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# Evidence for Involvement of Microtubules in the Action of Vasopressin in Toad Urinary Bladder

## III. Morphological Studies on the Content and Distribution of Microtubules in Bladder Epithelial Cells

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*Summary.* Colchicine and other antimitotic agents have been found to inhibit the action of vasopressin and cyclic AMP on transcellular water movement in the toad bladder; functional and biochemical studies suggest that the effect of these agents is due to interference with microtubule function. To further assess this hypothesis, a quantitative ultrastructural analysis of the content and distribution of microtubules was performed on epithelial cells of bladders exposed to colchicine, vasopressin, and cyclic AMP. The content (volume density) of microtubules was estimated by a point-counting stereological technique. The results indicate that the content of assembled microtubules in the granular epithelial cells is reduced in hemibladders exposed to colchicine; this effect is dose-dependent and is estimated to be half-maximal at a colchicine concentration of $1.4 \times 10^{-6}$ M. In contrast, the content of assembled microtubules in the granular cells is slightly ($\sim 30\%$) but significantly increased after exposure of hemibladders to vasopressin (100 mU/ml) or cyclic AMP (10 mM). The content of microtubules in mitochondria-rich cells was not found to be significantly altered after exposure to vasopressin. The combined results of functional, biochemical, and morphological studies provide evidence that cytoplasmic microtubules in the granular epithelial cells play a critical role in the action of vasopressin on transcellular water movement in the toad bladder. Precisely how microtubules are involved in the action of the hormone remains to be determined.

Colchicine and other antimitotic agents have been found to inhibit the action of vasopressin on transcellular water movement in amphibian urinary bladder [2, 14, 31, 36]. Functional and biochemical studies documented in the two preceding papers [30, 35] suggest that the inhibitory effect of these agents is due to their interaction with tubulin and consequent interference with microtubule function. In order to

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extend these investigations, a quantitative analysis of the content and distribution of microtubules and other cytoplasmic organelles in the bladder epithelial cells has been performed.

## Materials and Methods

### *Experimental Procedures*

Prior to preparation for microscopy, paired hemibladders from Colombian toads (*Bufo marinus*) were rinsed, mounted as bags on glass tubing, and suspended in Ringer's solution (Na 111.2, Cl 113.0, K 5.4, Ca 1.78, $HPO_4$ 4.8, $H_2PO_4$ 0.6 meq/liter; pH 7.3, 220 mOsm/kg $H_2O$). The bladders were mounted without an osmotic gradient across the tissue in order to avoid changes in epithelial cell volume which are known to accompany vasopressin-induced osmotic water movement [8, 21]. In the studies with colchicine, one member of each pair of hemibladders was exposed to colchicine $2 \times 10^{-6}$ to $8 \times 10^{-4}$ M in the serosal bathing medium for three hours [30] while its pair served as a control. In the initial studies with vasopressin (Table 1, Experiment I) and those with cyclic 3′5′-

Table 1. Effect of vasopressin on volume density of cytoplasmic microtubules in granular cells [a]

| Experiment | Volume density $\times 10^4$ | | | |
|---|---|---|---|---|
| | Animal | Control [b] | Vasopressin [b] | Percent Difference |
| I | 1 | 7.6 | 8.8 | +16 |
| | 2 | 6.8 | 8.4 | +24 |
| | 3 | 5.6 | 6.4 | +14 |
| | 4 | 7.2 | 9.2 | +28 |
| | 5 | 5.6 | 7.2 | +28 |
| | 6 | 8.3 | 11.2 | +35 |
| | 7 | 3.6 | 5.5 | +53 |
| | Mean ± SE | 6.4 ± 0.6 | 8.1 ± 0.7 | +28.3 ± 5.0 [c] |
| II | 1 | 5.1 | 11.9 | +133 |
| | 2 | 4.8 | 8.4 | +75 |
| | 3 | 6.2 | 9.7 | +56 |
| | 4 | 7.6 | 4.5 | −41 |
| | Mean ± SE | 5.9 ± 0.6 | 8.6 ± 1.6 | +56 ± 36 |

[a] Figures represent mean values for five cells from each hemibladder.

[b] Experiment *I*: Control hemibladders were suspended in Ringer's solution for 3 hr; vasopressin-treated hemibladders were suspended in Ringer's solution for $2\frac{1}{2}$ hr prior to exposure to vasopressin 100 mU/ml for 30 min.

Experiment *II*: Control hemibladders were suspended in Ringer's solution for 45 min; vasopressin-treated hemibladders were suspended in Ringer's solution for 15 min prior to exposure to vasopressin 100 mU/ml for 30 min.

