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Effects of Voltage Clamping on Epithelial Cell Composition in Toad Urinary Bladder Studied with X-ray Microanalysis

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Abstract. Toad urinary bladder epithelial cells were incubated in Na Ringer's with the serosal surface of the epithelium clamped at either +50 mV, 0 mV (short-circuited) or −50 mV with respect to the mucosal surface. Following incubation, portions of tissue were coated with an external albumin standard and rapidly frozen. Cryosections were freeze-dried and cell composition determined by x-ray microanalysis. Cell water and ion contents were unaffected when tissues were short-circuited rather than clamped close to their open-circuit potential difference (+50 mV). Incubation with vasopressin at +50 mV, and under short-circuit conditions, caused Na uptake without cell swelling or gain in Cl. Clamping at −50 mV resulted in uptake of water and ions, with considerable variation from cell to cell. These variations in cell composition were exacerbated by vasopressin. The greater the increase in water content, the greater the rise in cell Cl. However, there was no consistent pattern to the associated changes in cation contents. Most cells gained some Na. In some cells, this gain was accompanied by an increase in K. In others, the gain of Na was predominant and cell K content actually fell. At −50 mV with ouabain, many of the cells also gained water. As was found in our earlier study with ouabain under short circuit conditions (Bowler et al., 1991), there was considerable variation in the extent of the Na gain and K loss; some cells were largely depleted of K while in others the K content remained relatively normal. These results indicate differences between granular cells in the availabilities in the plasma membranes of ion pathways, either as a consequence of differences in the numbers of such pathways or in their control.

Key words: Toad bladder — Voltage-clamping — Vasopressin — Ouabain — Cell volume — X-ray microanalysis

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Introduction

Our understanding of transepithelial ion transport was revolutionized with the introduction of the short-circuit technique by Ussing and Zerahn (1951) in which the transepithelial voltage is clamped at 0 mV. In toad urinary bladder, this negates the serosa-positive spontaneous transepithelial potential difference and increases the driving force for Na entry to the cells from the mucosal medium with consequent increase in transepithelial Na transport. Despite this, short-circuiting appears to have no deleterious effects on epithelial function. This implies that the water and ion contents of the cells are maintained under such conditions. There is no histological evidence of cell swelling (Bobrycki et al., 1981); nor is ion composition (as determined by chemical analysis) altered in the short-circuited preparation (Robinson & Macknight, 1976).

Voltage-clamping the toad bladder at serosa-negative potentials, which reverses the direction of the normal spontaneous potential, further increases transepithelial Na transport. In a histological study of tissues voltage-clamped at 50 mV serosa-negative, this increase was associated with variable swelling of the granular cells, the dominant epithelial cell-type believed to play the central role in transepithelial Na transport (Bobrycki et al., 1981; DiBona et al., 1981). In contrast, basal and mitochondria-rich cells were apparently unaffected by the changed conditions. Granular cell swelling was prevented by prior exposure to mucosal amiloride and exacerbated by serosal vasopressin.

Serosa-negative voltage-clamping, by making the cell interior relatively more negative to the mucosal me-

dium, will steepen the electrochemical gradient for Na entry to the cells across the apical membrane. Bobrycki et al. (1981) and DiBona et al. (1981) suggested that the granular cell swelling was a consequence of the increased rate of Na entry to the cells saturating the (Na-K)-ATPase so that the Na pump could no longer keep up with the Na influx. Cells would thus accumulate Na and Cl (to preserve cell electroneutrality) and the gain of osmoles would result in the observed cell swelling. Amiloride, by blocking Na entry, would prevent the swelling, and vasopressin, by increasing apical membrane Na permeability, would exacerbate it. However, an alternative explanation would be that, although the Na pump can handle the increased Na influx, the basolateral membrane K permeability is insufficient to cope with the increase in K uptake. Cell swelling would then be associated with accumulation of K, together with Cl.

To determine the changes in cell composition that follow voltage clamping, we have used the technique of x-ray microanalysis, which allows analysis of the ion and water contents of individual cells. The results reveal that cell composition was similar when tissues were clamped at +50 mV serosa (i.e., the normal orientation of the transepithelial potential) or at 0 mV (short-circuited) with or without vasopressin. However, at −50 mV serosa there was considerable variability in composition from cell to cell. Most cells gained some Na. In some cells this gain was accompanied by an increase in K. In others, the gain of Na was predominant and cell K content actually fell.

Materials and Methods

Incubation Media

Na Ringer's contained (in mM): Na^+, 115; K^+, 3.5; Cl^-, 116.5; Mg^{2+}, 1; Ca^{2+}, 1; SO_4^{2-}, 1; glucose, 10; buffered to pH of 7.4 by HPO_4^{2-}, 1 mM. Ouabain (Sigma) was dissolved in the medium to a final concentration of 1 mM. Vasopressin (Sigma) was added to the serosal medium to a final concentration of 150 mU/ml.

The osmolalities of all solutions (220–230 mosmol/kg H_2O) were measured with a Wescor vapor pressure osmometer.

Animals and Incubation

Dominican toads (*Bufo marinus*) obtained from National Reagents, Bridgeport, CT were kept on wood shavings with free access to water and regularly force-fed with ground liver. The toads were double-pithed and the two hemibladders removed.

Hemibladders were pinned, mucosal side upwards, onto a sylgard-coated Petri dish filled with Na Ringer's solution. Plastic rings (area approx. 3 cm^2) coated with cyanoacrylate adhesive were slid under each hemibladder. Care was taken at every stage to prevent excessive stretching of the tissue and to avoid any contact with the mucosal surface. Rings with adhering tissue were inserted in modified Ussing chambers and, after 60 min incubation under short-circuit conditions, the tissue was clamped continuously at either +50 mV, 0 mV (short circuited) or −50 mV serosa for a further 60 min (except for brief 1 sec pulses of ±5 mV every 2 min to determine tissue resistance). In the ouabain experiments, tissues were first exposed for 60 min on their serosal surface to the glycoside (1 mM) under short-circuit conditions and then clamped at −50 mV with ouabain for a further 60 min. In the vasopressin experiments, tissues were incubated under short-circuit conditions for 50 min, exposed to vasopressin (150 mU/ml) for 5 to 10 min and then clamped appropriately with vasopressin for a further 60 min. [Note that transepithelial current provides a measure of active transport through the cell only in tissues clamped at 0 mV. This current is referred to in the text as the short-circuit current (*Isc*). Otherwise, transepithelial current is represented as *I* and includes current flowing through the paracellular pathway as well as through the cells.]

