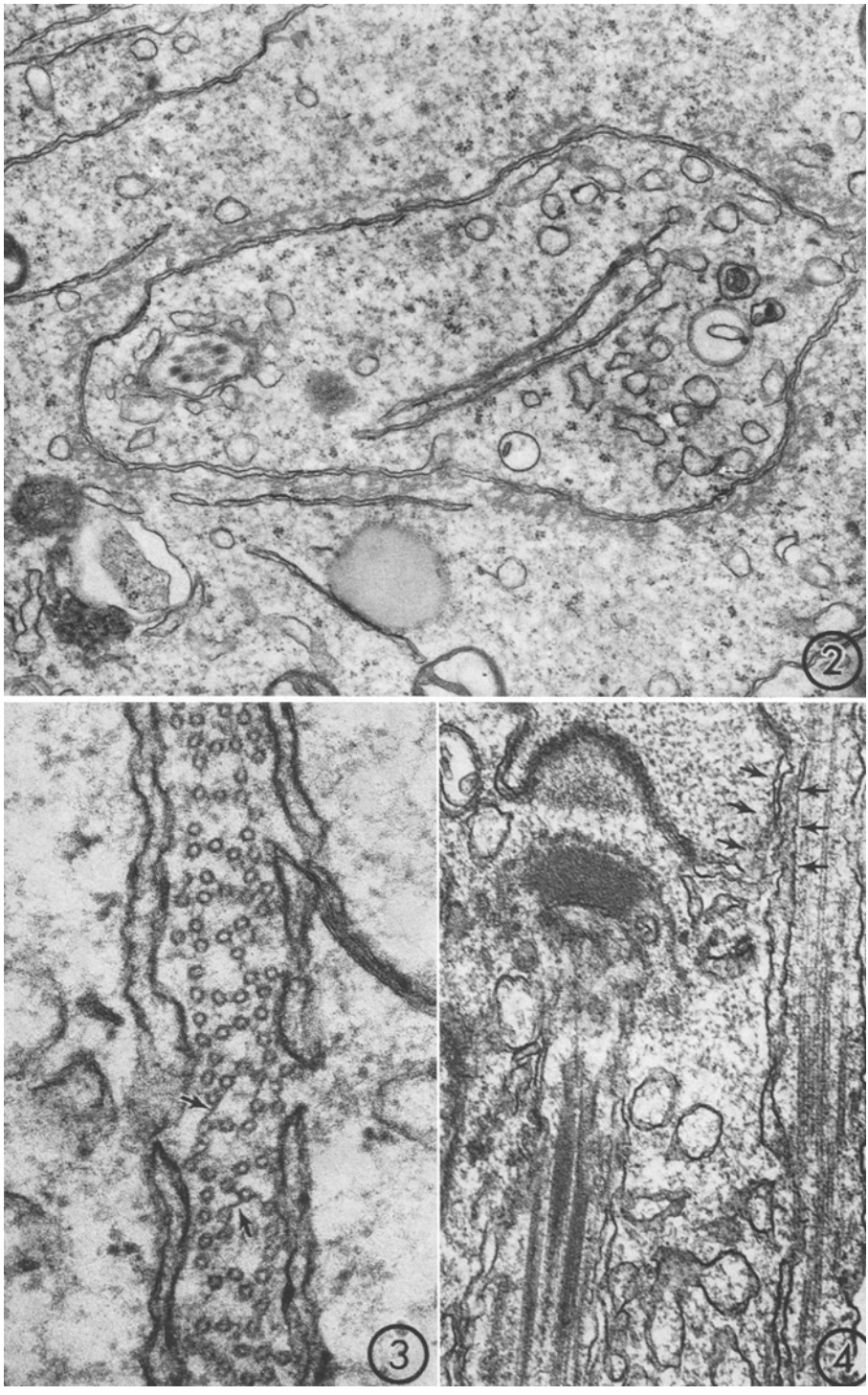


Fig. 2. Stage 13 spermatid cytoplasm in oblique section. The inner membranes appear to completely line the manchette microtubules whereas the outer membranes are sparse. × 8900

Fig. 3. Cross section of the caudal tube in an area where inner and outer membranes are present. Links (arrows) are obvious between microtubules. × 41000