[c] $P < 0.01$.

adenosine monophosphate (cyclic AMP), paired hemibladders were suspended in Ringer's solution for $2\frac{1}{2}$ hr and one member of each pair was then exposed to vasopressin 100 mU/ml, or cyclic AMP 10 mM, for 30 min while its pair served as a control. These incubation conditions were chosen so that all bladders were incubated for the same overall period of time. To determine whether the prolonged pre-incubation period had any effect on the cellular content of microtubules, a separate group of four bladders was pre-incubated for only 15 min before exposure to vasopressin (Table 1, Experiment II).

### *Tissue Preparation for Electron Microscopy*

Each hemibladder was gently stretched over a Lucite ring and floated, mucosal side downward, in a solution of 1% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.2, final osmolality 320 mOsm/kg $H_2O$; 24 °C); after 15–30 sec the same fixative solution was applied to the serosal surface of the hemibladder and the tissue was submerged overnight in the fixative solution. (Prefixation of the mucosal surface was employed to minimize the possibility that the hypertonic glutaraldehyde solution might draw fluid out of the bladder epithelial cells through their more permeable serosal surface, and thus decrease their volume prior to fixation [5]). Strips of tissue from the apex of the hemibladder were postfixed for $1\frac{1}{2}$ hr in 1% osmium tetroxide in Palade's veronal buffer (pH 7.0), stained en bloc with 0.5% uranyl acetate in veronal buffer (pH 5.5), dehydrated in a graded series of alcohols, and embedded in epon-araldite plastic. Thin sections (ca. 400 Å) cut perpendicular to the apical surface were stained with uranyl acetate and Reynolds lead citrate, and examined with a JEM-100 B electron microscope.

### *Stereological Analysis of Tissue Components*

Quantitative analysis of microtubules was performed on both granular and mitochondria-rich cells using the point-counting stereological technique of Weibel [33], as described in previous reports from this laboratory [23, 24]. Portions of five nucleated cells showing both apical and lateral intercellular membranes were randomly selected from thin sections of each of the paired hemibladders and were photographed at a magnification of 16,000×. Microtubules in both transverse and longitudinal profile were identified on photographic prints. A grid with a 2-mm lattice was used to estimate microtubule volume, and a grid with a 2-cm lattice was used to estimate cytoplasmic volume [23, 24]. The volume density of microtubules was calculated using the formula $\frac{(P)\text{ microtubules}}{(P)\text{ cytoplasm}} \times 100$, in which $(P)$ represents the number of lattice points falling on mictrotubules or cytoplasm, respectively. In order to avoid bias, all steps in these studies were carried out using blindfold techniques. The mean value for microtubule volume density in five cells from each treated hemibladder was compared with the mean value for its paired control. Paired data were analyzed using Student's *t*-test.

Estimations of the volume density of granules and mitochondria were obtained from the same prints that were used for the microtubule estimations, employing a 1-cm lattice to estimate both organelle and cytoplasmic volume.

### *Relationship of Microtubules to Other Cell Structures*

An attempt was made to determine whether microtubules in bladder epithelial cells show any specific, i.e., nonrandom, spatial relationship to other cell structures. This

possibility was assessed by estimating the proximity of microtubules to various cell structures, as compared with that of a series of random points.

In making measurements on individual micrographs, the center of a drawn circle (radius 3.5 or 7.5 mm) was placed over each identifiable microtubule (all cross sections and the centers of longitudinally oriented microtubules were used). Cell structures close enough to the microtubule to be included within the perimeter of its circle scored "hits", one "hit" being given to each separate structure, or part of a structure, enclosed by the circle. Certain arbitrary decisions were made as to the boundaries of certain structures (e.g., apical subplasmalemmal region, Golgi complexes); however, these decisions did not affect the final results since the same micrographs were used to compare the microenvironment of true microtubules with that of random points.

In order to compare the scores of true microtubules with scores from random points, a plastic sheet containing random circles of a given radius (3.5 or 7.5 mm) was placed over the same photographic prints and "hits" were recorded for enclosed cell structures precisely as described above, except in this instance the centers of the random circles took the place of true microtubules. The random circles used for this study were drawn by a computer from a random number program and were numbered in sequence. The number of random circle measurements made for each print corresponded to the number of real microtubule cross sections which had been identified on that specific print; the sequence in which the circles were used followed the numerical sequence in which the circles had been drawn by the computer. Random circles in which the centers hit areas which anatomically could not have contained microtubules (e.g., inside mitochondria or vacuoles) were excluded, and the next circle in numerical sequence was used. The total number of "hits" scored by a class of structures associated with true microtubules was computed and compared to the total number of "hits" scored by the same class of structures on the same prints using random circles as focal points.

## Results

The toad bladder epithelium is made up of four types of cells [4, 21]. While all four cell types contain cytoplasmic microtubules [31], our morphometric studies on the content and distribution of these organelles have been confined to the granular and mitochondria-rich cells [4, 21].

