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Intra- and extracellular element concentrations of rat renal papilla in antidiuresis

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Intra- and extracellular element concentrations of rat renal papilla in antidiuresis. The element concentrations in various intra- and extracellular compartments of the tip of the rat renal papilla were determined during antidiuresis using electron microprobe analysis. Urinary concentrations (means ± SEM) were: urea, 1509 ± 116; potassium, 268 ± 32; sodium, 62 ± 19 mmoles · l^{-1}; and osmolality, 2548 ± 141 mOsm · kg^{-1}. Electrolyte concentrations in the interstitial space were: sodium, 437 ± 19; chloride, 438 ± 20; and potassium, 35 ± 2 mmoles · kg^{-1} wet wt. The vasa recta plasma exhibited almost identical element concentrations. The values in the papillary collecting duct cells were: sodium, 28 ± 1; chloride, 76 ± 3; potassium, 135 ± 3; and phosphorus, 316 ± 7 mmoles · kg^{-1} wet wt. Similar concentrations were observed in the papillary epithelial cells. In interstitial cells potassium and phosphorus concentrations were virtually identical to those of the collecting duct cells, whereas sodium and chloride concentrations were higher by about 30 mmoles · kg^{-1} wet wt. The element composition of the various papillary cells is, thus, not substantially different from that of proximal tubular cells. This finding demonstrates that cellular accumulation of electrolytes is not the regulatory mechanism by which papillary cells adapt osmotically to their high environmental osmolality and sodium chloride concentration.

Concentrations élémentaires intra- et extra-cellulaires dans la papille rénale de rats en antidiurèse. Les concentrations élémentaires dans les différents compartiments intra- et extra-cellulaires de la pointe de la papille rénale de rats ont été déterminées pendant une antidiurèse, en utilisant une analyse à la microsonde électronique. Les concentrations urinaires (moyenne ± SEM) étaient: urée, 1,509 ± 116; potassium, 268 ± 32; sodium, 62 ± 19 mmoles^{-1}; et l'osmolalité de 2,548 ± 141 mOsm · kg^{-1}. Les concentrations d'électrolytes dans l'espace interstitiel étaient: sodium, 437 ± 19; chlore, 438 ± 20; et potassium, 35 ± 2 mmoles · kg^{-1} de tissu sec. Le plasma des vasa recta avait des concentrations élémentaires pratiquement identiques. Les valeurs dans les cellules du canal collecteur papillaire étaient: sodium, 28 ± 1; chlore, 76 ± 3; potassium, 135 ± 3; et phosphore, 316 ± 7 mmoles · kg^{-1} de tissu humide. Des concentrations identiques ont été observées dans les cellules épithéliales papillaires. Dans les cellules interstitielles, les concentrations de potassium et de phosphore étaient virtuellement identiques à celles des cellules du canal collecteur, tandis que les concentrations de sodium et de chlore étaient plus élevées d'environ 30 mmoles · kg^{-1} de tissu humide. La composition élémentaire des différentes cellules papillaires n'est, ainsi, pas substantiellement différente de celle des cellules tubulaires proximales. Ce résultat démontre que l'accumulation cellulaire d'électrolytes n'est pas le mécanisme de régulation par lequel les cellules papillaires s'adaptent osmotiquement à une osmolalité et à une concentration de chlorure de sodium ambiantes élevées.

The concentrating mechanism of the mammalian kidney is able to establish osmolalities in the papilla which are several times higher than in systemic plasma. In studies performed mostly in kidney tissue slices, vasa recta blood, the interstitium or tubular fluid, it has been demonstrated that the concentrations of various solutes, and thus the osmolality, increase continuously from the cortico-medullary boundary toward the tip of the papilla [1]. Correspondingly, the renal cells along the papillary axis are exposed to different osmolalities. The osmotic situation of the papillary cells is even more complex if it is considered that different diuretic states are characterized not only by different composition of the urine but also by that of the papillary interstitial fluid. In antidiuresis sodium concentration in the interstitium may rise to almost 450 mM whereas in diuresis it is almost identical to that of systemic plasma [2–4]. Furthermore, in the papillary tip, large differences exist between the electrolyte concentrations in the collecting duct fluid and the surrounding interstitium [2–4]. Therefore, in addition to the fact that all cell types in the papilla are exposed to variable osmolalities depending on the diuretic state, large electrolyte concentration gradients exist across collecting duct and papillary epithelium.