Fig. 4. In this section the redundant nuclear envelope (arrow) appears closely associated with the inner membranous sheath of the caudal tube. × 38000

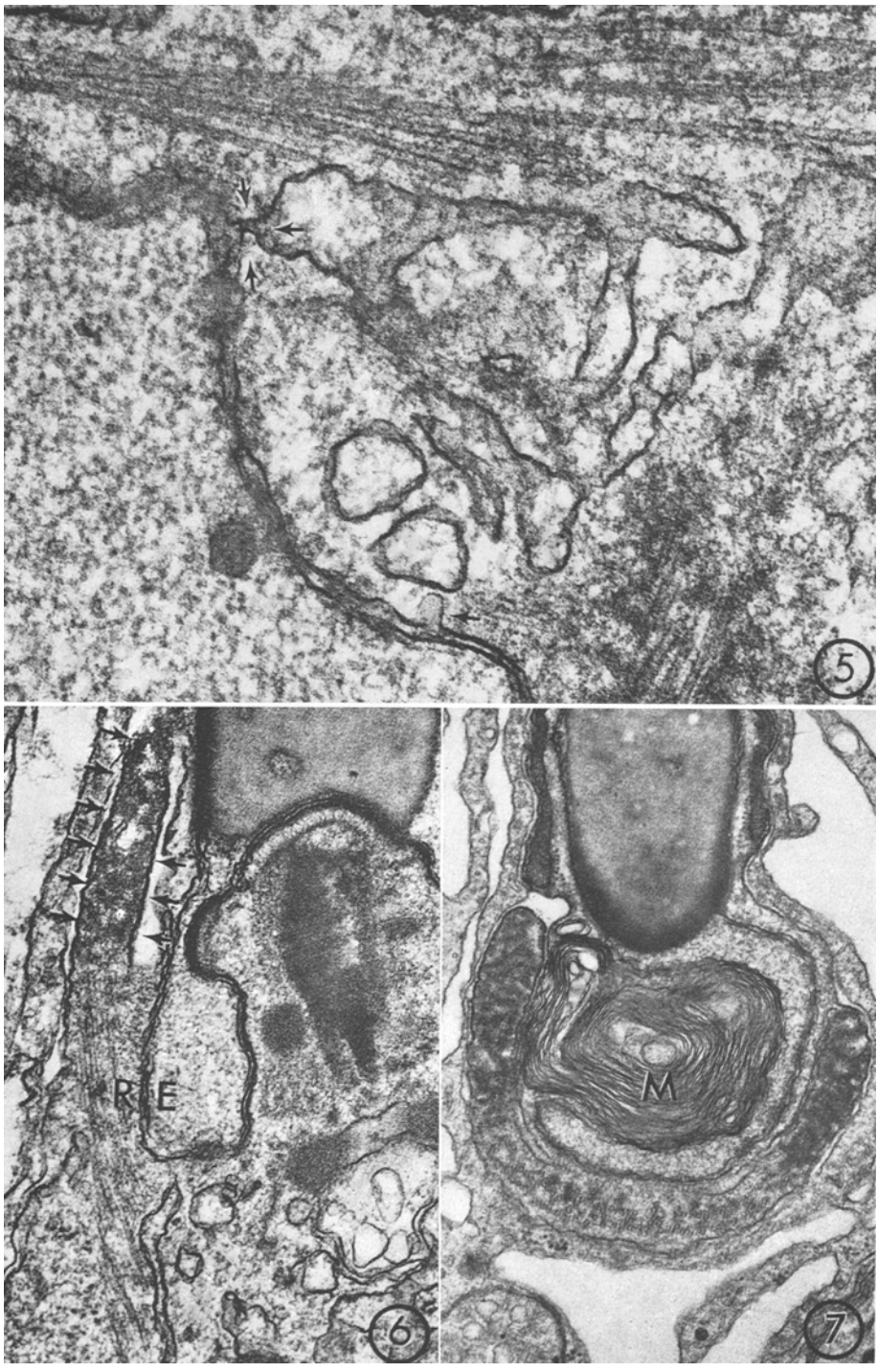


Fig. 5. Membrane apparently originating from the outer nuclear membrane (arrows) is closely associated with the manchette microtubules. Numerous nuclear pores are obvious in the redundant envelope. Another small outpocketing of the outer nuclear membrane is visible (arrow). × 85000