Figure 1 shows the fine structure of the apical portion of a typical granular cell from a control hemibladder. Short microvilli are evident and membrane limited granules are distributed throughout the apical cytoplasm; some granules are lined up in a characteristic manner under the apical membrane. Mitochondria and several strands of rough endoplasmic reticulum are present. Microtubules appear to be randomly oriented in the cytoplasm of these cells. Occasionally long lengths of microtubules can be seen, but most frequently microtubules are in the form of short fragments and cross sections. These microtubule fragments are sometimes found in close association with other cytoplasmic organelles, a relationship that we have attempted to analyze in this report. Microtubules are rarely observed in the filamentous subplasmalemmal

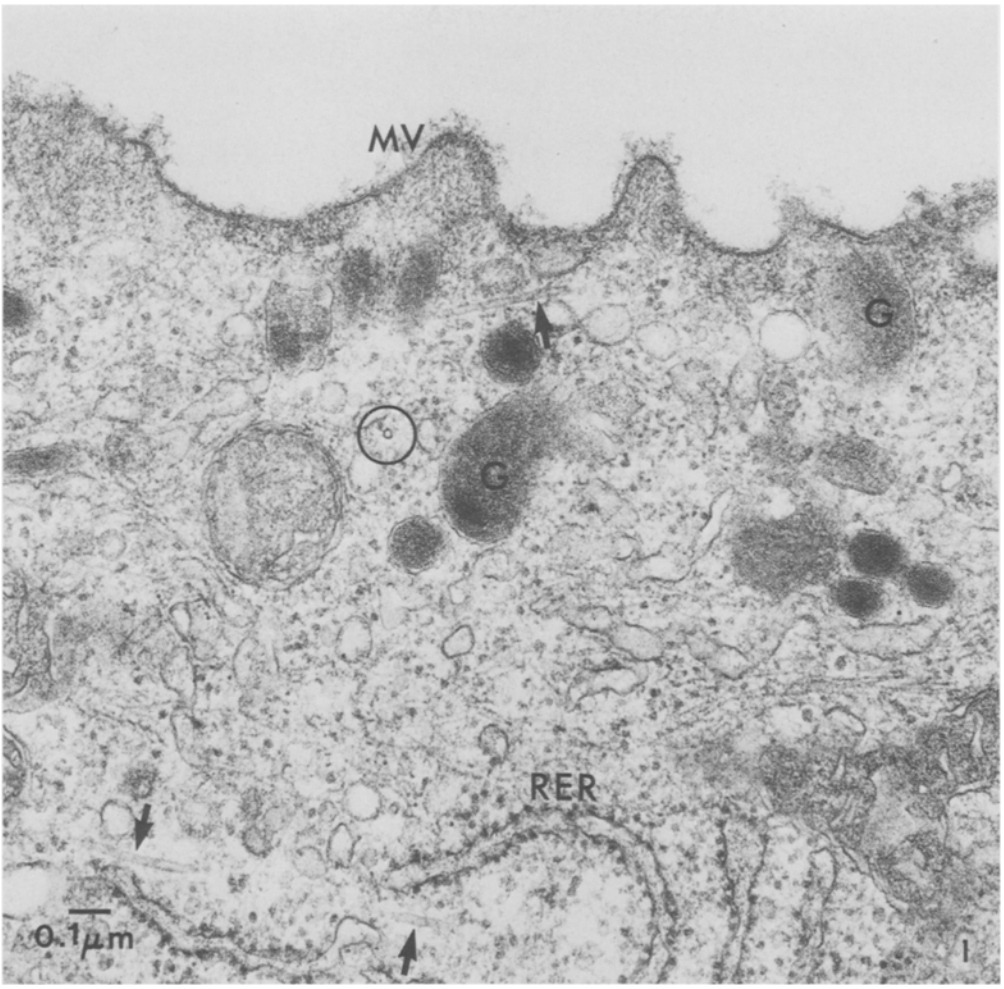


Fig. 1. Apical portion of typical granular epithelial cell from a control hemibladder. Short microvilli (*MV*), membrane-limited granules (*G*) (some of which are associated with the apical surface), and several distinct profiles of rough endoplasmic reticulum (*RER*) are seen. The distribution of microtubules is typical of granular cells in that several short longitudinal fragments (arrows) and a cross-section (encircled) are visible. 55,000×

region at the apical surface of the cells, and have not been seen to make direct contact with the apical membrane or with other cellular membranes.