Information regarding the mechanism of cellular osmotic adaptation in the mammalian papilla is relatively scarce and only a few attempts have been made to measure the intracellular element composition [4–7]. From data obtained with chemical analysis, it has been concluded that the osmotic adaptation of papillary cells is mainly achieved by the cellular accumulation of sodium. In the papillary tip of cats [4, 7] and rat papillae incubated in vitro [6], the calculated cellular sodium concentration increased to more than 400 mM with increasing osmolality. However, the interpretation of these data is complicated firstly by the fact that chemical analysis only provides mean concentrations for all the cell types present in the renal papilla and secondly because such data are derived indirectly by estimation of the extracellular space, a measurement which itself is fraught with difficulties. These problems could be overcome by a method in which the analysis is restricted to very small tissue compartments which can be accurately identified. Such a method is the electron microprobe analysis of papillary tissue cryosections. A recent study using this method [5] presented data for intracellular element concentrations which generally agreed with the results of chemical tissue analysis but were in complete conflict with preliminary data from this laboratory [8]. Thus, to clarify this issue electron microprobe analysis was

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used to measure element concentrations in various extra- and intracellular compartments at the tip of rat renal papillae, in vasa recta plasma, interstitium, collecting duct cells, papillary epithelial cells, and interstitial cells.

Methods

The experiments were performed on male Sprague-Dawley rats weighing between 50 and 120 g and kept on a standard diet (Altromin 1320; Altromin GmbH, Lage, West Germany). Antidiuresis was achieved by depriving the animals of food and water for 14 to 16 hr. Anesthesia was induced by the intraperitoneal injection of Inactin, 100 to 120 mg · kg^{-1} body wt (Byk-Gulden, Konstanz, West Germany). Body temperature was maintained between 37 and 38°C by a heated operating table. The trachea was cannulated, and polyethylene catheters were inserted into the jugular vein for the infusion of isotonic saline (3 to 4 ml · hr^{-1} · kg^{-1} body wt) and into the left femoral artery for monitoring BP. The left kidney was exposed through a flank incision, freed of adherent fat and connective tissue, placed in an aluminum cup, and continuously bathed with warm mineral oil at 38°C. The ureter was cannulated. After a 2-hr equilibration period, urine was collected under oil for 30 min and immediately analyzed for sodium, potassium, and osmolality. A 20 g% albumin solution with sodium and potassium concentrations and an osmolality similar to that of the sampled urine was then prepared by adding sodium acetate, potassium chloride, and urea to the albumin solution. Analysis of the urine and preparation of the albumin took no longer than 10 min.

Two different procedures were followed to remove the renal papilla. In one series of experiments the renal pelvis was opened, the ureter was removed, the albumin standard solution was dripped onto the exposed papilla, and the whole kidney was shock-frozen by pouring liquid propane cooled to −180°C over it. No more than 5 min elapsed between opening the renal pelvis and shock-freezing the kidney. In another series the whole kidney was removed from the animal; the papilla was excised, dipped in the albumin standard, and then snap-frozen in liquid propane. This procedure took an average of 12 sec.

After shock-freezing, the kidney or the excised papilla was transferred into liquid nitrogen. The papillae were then sandwiched between indium foils and mounted into clamp-type brass holders either directly, or after being broken from the kidney with a precooled scalpel blade. The brass holder was inserted into the cutting arm of a cryomicrotome (Om U2 or Om U3, Reichert AG, Vienna, Austria). Sections 1-μm thick were cut at −80°C, beginning 20 μm from the papillary tip and proceeding up to 200 μm. After cutting, the cryosections were freeze-dried at −80°C and 10^{-6} Torr. To avoid the contact with ambient air, the freeze-dried cryosections were transferred to the chamber of the scanning electron microscope in a dry nitrogen atmosphere.