Fig. 6. A portion of median section demonstrating the manchette insertion ring (arrows). *RE* redundant envelope. × 37000

Fig. 7. Saggital section demonstrating a portion of the manchette insertion ring which sits as a collar around the caudal regions of the nucleus. The height of the ring is reflected in the position of the microtubules in the central regions of the collar. *M* Myelin Figure. × 32000

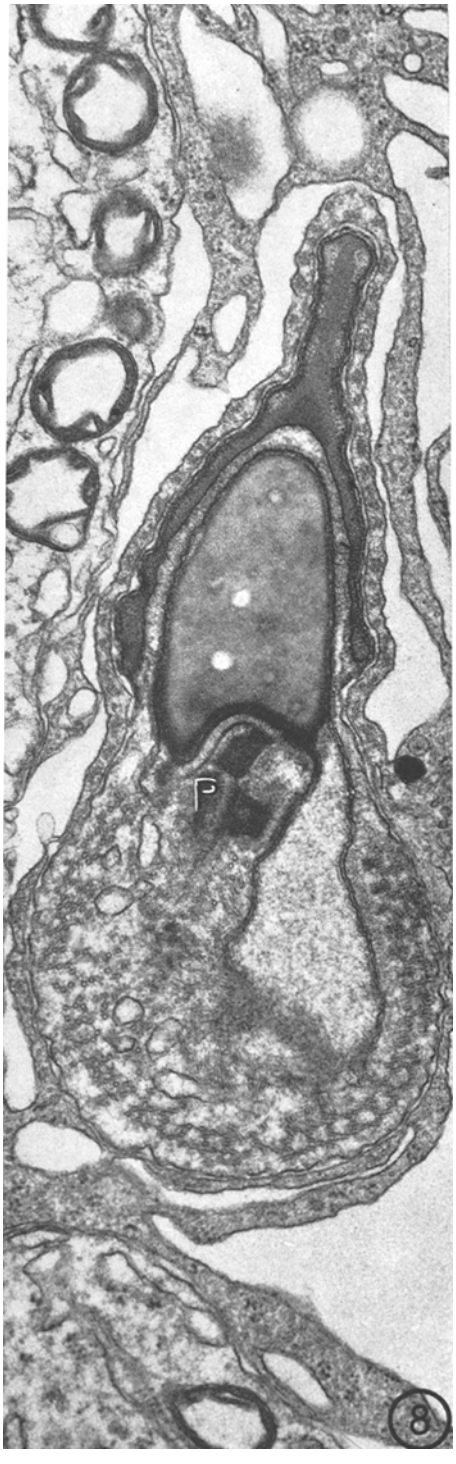


Fig. 8. Saggital section parallel to the length of the proximal centriole (*P*) demonstrating manchette microtubules in cross section. On the right side a caudal region in the insertion ring is evident. × 3100

associated with portions of the redundant nuclear envelope (Figs. 1, 4, 5). Occasionally sections in this region demonstrate continuity between the outer nuclear membrane and the inner portion of the membranous cylinder surrounding the microtubules of the manchette (Fig. 5).

Micrographs of those areas of spermatid nuclei near the redundant nuclear envelope reveal the insertion ring of the manchette microtubules, previously referred to as the nuclear ring (Figs. 6,7). This structure consists of electron dense material into which the microtubules of the manchette insert. In Stage 14 spermatids the ring in lateral regions inserts into pockets of plasma membrane approximately 0.01 μ in length and 0.003 μ in diameter (Fig. 6). Sagittal sections reveal that the height of the ring is not constant as the manchette microtubules appear to insert higher in central regions than lateral ones (Fig. 7). The ring at this developmental stage is positioned as a collar that surrounds those nuclear areas where redundant envelope is evident. Consequently, sagittal sections show the insertion ring as well as the microtubules below it to be incomplete (Figs. 7 and 8) as the redundant envelope involves only those regions of the nucleus which do not lie directly under the acrosome (see Fig. 13). In the apical regions of the redundant area myelin-like bodies are observed which are derived from nuclear membrane (Fig. 7).