Figure 2 represents a portion of a typical mitochondria-rich cell. The apical portion of the cell is narrow, has long upright microvilli and contains clusters of smooth-surfaced vesicles of all sizes; although few

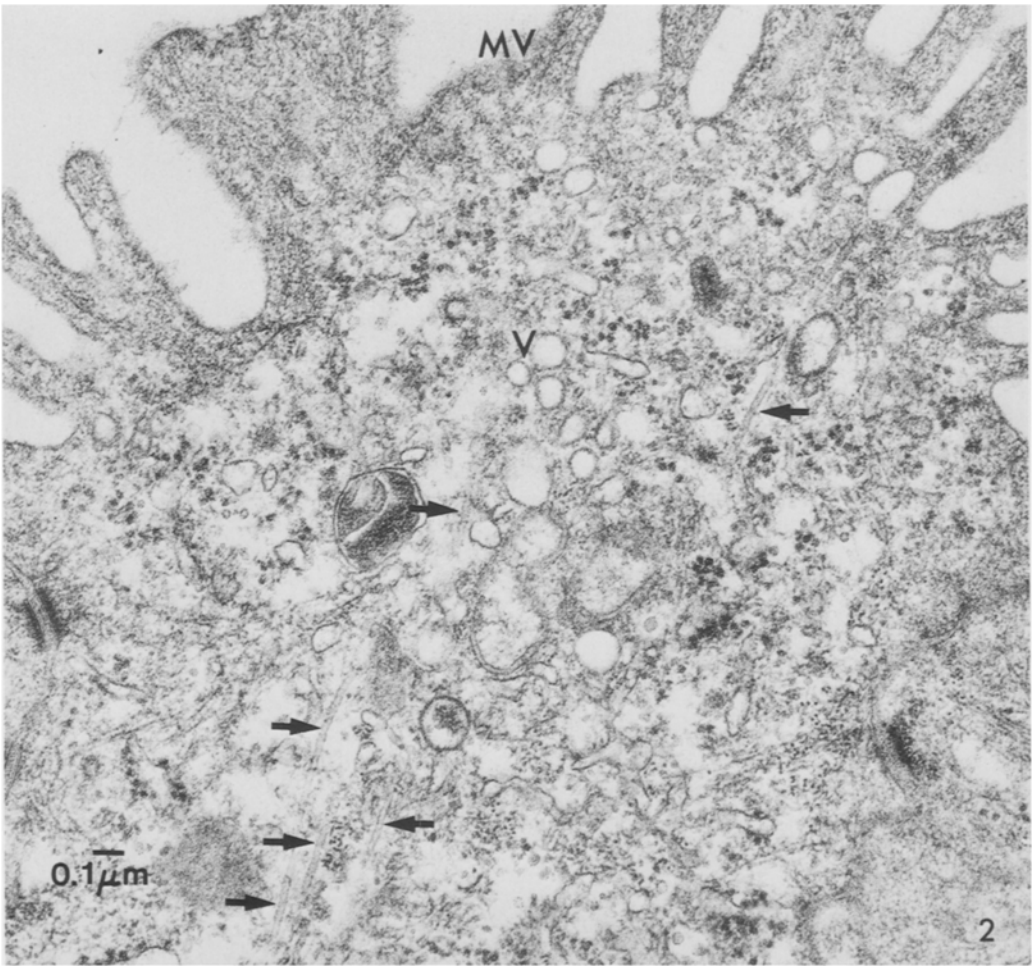


Fig. 2. Apical portion of a mitochondria-rich cell. This cell type is distinguishable from granular cells by its long, upright microvilli (*MV*), multiple vesicles (*V*), and few granules and/or mitochondria in the apical portion of the cell. The majority of microtubules in the apical cytoplasm of this cell type are oriented in the apical-basal axis of the cell (*arrows*). 43,000 ×

mitochondria are seen in this part of the cell, they are abundant in the sub-apical region. Microtubules are commonly seen in long lengths in the apical cytoplasm of mitochondria-rich cells and, in distinction to those in granular cells, are generally oriented perpendicular to the apical surface (Fig. 2).

### *Effect of Colchicine on the Microtubule Content of Granular Epithelial Cells*

To extend our previous studies with colchicine [30, 31, 35], we determined the effect of this agent on the fine structure of the granular epithelial cells. The granular cells were chosen for this study since they constitute the major cell type and, moreover, the movement of water induced by vasopressin appears to be confined to them [8, 27].

Exposure to colchicine in the concentration range $2 \times 10^{-6}$ to $8 \times 10^{-4}$ M for 3 hr resulted in no obvious conformational or morphological changes in the granular cells. However, microtubules appeared to be generally reduced in number. Quantitative analysis of apical portions of randomly-selected granular cells from 30 control hemibladders indicated that the mean volume density of microtubules was $6.4 \pm 0.7 \times 10^{-4}$, i.e., microtubules occupied approximately 0.06% of the apical cytoplasm of the cells. Although there was considerable variation between values for individual bladders, the volume density of microtubules in cells from colchicine-treated hemibladders was consistently lower than that in cells from their paired controls (*see* ref. [29]). The effect of colchicine on microtubule volume density (calculated as percent reduction for paired hemibladders) was concentration dependent. The dose-response relationships of the effect of colchicine on microtubule volume density and of its effect on vasopressin-induced water movement (determined in our previous functional studies [30]) are plotted together in Fig. 3. The dose-response curves are clearly similar. From a double reciprocal plot of the data the effect on microtubule volume density is estimated to be half-maximal at a colchicine concentration of $1.4 \times 10^{-6}$ M.

