# BUNDLES OF INTRACELLULAR TUBULES IN RENAL MEDULLARY INTERSTITIAL CELLS

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## INTRODUCTION

In the course of a study on the fine structure of rat renal medulla, occasional bundles of intracellular tubules have been seen in the interstitial cells. These tubules are probably the same as the "cyclindrical bodies" described by Bulger et al. (1). In the present study the bundles of tubules have been found in various experimental situations. The topography, connections, and possibly also the wall structure of the tubules seem to differ in some respects from the findings of Bulger et al. (1).

### METHODS

Male rats of approximately 250 g were killed under ether anesthesia, and the whole medullas were immediately removed, cut into small pieces, and fixed in 4% glutaraldehyde in a cacodylate buffer (containing 1% NaCl) at pH 7.4 for 2.5 h. The pieces were then washed in buffer, postfixed in 2% osmium tetroxide in cacodylate buffer at pH 7.4 for 2 h, dehydrated, and embedded in Epon. Most specimens were treated in the block with paraphenylenediamine (2) before dehydration. Sections were cut on a Reichert Om U2 ultramicrotome (C. Reichert, Vienna, Austria) and viewed in a Philips EM 300 electron microscope. Sections not treated with paraphenylenediamine were stained with lead citrate before being viewed. The area of the medulla examined was the center portion of the region where the papilla widens out to become the outer medulla.

## RESULTS

The tubule bundles were seen only in the interstitial cells. They were found in control rats, rats of the New Zealand genetic hypertensive strain (GH rats, 3), rats deprived of water for 36 h, and rats treated with frusemide (25 mg/kg for 3 h before killing), so that their distribution seems to be quite random, and not in any way connected with the abnormal situations existing in the experimental groups. The tubules were not found with any degree of regularity: in a total of 113 blocks (from 50 rats) scanned during a quantitative study for another purpose, tubules were seen in only 15 blocks (representing 13 rats). The tubules occurred in bundles consisting of up to 50 tubules. Their length is uncertain, but examples as long as  $11.2 \,\mu\text{m}$  were seen. They have a diameter of approximately 130–180 nm. The bundles can be seen in both longitudinal and transverse section within the same cell (Figs. la-d) so that clearly they run in different directions.

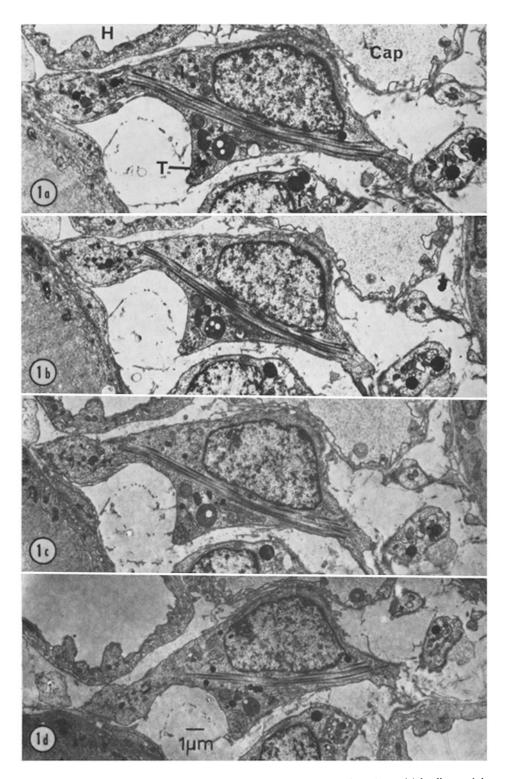
The communications of the tubules are at present not entirely clear. When the end of a tubule is seen, it often appears to communicate with a small irregular vesicle or tubule (Figs. 2, 3), both presumably part of the endoplasmic reticulum. In other cases the tubules appear to open into one of the membrane-bounded spaces which are a characteristic of the intersitial cells and which are probably extensions of the perinuclear space (Fig. 4). These communications in some cases seem to be completely open, while in others there is a suggestion of a fine membrane separating the lumen of the tubule from the lumen of the sarcoplasmic reticulum, as described by Bulger et al. (1).

The lumen of the tubules is filled with a material of greater density than that of the cytoplasm, and small ovoid bodies or vesicles are seen in the lumen (Figs. 2–5), in some instances causing the tubule to bulge (Fig. 5). Lipid granules have not been seen in the lumen of the tubules and indeed most of them are much larger than the diameter of the tubules.

The walls of the tubules appear to have a unit membrane structure (Fig. 5).