After all incubations, rings were removed and the tissues gently blotted with Whatman 542 filter paper on both the mucosal and serosal surfaces to remove excess solution. An external standard solution containing albumin (30%) was applied to the mucosal surface with a Pasteur pipette and the excess removed with filter paper.

Freezing and Cryosectioning

The techniques described recently (Bowler et al., 1991) were used. Briefly, the plastic ring with its tissue was plunged into a propane-isopentane mixture at liquid N_2 temperature within 20 sec of removal of the tissue from the incubation medium.

Sections were cut at −80° to −90°C with a modified Cryocut I (Burlington Scientific Instruments) fitted to a Sorvall MBT-2 ultramicrotome. Sections were transferred to Formvar-coated 3 mm nickel slot grids which were then placed face down on a Formvar film on an aluminium specimen holder. The sections were thus sandwiched between two films. Two or more blocks from each piece of frozen tissue were cut and several sections from each block analyzed. The sections were transferred into a scanning electron microscope (JEOL JSM-840) with a Hexland transfer device precooled with liquid nitrogen. The microscope stage was precooled (<−180°C). Sections were freeze-dried by warming the stage.

Analysis of Sections

Sections were imaged with a transmitted electron detector. Spectra were collected for 100 sec at 15 kV with a Tracor Northern X-ray 30 mm^2 detector connected to a Nucleus AD converter. The probe current (150–250 pA) was measured with a Faraday cup after each spectrum.

Spectra were collected from at least two relatively large areas of albumin above the cells. Data from these areas were pooled to provide an average value. To obtain average cellular values, a large area within each cell, including wherever possible cytoplasm and a portion of the nucleus, was scanned. Only one measurement was taken from each cell in any section.

Data analysis and quantification were performed using the approach of Rick, Dörge & Thurau, (1982) as described in detail for our laboratory elsewhere (Bowler et al., 1991). Data were obtained from 3 or 4 bladders. In any section all epithelial cells with a clearly definable nucleus and cell borders were analyzed.

Differences between groups were analyzed nonparametrically using the Kruskal-Wallis test. Unless stated otherwise, values are compared to those found under short-circuit conditions in the absence of vasopressin and significant differences are indicated in the figures by either * (< 0.01) or ** (< 0.001) above the conditions. These significances were calculated using Dunn's Multiple Comparison Test. Box plots are used to display the data. In these, the box depicts the data between the 25th and 75th percentiles with the 50th percentile (median) marked by the horizontal line within the box. The whiskers depict the

Table. Mean transepithelial current (I) under different voltage-clamp conditions

Final conditions	I_{sc} before changes	I_{sc} after drug	I after voltage clamp	
			Peak	After 60 min
+50 mV serosa				
Control (3)	8.57 ± 1.17		−0.77 ± 1.73	−3.10 ± 1.82
+ Vasopressin (3)	7.97 ± 0.70	9.10 ± 0.87[a]	9.70 ± 1.26	5.63 ± 1.23
0 mV				
Control (3)	6.60 ± 1.62			6.30 ± 1.92
+ Vasopressin (3)	6.53 ± 1.29	16.90 ± 3.11[a]		12.37 ± 0.98
−50 mV serosa				
Control (4)	12.38 ± 3.73		28.15 ± 9.54	27.10 ± 8.64
+ Vasopressin (4)	7.75 ± 1.41	16.55 ± 2.56[a]	40.55 ± 4.64	22.66 ± 5.65
+ Ouabain (4)	11.65 ± 1.75	1.80 ± 0.44[b]	9.70 ± 1.63	10.80 ± 2.43

Table shows mean ± SEM from the number of bladders in brackets. Tissues originally clamped at 0 mV and short-circuit current (I_{sc}) recorded for 60–120 min.

[a] Peak response within 5–10 min after vasopressin under short-circuit conditions.

[b] I_{sc} 60 min after ouabain under short-circuit conditions.

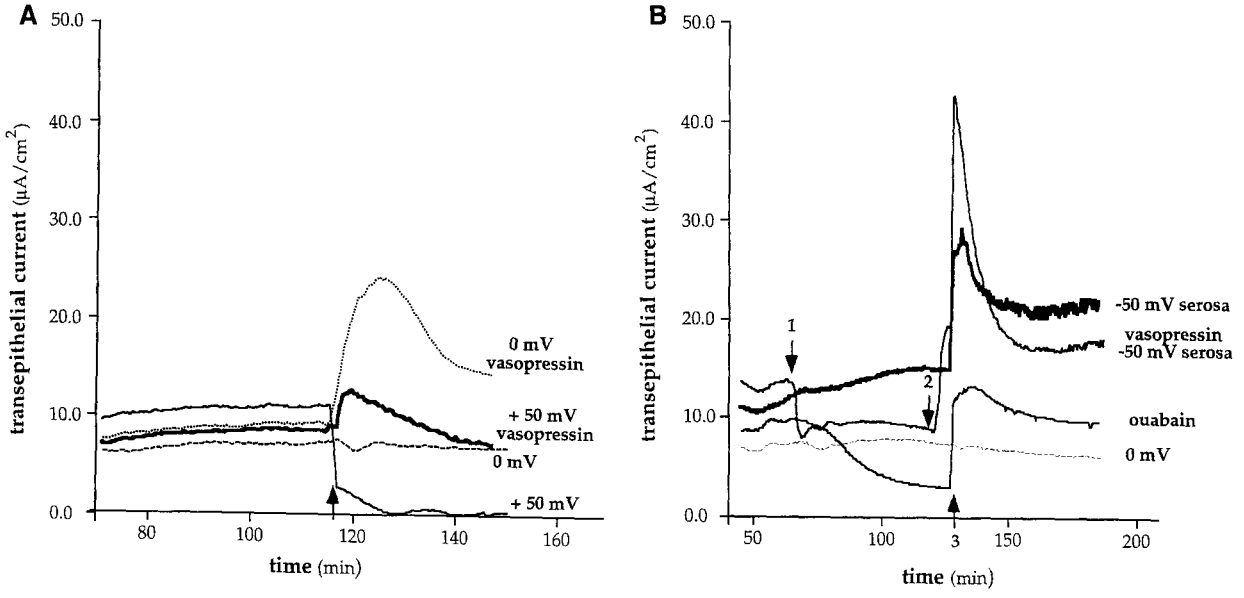

Fig. 1. Representative changes in transepithelial current. (A) The responses of 4 quarter bladders to voltage clamping at +50 mV serosa or at 0 mV (short-circuited). Initially all tissues were short-circuited. Two tissues received serosal vasopressin. After 3.5 min, as the current started to increase in response to vasopressin, one tissue treated with vasopressin and one untreated tissue were clamped at +50 mV serosa (arrow). The other two tissues remained at 0 mV. (B) The responses of 4 quarter bladders to voltage clamping at −50 mV serosa or at 0 mV (short-circuited). Initially all tissues were short-circuited. At arrow 1, one tissue was exposed to serosal ouabain (1 mM). At arrow 2, another tissue was exposed to serosal vasopressin. At arrow 3, three tissues were clamped at −50 mV serosa. The control tissue, which was not exposed to reagents, remained at 0 mV throughout.