Since a change in the value for microtubule volume density after exposure to colchicine could theoretically have resulted from a change in cytoplasmic volume, rather than from a change in microtubule volume, an estimate of the volume density of other cytoplasmic organelles was made from the same photographic prints used for the microtubule estimates. No consistent difference was found in the volume density of either mitochondria or granules in cells from colchicine-treated hemibladders, as compared with their paired controls. Thus, in a group of paired hemibladders in which one member of each pair had been exposed to colchicine $2 \times 10^{-4}$ M for 3 hr the mean volume density of mitochondria and granules was $9.8 \pm 0.7 \times 10^{-2}$ and $6.8 \pm 1.3 \times 10^{-2}$, respectively, in the colchicine treated hemibladders, and $9.3 \pm 1.0 \times 10^{-2}$ and $7.7 \pm 1.2 \times 10^{-2}$, respectively, in their paired controls ($n = 6$, NS).

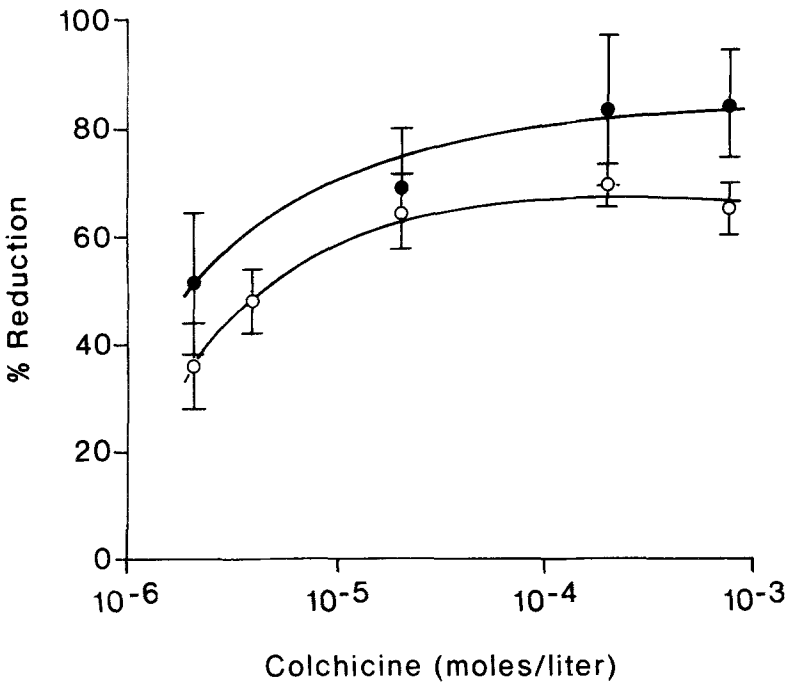


Fig. 3. Comparison of dose-response relationship of the effect of colchicine on (i) microtubule volume density in granular epithelial cells (●—●) and (ii) vasopressin-induced water flow across the isolated toad bladder (○—○). In the functional studies, one member of each pair of hemibladders was exposed to colchicine for 4 hr prior to addition of vasopressin (20 mU/ml); results are expressed as percent reduction in the response to vasopressin in colchicine-treated hemibladders relative to their paired controls measured over a 30-min period [31]

### *Effect of Vasopressin on Microtubule Content of Granular Epithelial Cells*

After exposure of hemibladders to vasopressin 100 mU/ml for 30 min, no obvious change in the content or distribution of microtubules in the granular cells was noted on preliminary inspection. However, quantitative analysis revealed that the volume density of microtubules in the apical cytoplasm of the hormone-treated cells was increased in comparison with controls. In the first group of bladders examined (preincubated for $2\frac{1}{2}$ hr before addition of vasopressin, Table 1, Experiment I), there was considerable variation, and indeed overlap, between the figures for individual bladders; however, in each instance, microtubule volume density was slightly greater in the hormone-treated cells than in cells from the paired controls, the mean percentage difference being $28 \pm 5\%$

($n=7$, $P<0.01$). In the second group of bladders (pre-incubated for 15 min before addition of vasopressin, Table 1, Experiment II), mean microtubule volume density in the granular cells was substantially greater in three out of four of the vasopressin-treated hemibladders than in their paired controls. There was no difference between the control values for microtubule volume density in Experiments *I* and *II* (Table 1), i.e., cellular microtubule content in the control situation did not vary significantly with the period of pre-incubation.