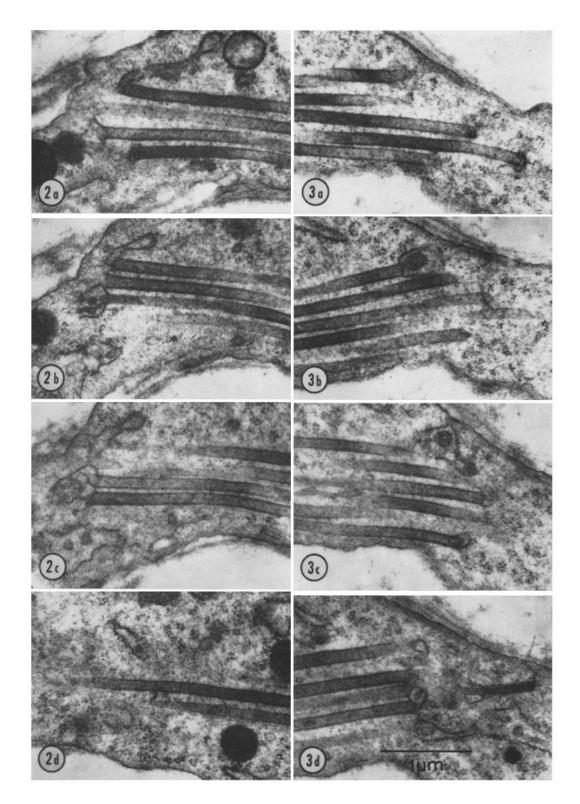
#### DISCUSSION

These tubular organelles appear to be the same as the cylindrical bodies described by Bulger et al. (1). However, in the present study they seem to be more regular and to have a denser lumen, and the walls appear to have a double leaflet, unit membrane, structure. Bulger et al. (1) found the lumen to have the same density as the cytoplasm, and they describe the walls of the organelles as consisting of helically arranged sheets of very



FIGURES 1a-d Serial sections (not necessarily fully consecutive) through an interstitial cell containing two bundles of tubules, one sectioned longitudinally, the other transversely (*T*). *I*, interstitial cells; *Cap*, capillary; *H*, parts of loops of Henle. Frusemide-treated rat. Lead citrate stain.  $\times$  4,500.

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FIGURES 2-3 Higher power views of parts of Fig. 1. Note apparent connections with vesicular and tubular elements, presumably parts of the sarcoplasmic reticulum. Communication at these points of connection may not be entirely free, as an occluding membrane appears to be present in some cases.  $\times$  24,300.

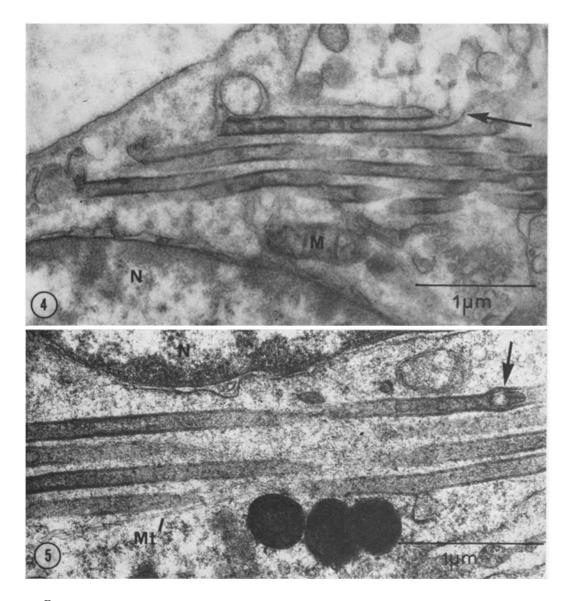


FIGURE 4 Example of open communication (arrow) between a tubule and a membrane-bounded area (interpreted as an extension of the perinuclear space). M, mitochondrion; N, nucleus. Frusemide-treated rat. Paraphenylenediamine in block.  $\times$  32,000.

FIGURE 5 High power view of part of Fig. 1 d. Note bulge around ovoid body in tubule (arrow). A microtubule is seen (Mt). N, nucleus. Lead citrate stain.  $\times$  39,000.

small tubules. These differences may well depend on differences in fixation, and certainly in the present study the structure of the tubule walls has not been resolved sufficiently well to enable any definite statement to be made.

Another difference concerns the incidence of the tubules. Bulger et al. (1) found them commonly

in dehydrated rats and only very seldom under conditions of water diuresis, and they considered them to be a unique response to dehydration. In the present study the tubules have been found to occur sporadically in all experimental groups mentioned and there was no greater incidence in rats deprived of water for 36 h. Possibly this discrepancy between our findings and those of Bulger et al. (1) could be due to a difference in the area of medulla examined, though this seems unlikely to be the whole explanation. Meantime, the function of the tubules remains uncertain.

# SUMMARY

Bundles of intracellular tubules were found in interstitial cells of rat renal medulla in about 12% of 113 blocks, derived from 50 animals. The tubules have a diameter of approximately 130–180 nm and are of uncertain length (examples as long as 11.2  $\mu$ m have been seen). They occur sporadically both in control rats and in rats exposed to various experimental situations. Their incidence was not increased in animals deprived of water. The material in the lumen of the tubules is more electron-opaque than the cytoplasm,

and ovoid bodies are quite frequently seen within the lumen. The function of the tubules is unknown.

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