

THE DEMONSTRATION OF CILIA IN CANINEMACULA Densa CELLS (1)

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**ABSTRACT** Although the presence of cilia in mammalian epithelia is common, the finding of cilia in the macula densa cell of the kidney has never been reported. In this paper are reported electron microscopic observations of cilia in canine macula densa cells. The cilia observed have the classic 9+2 configuration. The centriole, basal body, foot process, ciliary rootlet and the substructure of the latter also were observed.

Cilia in renal epithelia have been investigated previously. Chase ('23) observed a ciliated cuboidal epithelial lining at the junction of the glomerulus and the renal tubule in Necturus maculosus refinesque. White and Lucus ('23) described cilia in a similar location and inferred that their function was to aid the flow of filtrate in the kidney of Necturus. Bulger and Trump ('66) demonstrated the fine structure of cilia in the neck segment of the flounder nephron. Fine structural descriptions of cilia in the mammalian nephron are also available. Latta et al. ('61) observed the occasional occurrence of cilia in rat collecting duct cells. Meyers et al. ('66) found cilia in human collecting duct cells. Herein we describe for the first time cilia in canine macula densa cells.

**MATERIALS AND METHODS** Canine renal cortical tissue was obtained by an open biopsy technique with a Silverman needle. These samples were diced immediately while being fixed for two hours in a fixative composed of 1/2 strength Karnovsky fixative and 2% potassium pyroantimonate in 0.1 M

potassium phosphate buffer, pH 7.4. Fixation was followed by an eight-hour wash 0.1 M potassium phosphate buffer at pH 7.4. The tissue was then post-fixed for two hours in 1% OsO<sub>4</sub> (pH 7.4, 0.1 M potassium phosphate buffer). After fixation the tissues were rinsed for five minutes with 0.05 M maleic acid, pH 5.2, and stained for one hour in 0.5 M maleic acid containing 0.5% uranyl acetate at pH 5.2. The tissues were then washed with 0.5 M maleic acid, dehydrated in ethanol and embedded in Epon 812. Sections 1-2 μ thick were stained with methylene blue for the purpose of orientation.

Thin sections were stained with uranyl acetate and lead citrate, and examined at 75 KV in a Hitachi HU 11A electron microscope.

**OBSERVATIONS** The results from renal biopsies from both kidneys of two mongrel dogs are reported. Cilia in the canine macula densa cells are scattered and singly distributed (fig. 1). Some sections of a single cell show more than one cilium (fig. 1). Like other cilia they have the typical 9+2 axonemal structure (fig. 2). Sagittal and oblique sections of these cilia (figs. 3-6) reveal the characteristic ciliary rootlets, basal body and centrioles. In the longitudinal section shown in figure 4, the ciliary rootlet radiates from the center of the proximal end of the basal body at an obtuse angle and blends with the adjacent cytoplasm, whereas the denser foot process attaches perpendicularly to the long axis of the cilium. The ciliary rootlet is slender. It tapers gradually as it blends with the surrounding cytoplasm. Both the ciliary rootlet and the foot process have cross-bandings which resembles fibrous, long-spacing collagenous fibrils. The electron-opaque cross-bandings of the ciliary rootlet are narrower than those of the foot process. They are 310 and 360 Å wide, respectively. Contrary to the latter observation, the electron-lucent cross-bandings of the

ciliary rootlet are wider than those of the foot process. Their respective widths are 1370 and 770 Å. Observation at high resolution of the ciliary rootlet reveals filamentous substructures embedded in the electron-lucent cross-bandings. These filamentous substructures are 90 Å thick and can be readily differentiated from the homogeneous matrix. They are parallel to one another with the ends apposed perpendicularly to the electron-opaque cross-bandings.

DISCUSSION Cilia in the renal epithelia of fish and amphibia frequently have been described near the glomerulo-tubular junction (Chase, '23; Lucas and White, '32; Bulger, '66). The function of these cilia is thought to be related to propulsion of filtrate down the tubule, as rapid undulating movements have been observed (Lucas and White, '32). Contrary to the frequent observation of cilia in fish and amphibia, cilia in mammalian nephrons are less frequently encountered. Among the laboratory animals, cilia in rodents are more commonly observed. They occur singly in the distal tubule (Latta et al., '61; Rhodin, '58) and the collecting duct (Latta et al., '61) rather than at the glomerulo-tubular junction as in fish and amphibia. Because of the inconsistent occurrence and irregular distribution, it is unlikely that cilia play an important role in propelling the filtrate along the nephron. However, there are at least two aspects of interest relating to the occurrence of cilia in mammalian renal epithelia. The more obvious one is the evolutionary connotation of this cell organelle. The prevalence of renal cilia in fish and amphibia, but not in mammals, may indicate involution of these organelles secondary to environmental adaptation. Perhaps the cilium is a more common feature of the more primitive mammalian mesonephric or metanephric kidney.