10th and 90th percentiles, with values outside of these ranges plotted separately. In some plots where the scale allowed, the 95% confidence interval around the median is also shown (by the notch in the box).

Results

Voltage-clamping altered transepithelial currents in the predicted directions (Table; Fig. 1), with serosa-positive voltage reducing current, and serosa-negative voltage increasing current in untreated tissues. Vasopressin increased current substantially in short-circuited tissues and in those clamped at −50 mV serosa. In tissues clamped at +50 mV serosa, vasopressin prevented the initial marked fall in current that resulted from the voltage-clamping, and current was better sustained over the 60 min. The mean current in the control tissue at +50 mV was close to zero, as expected given that the mean open circuit potential for these three tissues was +47 mV.

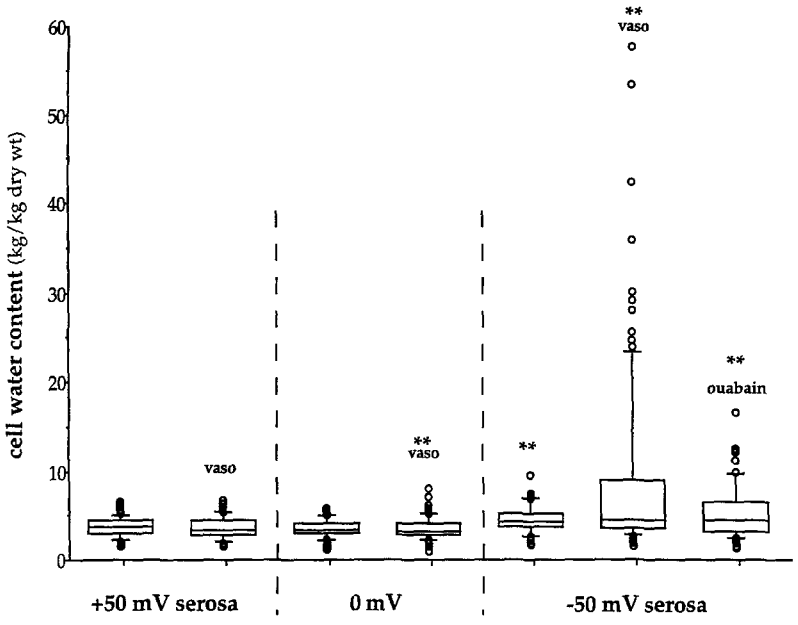

Fig. 2. Box plots of cell water contents in tissues clamped at +50 mV, 0 mV or −50 mV serosa. The stars indicate median values significantly different from the 0 mV control (not vasopressin) at either $P < 0.01$ (*) or $P < 0.001$ (**). For the +50 mV and 0 mV experiments, cells were obtained from tissues from 3 toads, with analysis of 85 individual cells for +50 mV, 72 individual cells for +50 mV with vasopressin, 192 individual cells for 0 mV and 87 individual cells for 0 mV with vasopressin. For the −50 mV experiments, cells were obtained from tissues from 4 toads, with analysis of 76 individual cells for −50 mV, 101 individual cells for −50 mV with vasopressin, and 90 individual cells for −50 mV with ouabain.

Ouabain inhibited *Isc* by some 80% over 60 min. In tissues clamped at −50 mV serosa, transepithelial current reflects both cellular and paracellular flow. The latter is driven by the transepithelial electrical gradient and facilitated by the decreased junctional resistance associated with junctional "blistering" induced by the increased current flow (Bindslev et al., 1974; Bobrycki et al., 1981). Much of the increased current in ouabain-treated tissues clamped at −50 mV serosa is a consequence of this passive paracellular flow. Such an increase was also found in amiloride-treated tissues clamped at this potential (Bobrycki et al., 1981).

Cell water contents expressed on a dry wt. basis were not altered significantly by clamping at +50 mV or 0 mV either in the absence of, or following exposure to, vasopressin (Fig. 2), median values being 3.74 kg/kg dry wt. without and 3.50 kg/kg dry wt with vasopressin at +50 mV, and 3.52 kg/kg dry wt without and 3.27 kg/kg dry wt with vasopressin under short-circuit conditions. There was also comparatively little scatter in these data (Fig. 2). In contrast, the water contents of tissues clamped at −50 mV serosa were all increased significantly ($P < 0.001$) compared to those of tissues incubated under short-circuit conditions with or without vasopressin, the median values being 4.44 kg/kg dry wt in the absence of drugs, 4.50 kg/kg dry wt following vasopressin and 4.63 kg/kg dry wt following ouabain. With vasopressin, in particular, there was also considerably greater scatter, some cells having very high water contents. Cell phosphorus (P) reflects the content of the normally nondiffusible P-containing molecules (e.g., phosphoproteins, phospholipids, nucleic acids and high energy P compounds). It is calculated by fitting to the P elemental peak. In contrast, cell mass (from which cell water content is derived) is measured from the continuum of the energy spectrum. If swelling of the cells simply dilutes the cell contents, then there should be a good correlation between these two variables. Despite the enormous swelling seen in some of the cells, this was indeed true, as illustrated for the vasopressin data (Fig. 3), with r = 0.910.

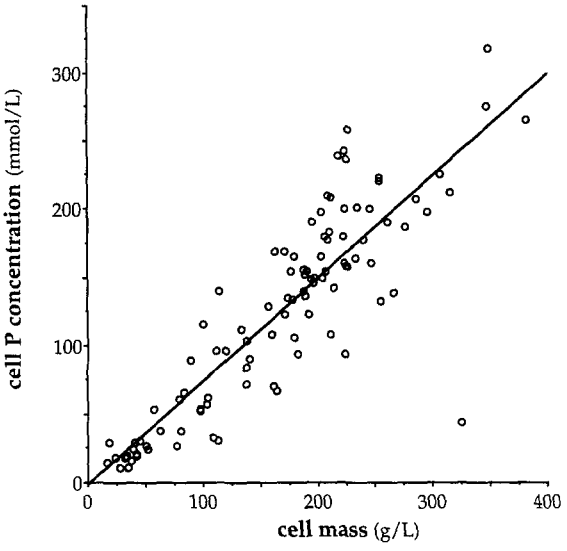

Fig. 3. Relationship between cell P concentration (mmol/L of cell) and cell mass (g/L of cell) in vasopressin-treated tissues clamped at −50 mV. y = 0.75 × −1.49, r = 0.8688.