As a test of the specificity of the increase in microtubule volume density after exposure to vasopressin, independent estimates of the volume density of mitochondria and granules were obtained. No consistent difference was found in the volume density of either mitochondria or granules in cells from the hormone-treated hemibladders, as compared with their paired controls; the mean volume density of mitochondria and granules was $9.4 \pm 0.8 \times 10^{-2}$ and $8.9 \pm 2.0 \times 10^{-2}$, respectively, in the vasopressin-treated hemibladders, and $8.4 \pm 1.1 \times 10^{-2}$ and $7.4 \pm 1.0 \times 10^{-2}$, respectively, in their paired controls ($n=7$, NS).

### *Effect of Cyclic AMP on Microtubule Content of Granular Epithelial Cells*

It is generally accepted that cyclic AMP is an intracellular mediator of the action of vasopressin [12]; the effect of the nucleotide on assembled microtubule content was therefore examined. Following exposure of hemibladders to cyclic AMP 10 mM, the mean volume density of microtubules in the granular cells was increased by $31 \pm 13\%$ ($n=11$, $P<0.05$) (Table 2). The effect of cyclic AMP was similar in extent, although it was not as consistent, as that seen with vasopressin.[1]

### *Relationship of Microtubules to other Cell Structures in Granular Epithelial Cells*

In an attempt to determine whether microtubules have any special association with granules, mitochondria, or other cell structures [*vis.* the nucleus, apical subplasmalemmal region, intercellular plasma membranes, rough endoplasmic reticulum (RER), unidentified smooth sur-

1 Variability in the effect of cyclic AMP on water and sodium transfer by the toad bladder has been noted by others (e.g., [6, 11]) and may be attributable to variable rates of metabolism of cyclic AMP by intact bladder tissue [11].

Table 2. Effect of cyclic AMP on volume density of cytoplasmic microtubules in granular cells[a]

| Volume density $\times 10^4$ | | | |
|---|---|---|---|
| Animal | Control[b] | cAMP[b] | Percent difference |
| 1 | 4.5 | 3.1 | − 31 |
| 2 | 3.7 | 7.6 | +105 |
| 3 | 7.5 | 11.0 | + 47 |
| 4 | 4.2 | 3.8 | − 10 |
| 5 | 6.7 | 13.7 | +105 |
| 6 | 8.6 | 7.4 | − 14 |
| 7 | 8.2 | 5.6 | − 32 |
| 8 | 4.8 | 4.4 | − 8 |
| 9 | 3.3 | 4.8 | + 45 |
| 10 | 4.6 | 7.9 | + 72 |
| 11 | 6.0 | 11.6 | + 93 |
| 12 | 11.5 | 16.9 | + 47 |
| 13 | 5.2 | 6.6 | + 27 |
| 14 | 8.0 | 7.5 | − 6 |
| Mean ± SE | 6.2 ± 0.6 | 8.0 ± 1.1 | + 31 ± 13[c] |

[a] Figures represent mean values for five cells from each hemibladder.
[b] Control hemibladders were suspended in Ringer's solution for 3 hr; cyclic AMP treated hemibladders were suspended in Ringer's solution for $2\frac{1}{2}$ hr prior to addition of cyclic AMP 10 mM for 30 min.
[c] $P < 0.05$.

faced membranes (SER), or the Golgi complex] analyses were made of the micro-environments of true microtubules and of random points as described in *Materials and Methods*. Under the specific conditions of this study, no special, spatial association between microtubules and any other cell structures was detected, either in control tissue (Table 3) or in vasopressin-treated hemibladders. The only significant correlation observed was a negative correlation between microtubules and the apical subplasmalemmal region (Table 3), indicating that fewer microtubules were actually associated with the apical cell surface than predicted on the basis of a random distribution.

### *Microtubule Content of Mitochondria-Rich Cells*

Mitochondria-rich cells do not appear to directly participate in the transcellular movement of water induced by vasopressin [8, 27], but

Table 3. Relationship of microtubules to other cell structures in granular cells[a]

| Cell structure | Mean (±SE) hits within microtubule micro-environment | Mean (±SE) hits within random point micro-environment | Significant differences |
|---|---|---|---|
| Granules | 2.1±0.9 | 2.3±0.6 | NS |
| Mitochondria | 3.6±1.0 | 3.7±0.8 | NS |
| Nucleus | 1.0±0.7 | 0.4±0.3 | NS |
| Apical subplasmalemmal region[b] | 0.6±0.4 | 1.1±0.5 | $P<0.05$ |
| Intercellular membrane | 0.4±0.4 | 0.3±0.3 | NS |
| RER | 3.0±1.0 | 3.0±0.8 | NS |
| SER | 10.1±3.1 | 13.3±4.3 | NS |
| Golgi complex | 11.0±1.8 | 9.9±1.4 | NS |

[a] In all cases (except measurements involving Golgi complex) figures represent mean values for the same prints of 4–5 cells from each of seven control hemibladders. Prints of Golgi complexes were from different cells specifically photographed to include Golgi complexes and represent values from only three hemibladders.