Sections in the region of the redundant envelope (Fig. 8) as well as those

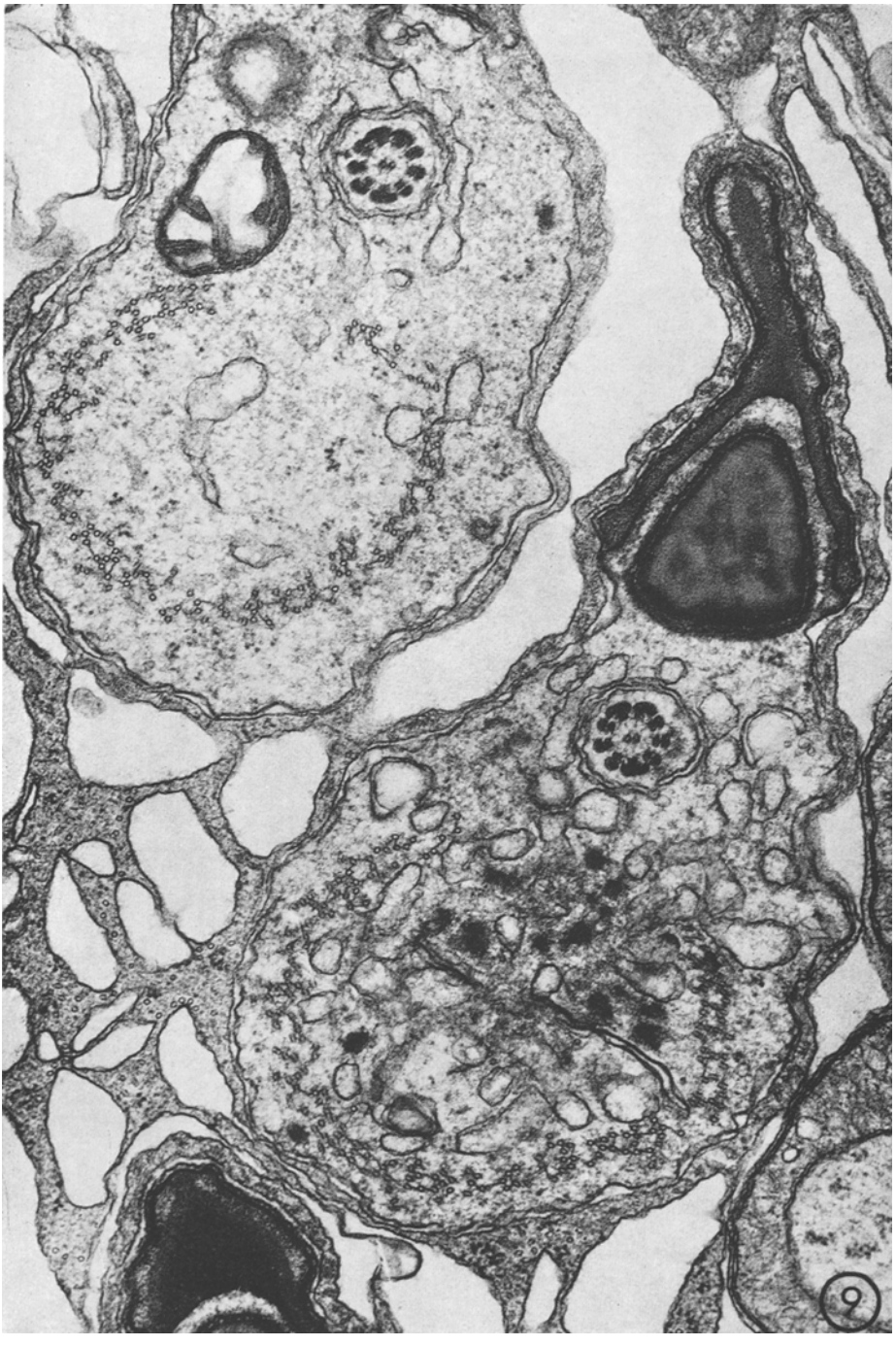
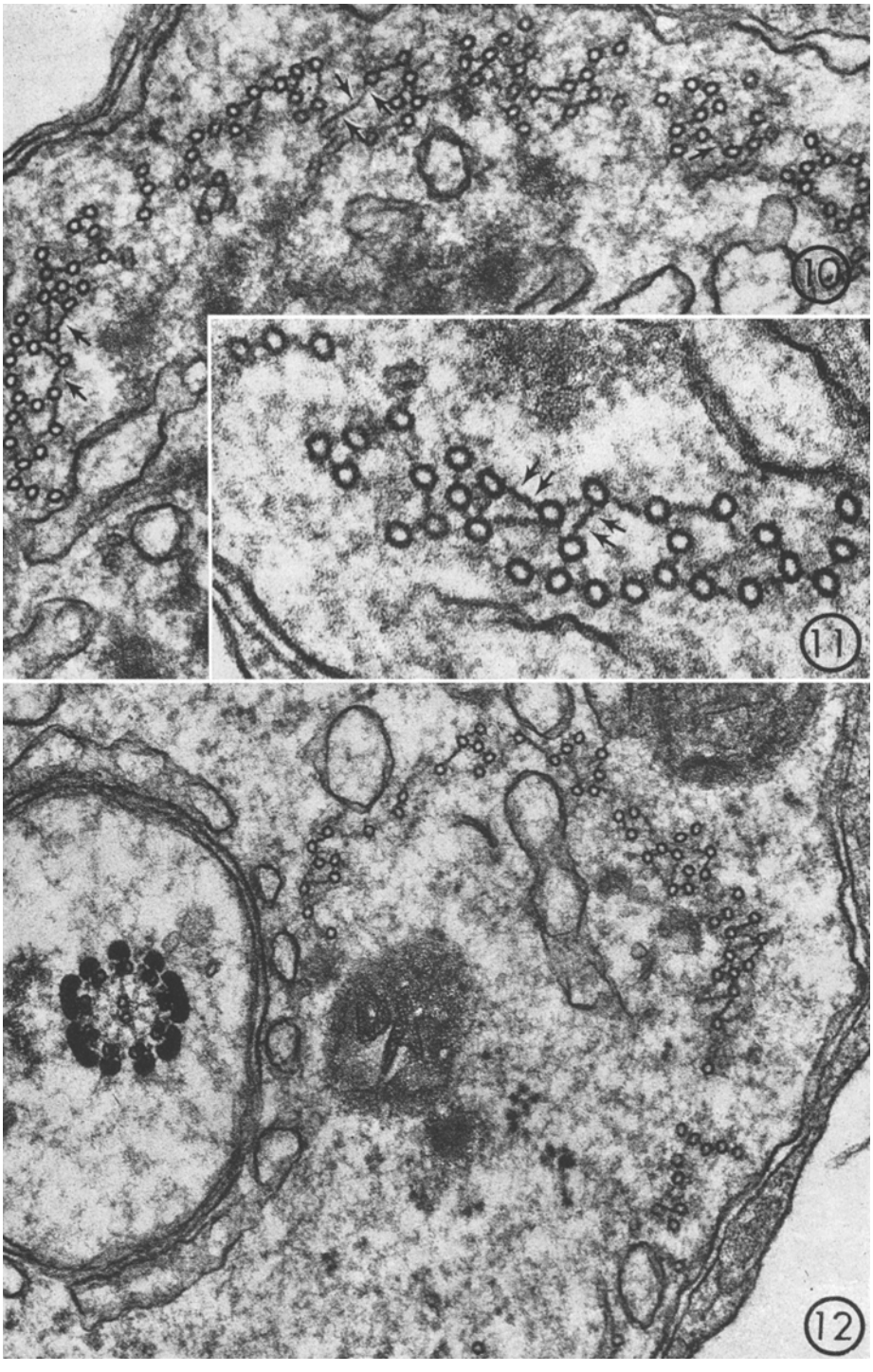


