# Ciliated Human Renal Proximal Tubular Cells

## *Observations in Three Cases of Hypercalcemia*

John L. Duffy, M.D., and Yasunosuke Suzuki, M.D.

MANY renal tubular epithelial cells possess at least one elongated structure which is indistinguishable from the flagella and cilia of all living things. In this paper we refer to them as cilia, even though they are not commonly found in large numbers. On the other hand, when many such structures are found to arise from a single cell, we will call that cell heavily ciliated. Although heavily ciliated cells are not uncommon in the renal tubules of lower forms of animal life, they are not expected in the renal tubules of man. Isolated cilia are probably normal in the apexes of collecting duct cells,[1] in proximal tubular epithelial cells,[2] and in the thin limb of Henle's loop[3] of the human kidney. Groups of such structures have been observed in a case of systemic lupus erythematosus, but no details are given.[4] This report is prompted by the finding of heavily ciliated renal proximal tubular cells in 3 adults with hypercalcemia presumably as the result of milk-alkali syndrome.

### Materials and Methods

Human renal cortical tissue was obtained from 60 patients (Table 1) by percutaneous needle biopsy. One portion was fixed in 10% formalin, dehydrated, and embedded in paraffin according to standard methods. Sections were cut at 3 $\mu$ and stained with hematoxylin and eosin, and/or the periodic acid-Schiff reagent. Small portions were cut into cubes less than 1 mm. in greatest dimension, fixed for 1 hr. in 1% phosphate-buffered osmic acid, pH 7.2–7.5, dehydrated in graded alcohols, and embedded in Epon 812. Sections were stained with uranyl acetate or double-stained with uranyl acetate and lead hydroxide. These sections were then examined at 50 and/or 100 kv with RCA 3-G and/or H electron microscope.

The 3 patients exhibiting heavily ciliated cells were extremely similar. They were all males, ages 35, 52, and 61 years, heavy drinkers or alcoholics, who had received considerable medical and surgical treatment for peptic ulcer disease. Each had ingested large quantities of milk and antacids and exhibited hypercalcemia

From the Department of Pathology and Laboratories, Meadowbrook Hospital, East Meadow, L.I., N.Y., and the Division of Renal Pathology, Department of Pathology, Mount Sinai School of Medicine, New York, N.Y.

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Address for reprint requests: Dr. Suzuki, Division of Renal Pathology, Mount Sinai Hospital, Fifth Ave. and 100th St., New York, N.Y. 10029.

Table 1. Diagnoses of Cases Examined

| Diagnosis | | No. of patients |
|---|---|---|
| Membranous nephropathy | | 16 |
| Glomerulonephritis, acute and chronic | | 8 |
| Systemic lupus erythematosus | | 6 |
| Foot process disease (lipoid nephrosis) | | 5 |
| Hypertension | | 4 |
| No significant change | | 3 |
| Lead poisoning | | 2 |
| Hypothyroidism | | 2 |
| Medullary sponge kidney | | 2 |
| Diabetes mellitus, tubular necrosis, cystinosis, and Henoch-Schoenlien purpura (1 each) | | 4 |
| Hypercalcemia | | 8 |
| Milk-alkali syndrome | 5 | |
| Hyperparathyroidism | 2 | |
| Sarcoidosis | 1 | |
| *Total* | | 60 |

(milk-alkali syndrome). In each case renal biopsy was performed because of disturbance of renal function.

## Results

### Light Microscopy

Examination of renal biopsy material processed for conventional light microscopy revealed similar findings in all 3 cases. Many glomeruli exhibited moderate periglomerular fibrosis, and obsolescent glomeruli were not infrequent. Varying, but usually mild, degrees of glomerular basement membrane thickening and mesangial widening were noted in most glomeruli. Many foci of tubular atrophy, tubular basement membrane thickening, and peritubular and interstitial fibrosis were also noted. This change involved proximal tubules primarily and was occasionally associated with focal round-cell infiltration. Multifocal deposits of amorphous crystalline appearing basophilic material, presumably calcium, were noted usually in or about proximal convoluted tubules. Careful search by light microscopy failed to reveal ciliated cells in any of these cases.