The patterns of the ion contents were consistent with those found for the water contents. In the absence of vasopressin, cell ion contents were not altered appreciably between +50 mV and 0 mV (Figs. 4–6). Under short-circuit conditions, mean Na rose from 13.5 ± 0.5 SEM mmol/kg wet wt to 18.9 ± 1.1 mmol/kg wet wt ($P <$ 0.001) and mean K fell from 104.7 ± 1.7 mmol/kg wet wt

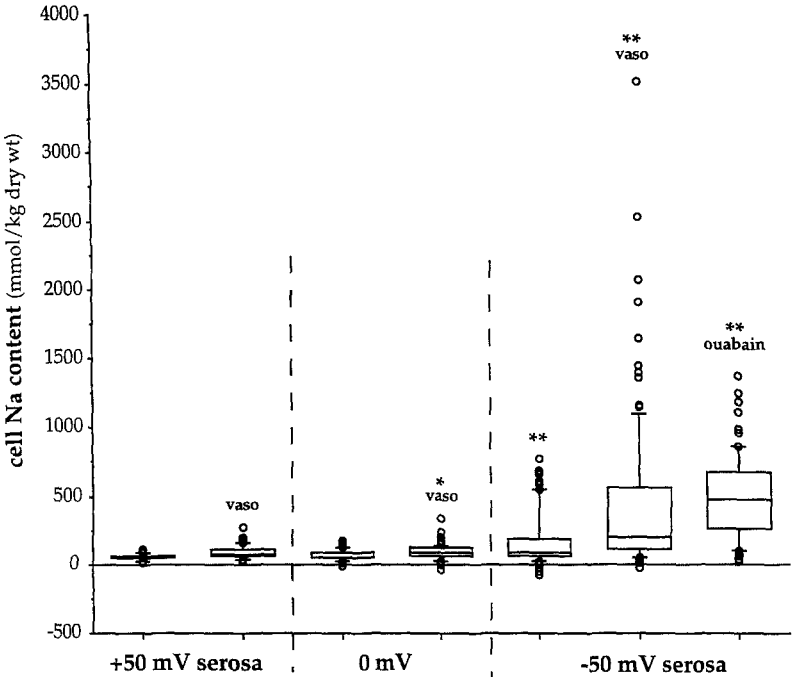

Fig. 4. Box plots of cell Na contents in tissues clamped at +50 mV, 0 mV or −50 mV serosa. In this, and in Figs. 4–6 and 8, the stars indicate median values significantly different from the 0 mV control (no vasopressin) with * indicating $P < 0.01$ and ** indicating $P < 0.001$.

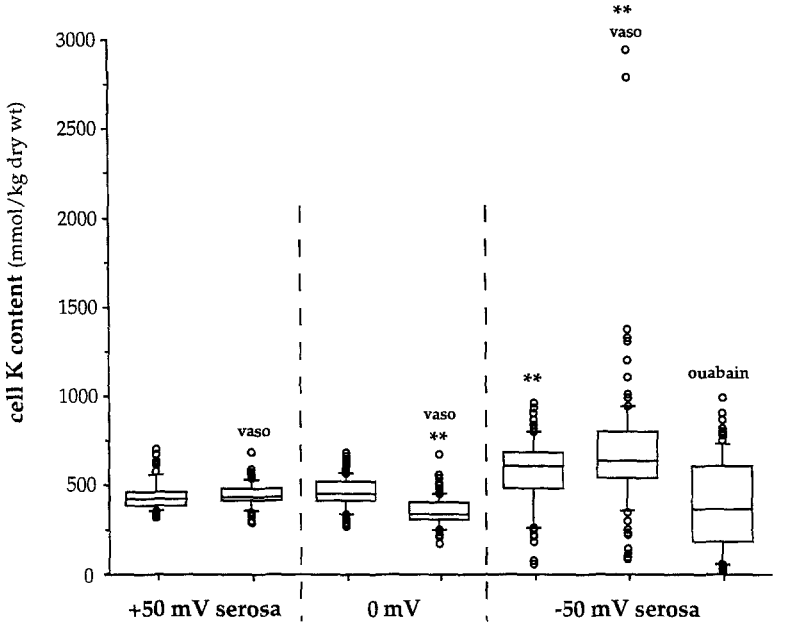

Fig. 5. Box plots of cell K contents in tissues clamped at +50 mV, 0 mV or −50 mV serosa.

to 81.5 ± 2.5 mmol/kg wet wt following vasopressin ($P < 0.001$). The same conclusion emerges if comparisons are made on a dry wt basis and whether parametric or nonparametric tests (comparing medians) are performed. In tissues clamped at +50 mV, cell Na was also significantly elevated after vasopressin, rising from 11.0 ± 0.7 to 19.7 ± 1.4 mmol/kg wet wt ($P < 0.001$), but, when expressed in this way, cell K was not affected (95.4 ± 2.8 without and 100.5 ± 3.0 mmol/kg wet wt after vasopressin, $P = 0.22$). However, the change in K would be relatively small compared to the variability in the K measurement from cell to cell. In part, this variability arises from differences in cell mass from measurement to measurement. Phosphorus, being a constituent of cell nondiffusible solutes, which carry net negative charge, correlates well with cell mass (Fig. 3). Potassium, as the dominant diffusible cell cation, is chiefly responsible for balancing these charges, and there is an excellent correlation from measurement to measurement between K and P (Fig. 7). Consequently, a more sensitive way to detect small differences in cell K between groups, is to normalize the data from each measurement to the P content for

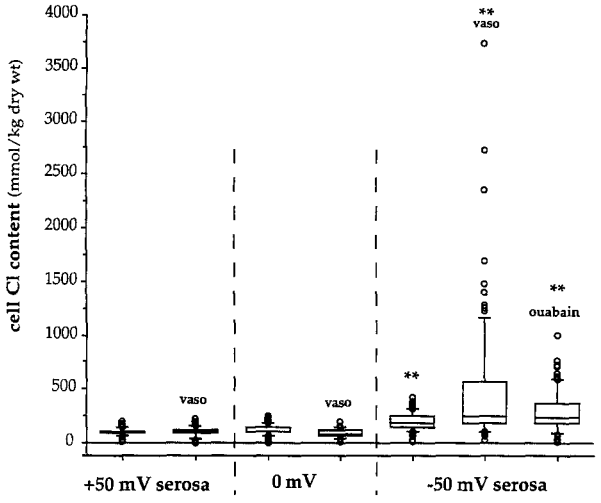

Fig. 6. Box plots of cell Cl contents in tissues clamped at +50 mV, 0 mV or −50 mV serosa.