Fig. 9. Section in the same plane and more caudal to that in Fig. 8. Redundant envelope is absent at this level. Manchette microtubules and links between them are obvious. The orientation of the manchette is eccentric with regards to the axial filament. The cytoplasm of the upper spermatid demonstrates microtubules without accompanying membranes. $\times 34000$



Figs. 10–12. Links between microtubules of the caudal tube. Fig. 10. Arrows point to long links which appear bent. × 83000. Fig. 11. Arrows point to long links in which two electron densities are evident. × 180000. Fig. 12. Section taken at right angles to the axial filament. The number of microtubules is less than that in sections taken closer to the nucleus (see Figs. 8 and 9). The position of the manchette tubules is eccentric with reference to the axial filament. × 64000

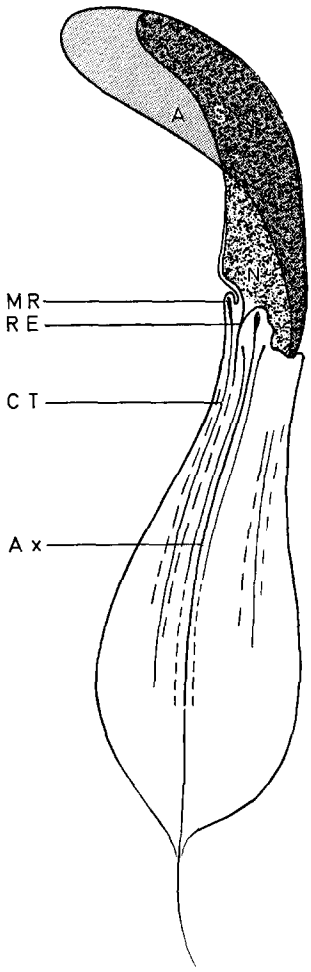


Fig. 13. Diagram of a Stage 14 spermatid somewhat modified from that of Clermont and Perry (1957). The membranous ring around the axial filament is not drawn in caudal portions of the spermatid cytoplasm. The manchette insertion ring (*MR*) is present only as a collar in areas above the redundant envelope, consequently it is depicted only the side opposite the acrosomal cap. As the redundant envelope encloses uncondensed chromatin, the nucleoplasm is not shaded in this region. The redundant envelope (*RE*) is extended on the inner side of the caudal tube (*CT*) to demonstrate at least partial continuity with the membranes surrounding the manchette microtubules. Although this diagram does not depict three dimensions, the paucity of caudal tube material on the right side of the diagram is meant to partially represent manchette eccentricity. The insertion ring is absent on the right side to depict that it does not extend behind the acrosomal cap. *N* nucleus, *AS* acrosomic system, *AX* axial filament

caudal to this region (Figs. 9–12) that are approximately parallel to the proximal centriole and at right angles to the distal centriole and axial filament demonstrate the majority of manchette microtubules in cross section. The position of the microtubules becomes increasingly eccentric, with reference to the axial filament, in a caudal direction from the nucleus (Figs. 8, 11). The number of microtubules in the caudal tube decreases in this direction (Figs. 10, 12).

Links are present between neighbouring microtubules in the manchette at this stage of development (Figs. 10–12). These links are ~35–40 Å in diameter and vary in length from ~70 to ~1000 Å (Figs. 10–12). Relatively long links do not appear to be straight but rather kinked, possibly coiled and occasionally bent (Figs. 10, 11). At higher magnifications two electron densities are often observed within these links (Fig. 11). On microtubules where two or more links are found, the angle between adjacent links has been observed to be a minimum of $30° \pm 1°$ or simple multiples of this minimum angle.

Discussion

Three new facets of rat spermatid architecture have been described. Caudal tube microtubules are partially enclosed by large sheets of smooth endoplasmic reticulum. These membranes appear to maintain continuity with the redundant envelope. Caudal regions of manchette microtubules are interconnected by links 35–40 Å in diameter and of variable length, shape and electron density. Although this report is limited to a detailed analysis of one stage of rat spermiogenesis, these results would appear to be of general interest in an understanding of spermatid cytology.