### Electron Microscopy

Since the purpose of this report is to describe the finding of heavily ciliated renal tubular cells, glomerular findings will not be presented here. The tubules involved were in each case pathologically altered but could be easily identified as portions of proximal renal tubules. The tubular basement membranes in these areas were markedly and ir-

regularly thickened and laminated and they frequently contained multiple small, irregular deposits of osmiophilic material. The usually abundant brush border, although easily observable, was markedly decreased in some cells. These particular cells usually abutted on pathologically altered basement membranes and exhibited moderate flattening and a marked decrease in basal infoldings. Intracellular aggregates of small, uniform particles, presumably glycogen, were prominent. The mitochondria of these cells appeared sparse and were usually found free in the cytoplasm rather than within basal infoldings. These findings are considered to represent atrophy. It was usually these cells which could be shown to give rise to numerous cilia. The ultrastructure of these cilia was found to be identical to that described throughout the animal kingdom—that is, a longitudinal bundle of filaments surrounded by a membrane. The filaments numbered eleven, with two central single filaments and nine peripheral double filaments. The cilia were embedded in the cells by typical ciliary rootlets. The cilia measured approximately 0.2 $\mu$ in diameter and were found to be as close as 0.5 $\mu$ from center to center. The ciliary filaments measured approximately 200 Å in diameter. Cilia were found in up to 5 cells per tubule per section, and up to 12 ciliary attachments per cell per section were noted. In one micrograph, over 90 cilia were found in a single cross section of tubular lumen.

Random examination of renal tubules in the 57 other patients did not reveal a single heavily ciliated cell. On the other hand, it was relatively easy to find isolated cilia when a very careful search was made for them. In a case of sarcoidosis with hypercalcemia, up to 15 cilia were found within a single tubular lumen. However, serial sections failed to reveal the rootlets of these structures. We have no way of determining whether these structures arose from heavily ciliated cells.

## Discussion

Ciliated cells are abundant in the excretory organs of lower animals. The cell rosettes of the ctenophores, the flame bulb protonephridia of the flat worms and the nemertines, and the metanephridia of the annelids all have cilia as an important functional component. The amphioxus, a chordate, has a similar excretory system.[5] Recent studies describe ciliated renal tubular cells in the urodele [6] and rat.[7] It is apparent, therefore, that the potential for human renal tubular epithelial ciliation exists, and one could postulate reversion to a more primitive cell type, either as a result of irritation or injury or in response to the process of repair, as an initiating factor. However, we were unable to

find cilia in various other conditions, many of which were associated with tubular injury. These cases include several patients who had both tubular injury and one finding in common with the cases reported here—i.e. hypercalcemia. It would seem, therefore, that we should look beyond irritation or injury and hypercalcemia as the causal agents of renal tubular epithelial ciliation in these cases.

Marshall [8] notes that the tubules of many lower vertebrates have ciliated neck segments leading from the glomerulus and that these cilia are not usually found in animals with relatively high blood pressure. He suggests that this association with low blood pressure might indicate that cilia are needed to aid in the relatively low filtration pressure. Kempton,[9] in studying the lengthy complex tubules of the spiny dogfish, suggests that cilia may aid in overcoming the tubular resistance to the passage of fluid. He notes further that this would increase the glomerular filtration pressure. Certainly it must be this kind of function that is important in those primitive systems which lack glomeruli. It has long been known that cilia can exert considerable pressure in the tubule.[10] However, none of our cases suffered from low blood pressure. One might postulate that cilia would aid in propelling fluid down the damaged tubule, but as already stated, many other cases with tubular damage failed to reveal ciliation. On the other hand, in experimental hypercalcemic nephropathy, sloughing of necrotic portions of renal tubular epithelial cells with tubular obstruction has been observed.[11, 12] Perhaps the multiple stimuli of injury, repair, and the need to remove extremely viscid material from the tubular lumen act together to initiate the development of cilia.

Other possibilities must be considered. When one is able to study varieties of the same animal as are found in fresh water and salt water, one finds that whereas the fresh water forms need a rather extensive excretory system, the system of the salt water forms is much less developed.[13] It would seem that more extensive systems, including ciliated portions, are important in the maintenance of the tonicity of body fluids as opposed to the environment. In man, this problem is normally just the opposite—i.e., the problem is to protect against concentration rather than dilution.

There are several aspects of these cases which could affect the tonicity of body fluids. Perhaps the most important are hypercalcemia, marked hypochloremia, and metabolic alkalosis. Since these patients suffer from repeated vomiting [14] and were heavy drinkers,[15] magnesium deficiency may also have existed. The recently described association of phosphorus depletion and use of antacids [16] must be mentioned as a further possibility for inducing such complications.

We must conclude that although there are several possible explanations for the development of heavily ciliated renal tubular epithelial cells in humans, present knowledge is insufficient to indicate which, if any, of those suggested is primarily important.