[b] In this study the apical subplasmalemmal region includes the microvilli and the very narrow region under the apical plasma membrane which contains a reticulum of microfilaments.

A circle of 7.5 mm radius was used for these studies (*see*, Materials and Methods); when the measurements were repeated for granules and mitochondria using a circle of 3.5 mm radius, similar results were obtained.

there is evidence that they respond to the hormone, as indicated by an increase in adenylate cyclase activity [13] and in cyclic AMP content [10, 25]. The microtubule content of mitochondria-rich cells from control hemibladders was accordingly quantitated for purposes of comparison with granular cells from the same tissues. Table 4 shows that the mean volume density of microtubules in the apical cytoplasm of mitochondria-rich cells from five control hemibladders was $13.8 \pm 0.9 \times 10^{-4}$, as compared to $6.8 \pm 0.8 \times 10^{-4}$ for granular cells [paired difference $= 116 \pm 29\%$ ($P<0.01$)].

Also shown in Table 4 are values for the microtubule content of mitochondria-rich cells from the paired hemibladders which had been exposed to vasopressin. Mean volume density of microtubules in the apical cytoplasm of these cells was $12.7 \pm 2.0 \times 10^{-4}$; the mean value for the paired control hemibladders was $14.6 \pm 1.5 \times 10^{-4}$ (paired difference $= 5.0 \pm 22\%$, $n=5$, NS).

Table 4. Microtubule volume density in mitochondria-rich cells

| Experiment | Volume density $\times 10^4$ | | | |
|---|---|---|---|---|
| | Animal | Granular cell | MR cell[a] | Percent difference |
| I | 1 | 8.2 | 15.6 | + 90 |
| | 2 | 6.8 | 12.3 | + 81 |
| | 3 | 8.6 | 12.2 | + 42 |
| | 4 | 5.6 | 16.6 | +196 |
| | 5 | 4.6 | 12.4 | +170 |
| | Mean ± SE | 6.8 ± 0.8 | 13.8 ± 0.9 | +116 ± 29[b] |
| | Animal | Control-MR cell[a] | Vasopressin-MR cell[a] | |
| II | 1 | 10.0 | 13.0 | +30 |
| | 2 | 12.3 | 20.0 | +63 |
| | 3 | 16.6 | 11.1 | −33 |
| | 4 | 15.6 | 11.5 | −26 |
| | 5 | 18.3 | 7.8 | −57 |
| | Mean ± SE | 14.6 ± 1.5 | 12.7 ± 2.0 | − 5 ± 22[c] |

[a] MR cell = mitochondria-rich cell, conditions same as in Table 1. In Experiment *I*, granular cells and MR cells are from same tissue block; in Experiment *II*, control and vasopressin MR cells are from paired hemibladders.
[b] $P < 0.01$.
[c] NS.

## Discussion

The present studies indicate that assembled microtubules are sparsely and apparently randomly distributed and oriented in the cytoplasm of the granular epithelial cells of the toad bladder. However, microtubules are seldom seen in the subplasmalemmal region at the apical surface of these cells, and statistical analysis reveals that they are in fact less frequent in this region than predicted on the basis of a random distribution.

These studies provide morphometric evidence that exposure of the toad bladder to colchicine results in a dose-dependent decrease in the content of assembled microtubules in the granular epithelial cells. Comparison of the dose-response relationship of the effect of colchicine on granular cell microtubule content with that of its inhibitory effect on the response to vasopressin in the intact bladder [30] reveals a close parallelism between the two (Fig. 3). Thus, exposure of hemibladders to given concentrations of colchicine evidently induces proportional re-

ductions in the cytoplasmic microtubule content of the granular cells, and in vasopressin-induced water movement. Furthermore, comparison of data obtained in our previous biochemical studies on isolated bladder epithelial cells [35] with that obtained in the morphological and functional studies [30] on intact bladders reveals a remarkable correlation between the concentrations of colchicine required for (i) half-maximal binding to tubulin in the soluble fraction of isolated bladder epithelial cells ($1.0 \times 10^{-6}$ M) [35], (ii) half-maximal reduction in cytoplasmic microtubule content of the granular cells ($1.4 \times 10^{-6}$ M) and (iii) half-maximal inhibition of vasopressin-induced water movement ($1.8 \times 10^{-6}$ M) [30]. The combined evidence supports the view that the inhibitory effect of colchicine on vasopressin-induced water movement is indeed due to its interaction with tubulin and consequent interference with microtubule integrity and function, rather than to a direct action on the cell membrane [20, 34].