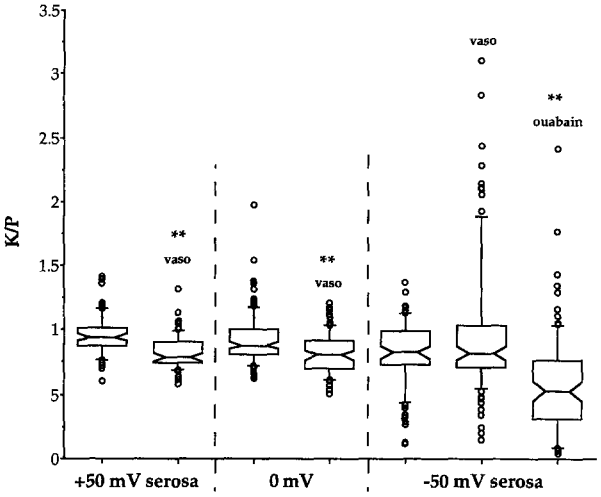

Fig. 8. Box plots of the ratio cell K/cell P in tissues clamped at +50 mV, 0 mV or −50 mV serosa.

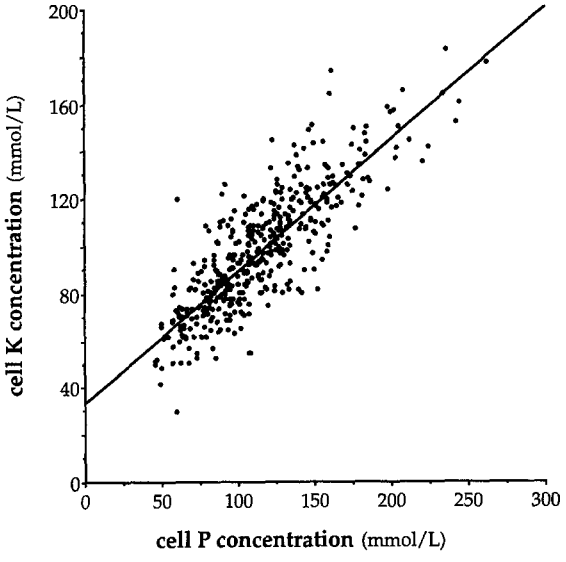

Fig. 7. Relationships between cell K (mmol/kg wet wt) and cell phosphorus (P) (mmol/kg wet wt) for all cells from tissues clamped at either +50 or 0 mV serosa. y = 0.56× + 33.56, r = 0.8307.

that measurement (Fig. 8). This also corrects for any variations arising in the albumin standard which is not required for data expressed in ratio form. After vasopressin at both +50 mV and 0 mV the K/P ratio decreased significantly ($P < 0.001$), using either parametric or non-parametric testing.

As with the water contents, the scatter in the Na, K and Cl contents (and in K/P) in tissues clamped at −50 mV was much greater than in the tissues incubated at +50 mV or under short-circuit conditions (Figs. 4–6, and 8). Clamping at −50 mV with or without vasopressin increased Na, K and Cl contents expressed on a dry wt basis significantly ($P < 0.001$) compared to values under short-circuit conditions without vasopressin. Incubation with ouabain at −50 mV resulted in a significant increase in Na and loss of K compared to −50 mV alone ($P < 0.001$), but, compared to 0 mV alone, although the gain in Na was highly significant ($P < 0.001$) (Fig. 4), the loss of K was not (Fig. 5). Analysis of the K data corrected for variations in P between groups (Fig. 8) reveals that, following clamping alone or with vasopressin, the K/P ratio was not significantly different from that of the 0 mV controls. However, there was a significant decrease ($P < 0.001$) in this ratio in tissues exposed to ouabain.

The greatest changes occurred with the combination of vasopressin and serosa-negative voltage clamping. As is apparent in a freeze-dried cryosection cut from a tissue clamped at −50 mV and exposed to vasopressin (Fig. 9), not all granular cells were affected equally by the conditions. Some cells are markedly swollen while others appear to be of relatively normal volume. The same observation was made with conventional histological techniques by DiBona et al. (1981). As illustrated in the box plot (Fig. 2), many of the cells incubated under these conditions gained water; some swelled enormously. Whereas 50% of the cells incubated under short-circuit conditions without vasopressin had water contents below 3.50 kg/kg dry wt (with a range of 1.15 to 5.94 kg/kg dry wt), this was true for only 27% of the cells clamped at −50 mV following vasopressin, and values ranged from 1.61 to 57.75 kg/kg dry wt.

The increased cell water content was associated with the uptake of diffusible cations (Fig. 10*A*). However, the relationship between the gain of Na or K and water was more variable from cell to cell (Fig. 10*B* and *C*). To display the relationship between Na and K for each individual cell on a dry wt basis, (cell K − cell Na) was plotted against cell water content (Fig. 11). A normal, positive value for (K − Na) indicates that cell K exceeds cell Na. A negative value means that cell Na is greater than cell K. In tissues clamped at +50 or 0 mV, cell Na

Fig. 9. An electron micrograph of a freeze-dried cryosection from toad bladder epithelium after voltage-clamping at −50 mV serosa negative with vasopressin.

and K showed relatively little variability from cell to cell with cell K greater than cell Na (Fig. 11*A* and *B*), although, as indicated by the tendency for cells incubated with vasopressin to gain Na and lose K, there are more lower values for (K − Na) in the vasopressin treated cells. In contrast, at −50 mV there was considerable variability, even with relatively modest swelling (water content < 10.0 kg/kg dry wt), and cells with much the same degree of swelling often differed markedly in their Na and K (Fig. 11*C*). Grossly swollen cells following exposure to vasopressin showed the same variability (Fig. 11*D*). Indeed the variability in (K − Na) was not related to the degree of cell swelling.

In cells clamped at −50 mV with ouabain, there was also a good correspondence between the amount of Na + K gained and the gain in water content (Fig. 12*A*), but no clear pattern to the associated changes in Na or K in individual cells (Fig. 12*B*); some cells with little or no increase in water content nevertheless lost considerable amounts of K, others gained water, with their K content (but not concentration) remaining within normal limits.