MacCallum, Conn and Baker (personal communication) have found cilia in a juxtaglomerular cell tumor from a patient with hypertension. This association supports the concept that tumor cells are more primitive in nature and reflects the possible relation of ciliated macula densa cells and renal tumors with hypertension. Although there is no evidence that renal cells possessing cilia are more likely to develop tumors, the possibility of such a correlation should be considered.

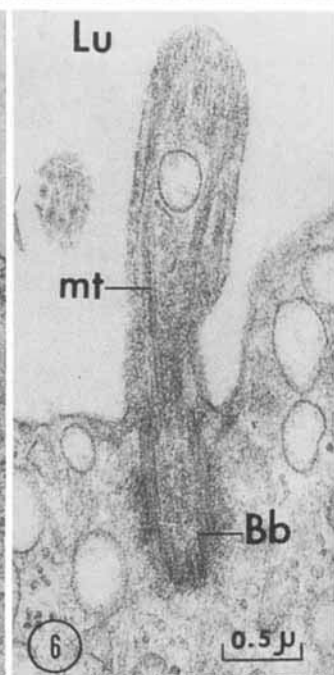
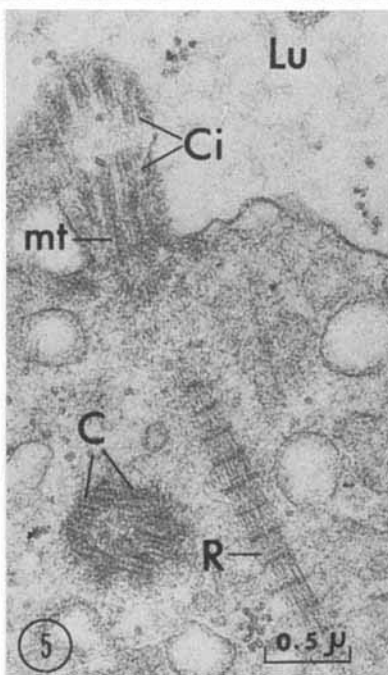
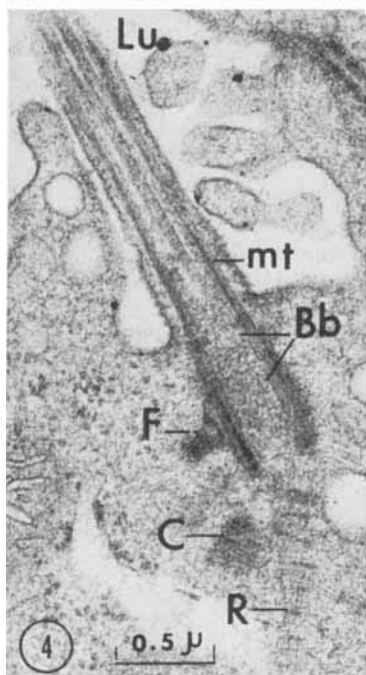
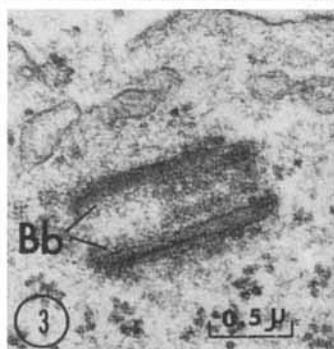
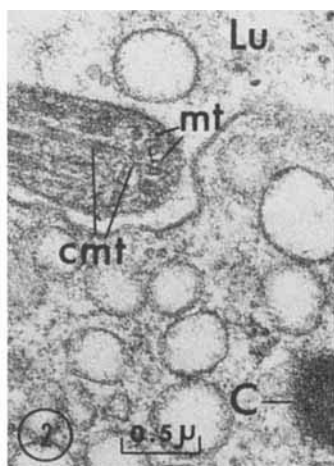
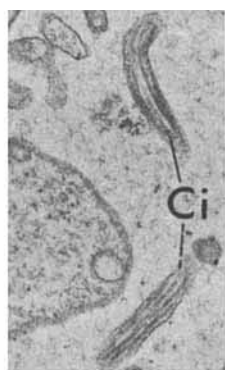
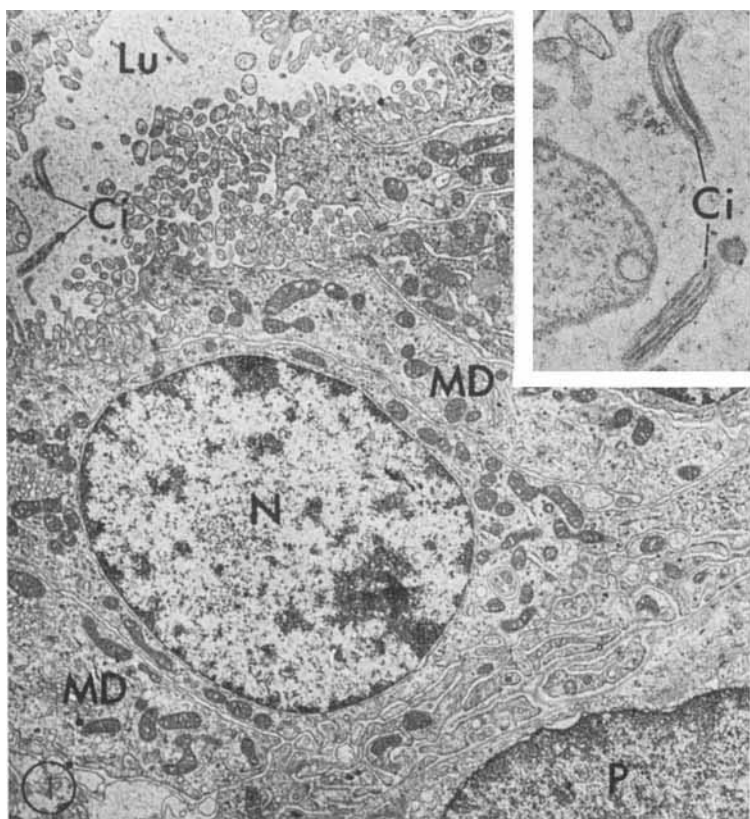
Our work indicates macula densa cells with cilia are common in the canine kidney. One wonders if such cilia represent involution of a functional organelle of lower forms, which have lost their original function, or if