## Summary

Tubules from 60 percutaneous human renal biopsies were examined for cilia by electron microscopy. Heavily ciliated cells were found in pathologically altered proximal tubules of 3 patients with hypercalcemia due to milk-alkali syndrome. Multiple cilia were found within a proximal tubular lumen in a single case of sarcoidosis with hypercalcemia, but they could not be shown to arise from heavily ciliated cells. Several possible stimuli for the development of heavily ciliated cells in these cases are discussed, but it must be concluded that their existence remains unexplained.

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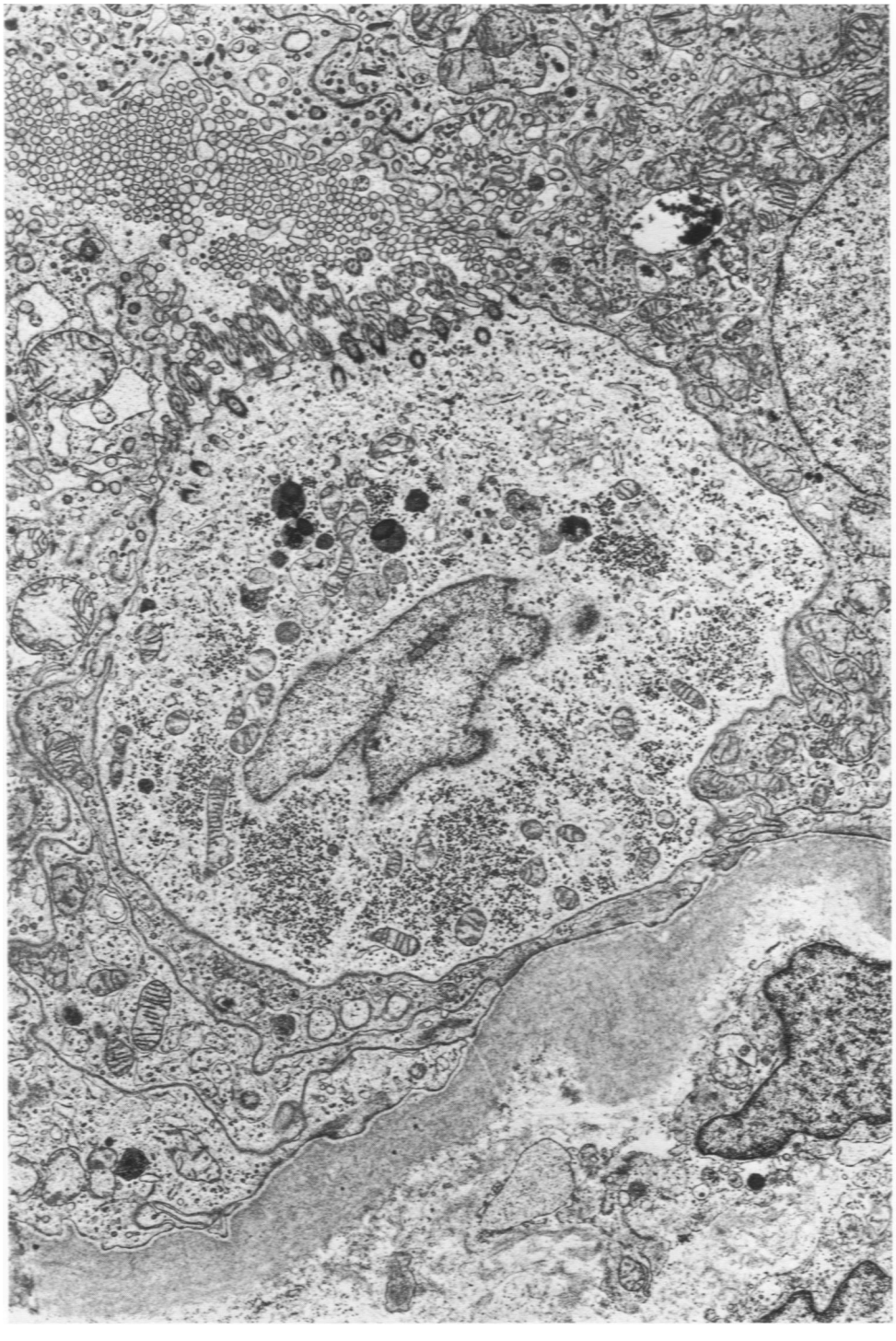
## Legends for Figures

**Fig. 1.** Ciliated proximal tubular cell from a 62-year-old male with hypercalcemia. At least 10 distinct rootlets may be seen. Although the cell does not abut upon the basement membrane in this section, tight junctions with neighboring cells may be clearly seen. Uranyl acetate and lead hydroxide. × 7800.