The few mitochondria-rich cells that were identified were unaffected by the clamp conditions. Also, we never detected appreciable cell swelling in basal cells in tissues clamped at −50 mV, even following exposure to vasopressin. These findings are in agreement with those of Bobrycki et al., 1981 and DiBona et al., 1981.

Discussion

The present results reveal that toad bladder epithelial cells tolerate voltage clamping between +50 mV serosa and 0 mV well, with no appreciable changes in ion or water contents (Figs. 2, 4–6, 11*A*), in agreement with earlier histological studies (Bobrycki, et al., 1981) and with results from chemical analysis (Robinson & Macknight, 1976). In contrast, clamping at −50 mV serosa, which provides a greater driving force for Na entry to the cells across the apical membrane, results in granular cell swelling, with considerable variability from cell to cell both in the extent of the swelling (Fig. 2) and in the pattern of the ion changes (Figs. 4–6, 11*C* and *D*). This variability was most marked in tissues exposed to vasopressin, with cells of relatively normal volume intermingled with grossly swollen cells (e.g., Fig. 9). It must be emphasized, however, that the majority of the cells, even after vasopressin, had comparatively modest increases in water content and that only a few cells had enormous water contents, the highest values being 53.6 and 57.8 kg/kg dry wt. Water content is calculated from cell mass which is derived from the continuum of the energy spectrum. The inaccuracy of the measurements at these extreme values is such that too much emphasis should not be placed on the estimated values. However, the excellent correlation (Fig. 3) between cell P expressed in mmol/L of cell and cell mass (g/L cell), which are measured differently but which both reflect the cell solids, makes us confident that some cells had indeed gained much water. Also, comparison of the sizes of cells in Fig. 9 indicates that an increase in water content of these magnitudes is certainly possible in the grossly swollen cells.

There was a significant correlation between gain of (Na + K) and cell swelling (Fig. 10*A*). Also, for most cells, the greater the swelling the greater the Na content (Fig. 10*B*). The relationship between cell K content and water content was much more variable (Fig. 10*C*). Some cells that were only slightly swollen had nevertheless lost much K in exchange for Na, whereas other grossly swollen cells had preserved the relationship between these cations relatively well (Fig. 11*C* and *D*).

The same variability in swelling was seen in cells exposed to ouabain. Ouabain blocks the (Na − K)-

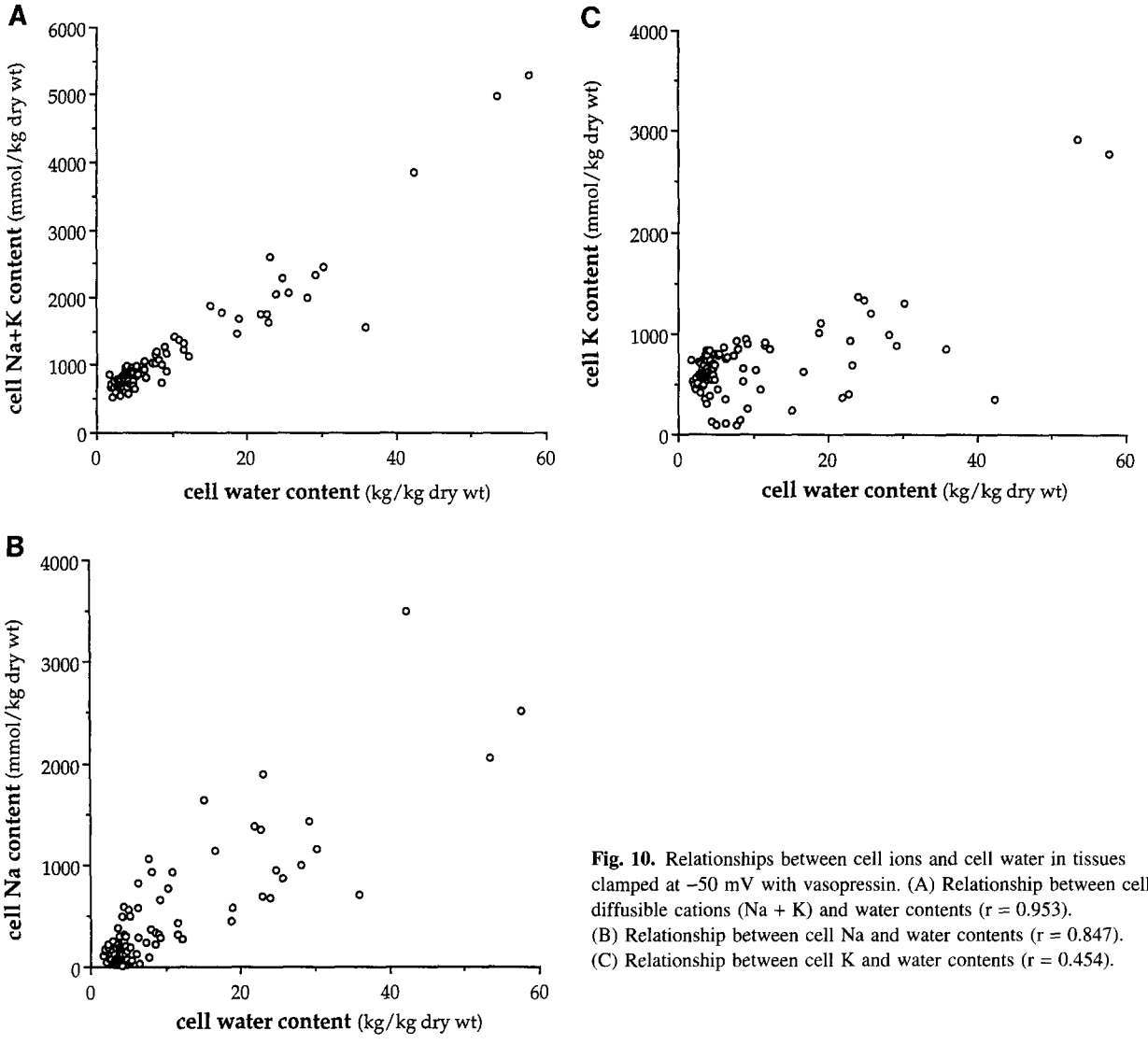

Fig. 10. Relationships between cell ions and cell water in tissues clamped at −50 mV with vasopressin. (A) Relationship between cell diffusible cations (Na + K) and water contents (r = 0.953). (B) Relationship between cell Na and water contents (r = 0.847). (C) Relationship between cell K and water contents (r = 0.454).