The demonstration that exposure to colchicine leads to disruption of cytoplasmic microtubules in the granular epithelial cells of the bladder constitutes evidence that the alkaloid enters these cells and there interacts with tubulin. However, this does not in itself indicate that assembled microtubules play an integral role in the action of vasopressin; clearly the disruption of cytoplasmic microtubules could be coincidental to the inhibition of vasopressin-induced water movement. In this context, the finding that the content of assembled microtubules in the granular epithelial cell is increased following exposure of the bladder to vasopressin, and to cyclic AMP, is of critical importance in that it suggests that microtubules participate in the cellular response to the hormone in the toad bladder. Assembled cytoplasmic microtubules are believed to exist in a state of dynamic equilibrium with a cellular pool of soluble tubulin [15, 16]. The observed change in granular cell microtubule content suggests that a small, but definite, shift in the equilibrium of the microtubule assembly/disassembly process is associated with the action of vasopressin and cyclic AMP on these cells. However, the relatively modest increase in cellular microtubule content in response to the hormone, taken together with the functional and morphometric findings with colchicine, suggests that the physiological response to vasopressin is largely a function of the content of assembled microtubules, and not to the process of microtubule assembly per se.[2]

2 The change in microtubule content after vasopressin and cyclic AMP may indeed reflect a decrease in the rate of microtubule disassembly, rather than an increase in the rate of assembly [28].

It is noteworthy that microtubules in the apical cytoplasm of the mitochondria-rich cells appear in general to be oriented perpendicular to the apical cell surface in contrast to the situation in granular cells. The finding that the content of microtubules in the apical region of the former cells is twice that in the latter may merely reflect a difference in microtubule orientation in the two cell types. However, the content of assembled microtubules in mitochondria-rich cells is not apparently influenced by vasopressin. Taken together, these findings suggest that microtubules may play a different functional role in the two cell types.

How microtubules in the granular cells of the toad bladder may participate in the vasopressin response has yet to be determined. Microtubules have been implicated in the transport and release of secretory products in a number of tissues (*see* [26]). We have previously postulated that in the toad bladder, under the influence of vasopressin, microtubules may participate in the translocation of secretory material and/or new membrane components (*cf.* [19, 22]) from the interior of the granular cells to their apical surface [29, 31]. However, analysis of the spatial distribution of microtubules within the granular cells in the present study has yielded no evidence to support, or to exclude, this hypothesis; although microtubules are occasionally seen in close relationship to granules [29], no specific, nonrandom association of microtubules with granules or with any other cellular organelles, in either control or vasopressin-treated hemibladders, was detected. It would appear that if microtubules are in special physical juxtaposition to other structures of the granular cell, the association is too subtle to be detected by our present methods.[3]

Microtubules, together with microfilaments, have been implicated in the control of the spatial distribution of surface receptors in several cell types [9, 32]. Recently, Bourguet and co-workers [1, 3] and Kachadorian *et al.* [18] have demonstrated that vasopressin and cyclic AMP [1] induce an aggregation of membrane-associated particles in the granular cells of amphibian bladder, and have suggested that this event underlies the permeability change induced by the hormone. Colchicine has been found to interfere with this vasopressin-induced particle aggregation [17]. Hence, it is tempting to speculate that microtubules play a role in the action of vasopressin through their involvement, with microfilaments [2, 6, 7, 28, 31], in the control of the distribution of

3 The validity of our results obviously depends on the assumption that glutaraldehyde fixation of the bladder epithelial cells preserves the original content of assembled cytoplasmic microtubules and does nothing to alter their cellular distribution.

intramembranous particles at the apical surface of the granular epithelial cells [17, 28]. It must be emphasized, however, that microtubules have never been seen to make direct contact with the apical plasma membrane of these cells, and are infrequently seen in the region of the subplasmalemmal microfilament network.

In summary, functional [31] and biochemical [35] studies suggest that the inhibition of vasopressin-induced water movement by colchicine and other antimitotic agents in the toad urinary bladder is related to the interaction of these agents with tubulin and the depolymerization of microtubules. The morphological data obtained in the present study provide further support for this view. The demonstration that the content of assembled microtubules in the granular cells is increased after exposure of toad bladders to vasopressin and to cyclic AMP provides evidence that cytoplasmic microtubules participate in the response to the hormone. The combined studies support the concept that assembled microtubules play a critical role in the action of vasopressin on transcellular water movement in the toad bladder. Precisely how microtubules are involved in the action of the hormone, and whether and how microtubule function is related to microfilament function [2, 6, 7, 28, 31], remain to be determined.

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