The redundant nuclear envelope has been considered to represent accommodation of the nuclear envelope to reduction in nuclear volume (Fawcett, 1965) and perhaps, separation of interchromosomal nucleoplasm from condensing chromatin in spermatid nuclei (Franklin, 1968). Related to these, another function may be indicated as the redundant envelope would appear to be at least structurally implicated in elaboration of, and continuity with, the sheath partially surrounding caudal tube microtubules. It may be significant that manchette microtubules and redundant envelope, both of which have been separately related to the phenomenon of volume reduction in spermatid nuclei, appear to be intimately related at this developmental stage. Apart from any consideration of the mechanics of volume reduction and elongation, it should be stressed that at this developmental stage the caudal tube consists of two recognizable components, both having their origin near caudal regions of spermatid nuclei. As such, the anterior portions of the caudal tube along with those of the developing axial filament partially subdivide the spermatid cytoplasm into two compartments. The inner compartment, closed anteriorly and progressively open laterally and posteriorly, is bounded by the inner membranes of the caudal tube and perhaps the membranous sheath surrounding the apical regions of the axial filament. The significance of these structures in relation to function remains obscure. It might be possible that during subsequent elongation the position of other organelles in developing spermatids is thus restricted to the general proximity of the axial filament. Other functional correlates are possible, all equally tenuous. However, the existence of continuity of the membranes of the caudal tube with those of the redundant envelope, the close association of these membranes with manchette microtubules, and the high degree of structural order that seems to exist between the microtubules themselves, all seem to imply a greater degree of complexity in structural integration in elongating spermatids than has been previously indicated.

The structure of the caudal tube in Stage 14 rat Spermatids may not be unique Vesicles in close association with manchette microtubes are evident in numerous reports on spermatid fine structure (Bedford and Nicander, 1971: Burgos *et al.*, 1970; Franklin, 1968; Horstmann, 1961, 1965; Susi and Clermont, 1971). Membranous sheaths similar to those in the rat are present in elongating mouse spermatids (Go, personal communication, 1971). Whether membranous elements in proximity to caudal tube microtubes noted in spermatids of other mammalian species are derived in the same manner as those in the rat has yet to be determined.

A high degree of order exists in the position of the microtubules of the manchette at this stage of development. This order appears to be a partial function of the

eccentricity of the manchette insertion ring and perhaps the links observed between adjacent microtubules, particularily prominent in spermatid postnuclear regions. The location of two or more links on the surface of a single microtubule is remarkable as they appear to be positioned in such a way that an angle of 30° or a multiple thereof separates them. Furthermore the position of neighbouring microtubules appears to correspond to the length and direction of links, although some are apparently incomplete. As such, the orientation of these links resembles closely that of those suggested and demonstrated to exist between microtubules in axonemes of *Actinosphaerium* (Tilney, 1968, 1969) and would appear support the contention that the location of links between microtubules is a function of the position of microtubule subunits. That links are associated with structural order in microtubule assemblies seems to be dependent upon coincidence between observed order and demonstrable links: To date direct experimental proof of the hypothesis is absent. However, a body of associative evidence exists (see Tilney, 1969) with which the present results seem to concur.

The large links between adjacent microtubules are unusual in that their morphology is extremely variable. The electron density of these links is similar to that usually associated with relatively short links, for example those observed between microtubules in fowl spermatids (McIntosh and Porter, 1967) or insect spermatids (Kessel, 1966, 1967; Hoage and Kessel, 1969). The possible presence of two electron densities and irregular contour suggests twist or coiling and the possible existence of two subunits in link substructure. While it is possible that two electron densities are the result of staining artifact and although different methods of preparation would be necessary to conclusively demonstrate their presence, a similar interpretation of link images could be made for some of those demonstrated between microtubules in *Actinosphaerium* (Roth *et al.*, 1970).

Links between microtubules have been ascribed two functions: motility and order (McIntosh and Porter, 1967; Tilney, 1969). The question arises as to which modality these unusually shaped links might subserve. Should coiling and bending not be artifactual in nature an intriguing possibility exists in that it may be possible that such links accommodate lateral movement. However, unlikely, if this were true one might expect to find link images in apparent stages of contraction or compaction. Obviously, alternate methods of analysis are necessary in order to determine either the degree of coiling or the true length of the links depicted in Fig. 10–12.

To our knowledge no previous report exists concerning links between manchette microtubules in mammals although one such structure may be apparent in micrographs of the manchette by Burgos and Fawcett (1955). In this regard one must give credit to the early fixation procedures which have been the basis for extensive modifications in technique during the past two decades. Similar links thus may exist between manchette microtubules in other mammalian species in specific stages of elongation.

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Dr. E. A. MacKinnon
Assistant Professor
Anatomy Department
Queen's University
Kingston, Ontario, Canada