ATPase and one would expect cells exposed to this glycoside to gain Na, lose K and eventually swell. Although this is true on average in toad bladder cells under short-circuit conditions (e.g., Rick, et al., 1978), these changes in granular cell ions are not uniform (Bowler et al., 1991). Some granular cells are little affected whereas others gain considerable amounts of Na and lose much K. Similarly, in tissues exposed to ouabain under short-circuit conditions and then clamped at −50 mV in the continuing presence of the drug, ouabain resulted in some cells losing virtually all of their K and gaining Na, whereas others maintained high K contents (Figs. 4, 5 and 12*B*). We previously suggested (Bowler et al., 1991) that the failure of cells to gain Na and lose K after ouabain under short-circuit conditions might reflect a low apical membrane Na permeability. Since Na is gained predominantly across this membrane after ouabain (Macknight, Civan & Leaf, 1975), this would limit K loss even with the pump inhibited. If this were true, then cells which fail to lose K should be more resistant to clamp-induced swelling. Conversely, cells which swell should have gained Na (with Cl) and have lost K. Neither of these expectations was met, for, although there was excellent correlation between the uptakes of (Na + K) and water (Fig. 12*A*), there was no correlation between the extent of the K loss and the swelling (Fig. 12*B*). In addition to its direct effect on the pump, there is evidence that ouabain secondarily inhibits apical Na permeability (Erlij & Smith, 1973; Helman, Nagel & Fisher, 1979; Palmer, Edelman & Lindemann, 1980; Chase & Al-Awqati, 1981; Thomas & Ehrenfeld, 1988). Nevertheless, cells swelled on average a little more with ouabain than they did when clamped at −50 mV alone.

The changes in cellular cations following clamping at −50 mV with or without vasopressin can not be explained solely on the basis of either of the hypotheses advanced in the Introduction. Serosa-negative voltage-clamping increases the electrochemical gradient for Na

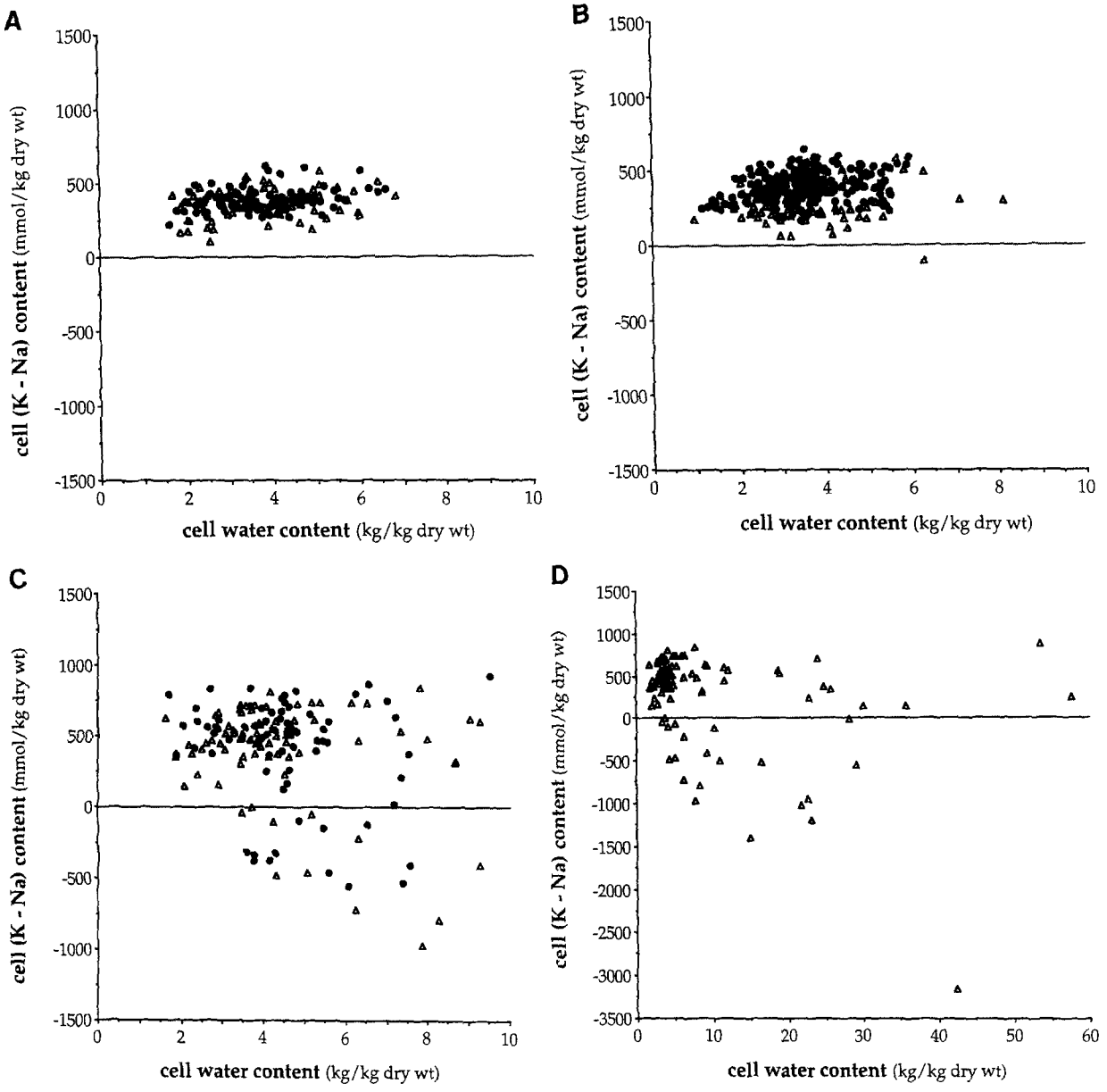

Fig. 11. Effects of voltage-clamping on the relative cell Na and K contents. (A) Individual cell (K – Na) against cell water content for all tissues clamped at +50 mV. [● – +50 mV without vasopressin, △ – +50 mV with vasopressin] (B) Individual cell (K – Na) against cell water content for all tissues clamped at 0 mV. [● – +50 mV without vasopressin, △ – +50 mV with vasopressin] (C) Individual cell (K – Na) against cell water content over the range 0 to 10.0 kg/kg dry wt, for tissues clamped at −50 mV with (△) or without (●) vasopressin. (D) Individual cell (K – Na) against cell water content for all cells from tissues exposed to vasopressin and clamped at −50 mV serosa.

entry to the cells across the apical membrane. Vasopressin increases apical membrane Na permeability, and the combination of this agent with the clamping at −50 mV would increase Na influx to the cells markedly. However, granular cells did not accumulate only Na and Cl, as would have occurred if the swelling was simply a consequence of the increased rate of Na entry to the cells saturating the (Na – K)-ATPase with the rate of basolateral K loss matching the increased pump activity. Neither could the findings be explained on the basis that the (Na – K)-ATPase could adjust to the increased rate of Na entry from the mucosal medium but that the basolateral membrane K permeability was now insufficient. Cell swelling would then have been associated with accumulation of K, together with Cl.