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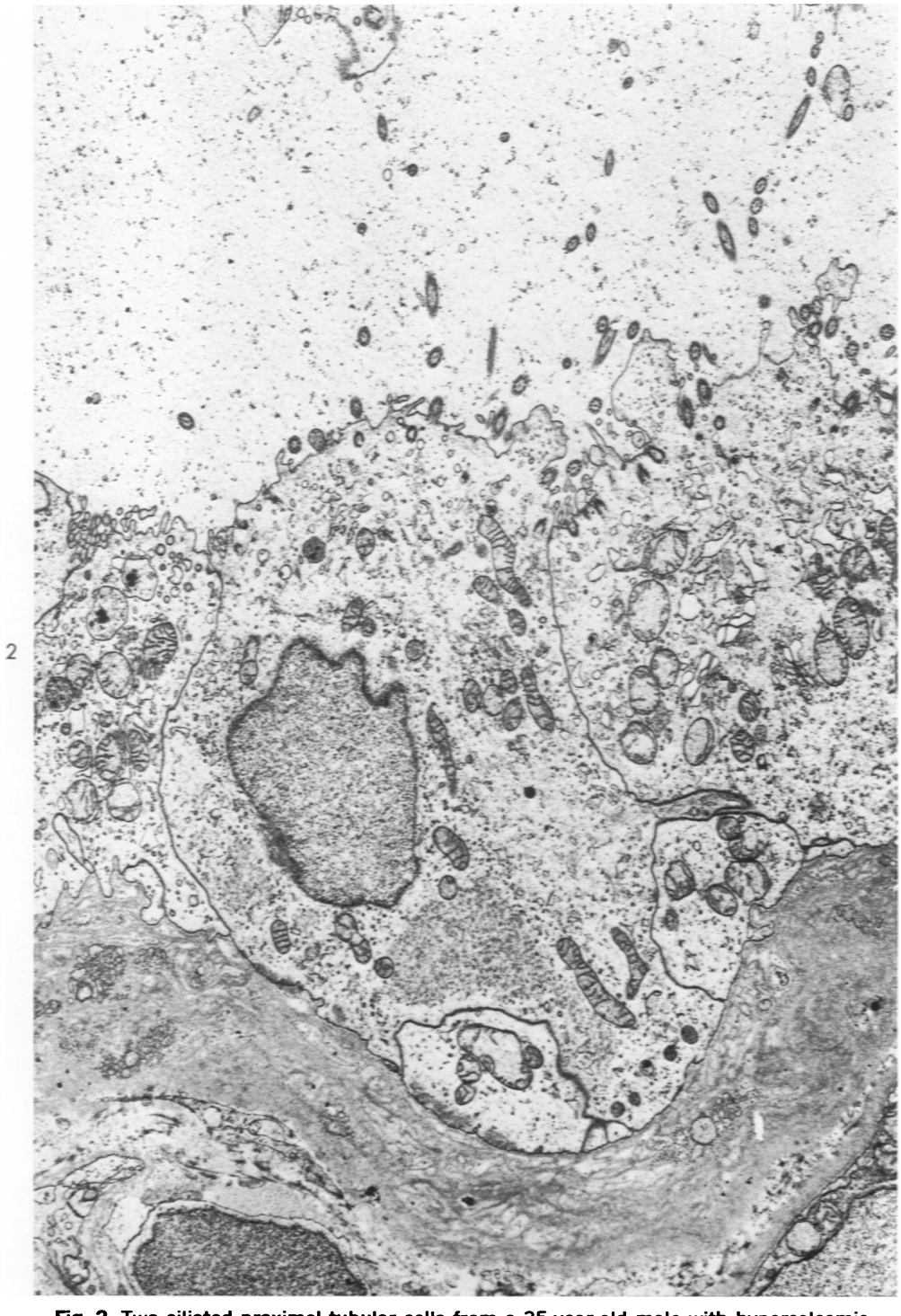

Fig. 2. Two ciliated proximal tubular cells from a 35-year-old male with hypercalcemia. The basement membrane is thickened and laminated and contains small osmiophilic deposits. Mitochondria appear sparse. Uranyl acetate. × 7800.