#### FIGURE LEGENDS

The electron micrographs were taken at magnification X 10,000-60,000 and printed with slight enlargement. The following code of labelling was used in all figures.

Lu -----	lumen	N -----	nucleus
Mt -----	microtubule	MD -----	macula densa cell
C -----	centriole	Ci -----	cilium
F -----	foot-process	P -----	polkissen cell
R -----	ciliary rootlet	Cmt -----	central microtubule
Bb -----	basal body		

1. Macula densa cells (MD) with cilia (Ci) in the lumen. Polkissen cells (P) were found at the hilum of the glomerulus between the macula densa cells and the Bowman's capsule. Inset: higher magnification of a tangential section of cilia. X 12,000.
2. An oblique section of a cilium at the lumen. Microtubules (mt) and central microtubules (cmt) within the cilium were present. The centriole (C) was also depicted. X 67,000.
3. The basal body of a cilium located near the luminal border of a macula densa cell. X 67,000.
4. A section of the macula densa cell luminal border. Foot process (F) and ciliary rootlet (R) radiate from the basal body (Bb) of a longitudinally sectioned cilium. Microtubule (mt) of the cilium and the adjacent centriole (C) are also present. X 65,000.
5. An oblique section of a cilium (Ci) with microtubules (mt), ciliary rootlet (R) and the adjacent centriole (C). X 65,000.
6. An obliquely sectioned cilium projecting into the lumen of a macula densa cell. Microtubules (mt) and the basal body (Bb) are also demonstrated. X 65,000.



they serve some purpose other than that usually assigned to cilia. Because the macula densa cells are intimately concerned with the control of secretion of renin, one might ask whether the cilia are involved in any manner in hormonal regulation of renin by the kidney or if they influence sodium uptake by the macula densa cells? The macula densa cells appear to sense the intracellular concentration of sodium and determine secretion rates of renin, (Vander '67).

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