The absence of appreciable changes in cell composition between the open-circuited and short-circuited state indicates that, despite the increased Na entry cellular transport processes are able to adapt in such a way as to preserve steady state cell composition. This may be as-

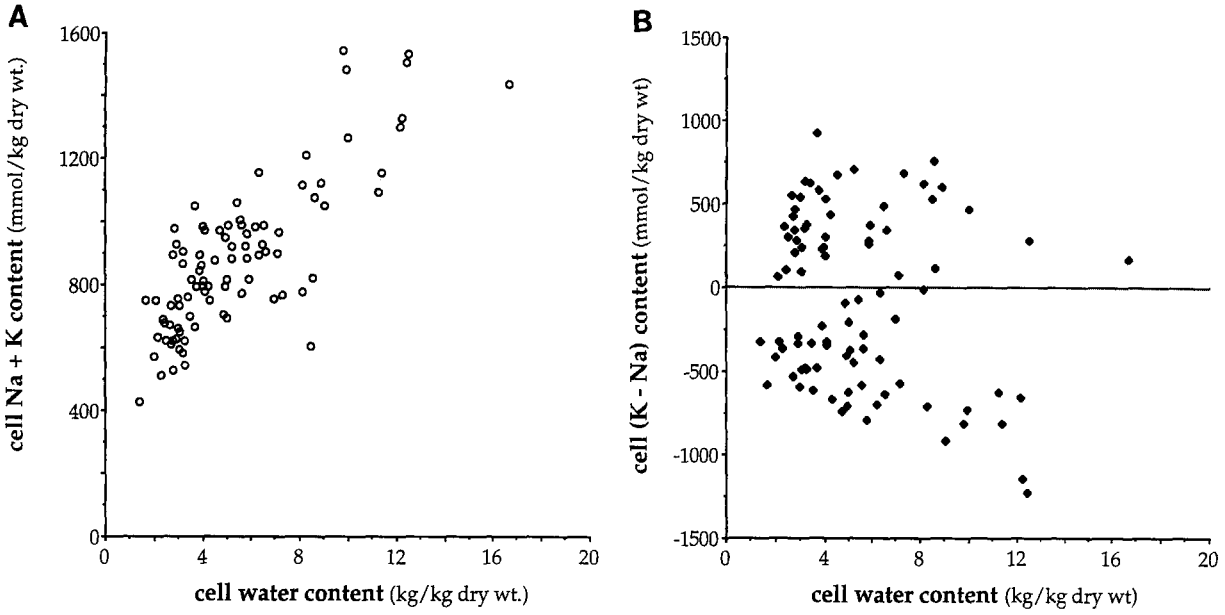

Fig. 12. Relationships between cell cations and water contents in tissue voltage-clamped at −50 mV in the presence of ouabain. (A) Relationship between cell diffusible cations (Na + K) and water contents (r = 0.805). (B) Individual cell (K − Na) against cell water content for all cells from tissues exposed to ouabain and clamped at −50 mV serosa.

sisted by coupling of cells through low resistance gap junctions. This would allow cells, that might otherwise have their transport pathways overloaded, to share the load with adjacent cells and thus preserve their composition. However, the large individual variations in cell swelling and cell cation content in tissues clamped at −50 mV serosa indicate that under these conditions there must be considerable differences between granular cells in the availabilities in the plasma membranes of ion pathways, either as a consequence of differences in the numbers of such pathways or in their control. In particular, vasopressin increases the availability of apical membrane Na channels, yet some cells hardly swelled while others were grossly swollen. It is possible that the situation is exacerbated by the closure of gap junctions between cells that are having difficulty in maintaining their composition and their neighbors. This would contribute to preserving the integrity of the adjacent cells while sacrificing cells that became unable to cope. We can not say from our data whether the gross swelling resulted from the opening of more Na channels than occurred in the less swollen cells (perhaps reflecting different cell sensitivities to vasopressin) or was a consequence of the failure of basolateral membrane pathways to adapt to the increased Na influx. For cells to swell there must be a net gain of cation associated with uptake of Cl to preserve cellular electroneutrality (Fig. 10*B*). It is possible that what determines the extent of the swelling of any individual cell is its basolateral Cl permeability. Gain of Na by cells with low Cl permeability would be associated with K loss, rather than with Cl gain and swelling would be limited. Cells whose Cl permeability was high, however, would gain Cl with the Na, and the K content could be relatively preserved despite the appreciable swelling.

The differing abilities of cells to accommodate to the increased Na influx induced by clamping at −50 mV may be related to cell age. A similar explanation was proposed for the variability in response to K-free media and to ouabain (Bowler et al., 1991). For example, mature cells about to be shed from the bladder wall may be in the process of modifying their transport pathways.

Finally, the increased Na and decreased K with vasopressin under short-circuit conditions, without cell swelling or uptake of Cl, confirms the findings of Rick et al. (1988) and Rick & DiBona (1987) who reported that ADH increased cell Na and decreased cell K (both expressed on a wet wt basis) in toad bladder epithelial cells incubated under short-circuit conditions in isosmotic media. Cell Cl was unaffected (Rick, et al., 1988). Similar changes occurred in our study in tissues exposed to vasopressin and clamped at +50 mV. The raised cell Na concentration would stimulate Na pump activity and contribute to the increased transepithelial current. Since cell K content and concentration fell, loss of K from the cell must be greater than the increased K influx that would accompany the increased (Na − K)-ATPase activity. This loss of cell K may reflect a less negative intracellular basolateral membrane potential or the activation of additional basolateral K channels, or both.

In summary, clamping cells at +50 mV or at 0 mV was not associated with any appreciable changes in cell

composition. However, clamping at −50 mV causes variable cell swelling and cation changes. Clearly, depending on the number of transporters and their sensitivity to regulation following changes in cell composition, a variety of responses to voltage-clamping is possible. These results thus show that the granular epithelial cells are not uniform in their ion transporting capacities and it is clearly not sufficient to regard these cells as constituting a homogeneous functional epithelium under all experimental conditions.

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