Electron microprobe analysis was performed in a scanning electron microscope (Stereoscan S150, Cambridge Instrument Co. LTD, Cambridge, United Kingdom) with an energy dispersive x-ray detector (LINK). The acceleration voltage was 20 kV, and the probe current was 0.3 nA. Areas of about 1 μ^2 were scanned for 100 sec. The emitted x-rays were analyzed in the energy range between 0.6 and 20.0 keV. Phosphorus, which was absent in the standard, was quantified by comparing the phosphorus signal in the cell with the chloride signal in the standard, considering the experimentally determined different x-ray yields. Using an acceleration voltage of 20 kV, it could be shown that in 1 μm thick freeze-dried standard sections the characteristic x-ray peaks of all the elements measured are related linearly to the element concentrations. From the element profiles measured for sodium, potassium, and phosphorus across cellular boundaries, a spatial resolution of about 0.5 μm could be calculated for this method. Element concentrations were evaluated by comparison of the characteristic x-ray intensities with those obtained in the adherent albumin standard layer. The quantification procedure used assumes that the analyzed standard and tissue areas were cut at the same thickness and have not undergone differential shrinkage. To minimize errors due to inhomogeneities of the thickness within a section, tissue and albumin standard regions were selected for comparison which had been cut at the same instant in time. Since the albumin standard matrix is 20 g%, as is the tissue, it seems probable that shrinkage is very similar for both. Furthermore, since shrinkage will predominantly affect the thickness of the section, it will have little influence on the microprobe data. The dry weight content was estimated by comparing the white radiation intensities in specimen and albumin standard. However, urea appears to be lost during analysis, since measurements on albumin standard sections revealed that the addition of urea did not lead to an increase in the white radiation intensity. Thus, the values calculated in this manner mainly reflect the urea-free dry weight. Details of the preparation of freeze-dried cryosections for microanalysis and the quantification procedure have been published previously [9, 10]. In general, cellular measurements were performed in the nuclei, but in some cases additional measurements were also made in the apical cytoplasm of the papillary collecting duct cells close to the nucleus.

The urine and the albumin standards were analyzed for sodium and potassium by flame photometry (IL), for chloride by chloridometry (Eppendorf, Gerätebau, Hamburg, West Germany) and for osmolality by the depression of vapor pressure (Wescor Inc., Logan, Utah). Urea was measured by photometry using a commercial diagnostic kit (Boehringer Mannheim GmbH, Federal Republic of Germany). The dry weight of the albumin standard was determined by drying to a constant weight at 95°C.

The element concentrations are expressed in mmoles · kg^{-1} wet wt and presented as mean ± SEM. Statistical evaluation of the data was performed with Student's t test for related or unrelated samples, as appropriate, and differences were considered to be statistically significant if the two-tailed probability was 0.05 or less. Linear regression analysis was performed by standard techniques.

Table 1. Urine flow rate and urinary composition in antidiuretic rats[a]

Urine flow rate, $\mu l \cdot min^{-1} \cdot 100\ g\ body\ wt^{-1}$	0.94 ± 0.13
U_{Osm}, $mOsm \cdot kg\ H_2O^{-1}$	2548 ± 141
U_{urea}, $mmoles \cdot l^{-1}$	1509 ± 116
U_K, $mmoles \cdot l^{-1}$	268 ± 32
U_{Na}, $mmoles \cdot l^{-1}$	62 ± 19

[a] Data are presented as mean ± SEM; N = 12.

Results

The urinary composition of the antidiuretic animals is shown in Table 1. Urea accounts for almost two thirds, and sodium,

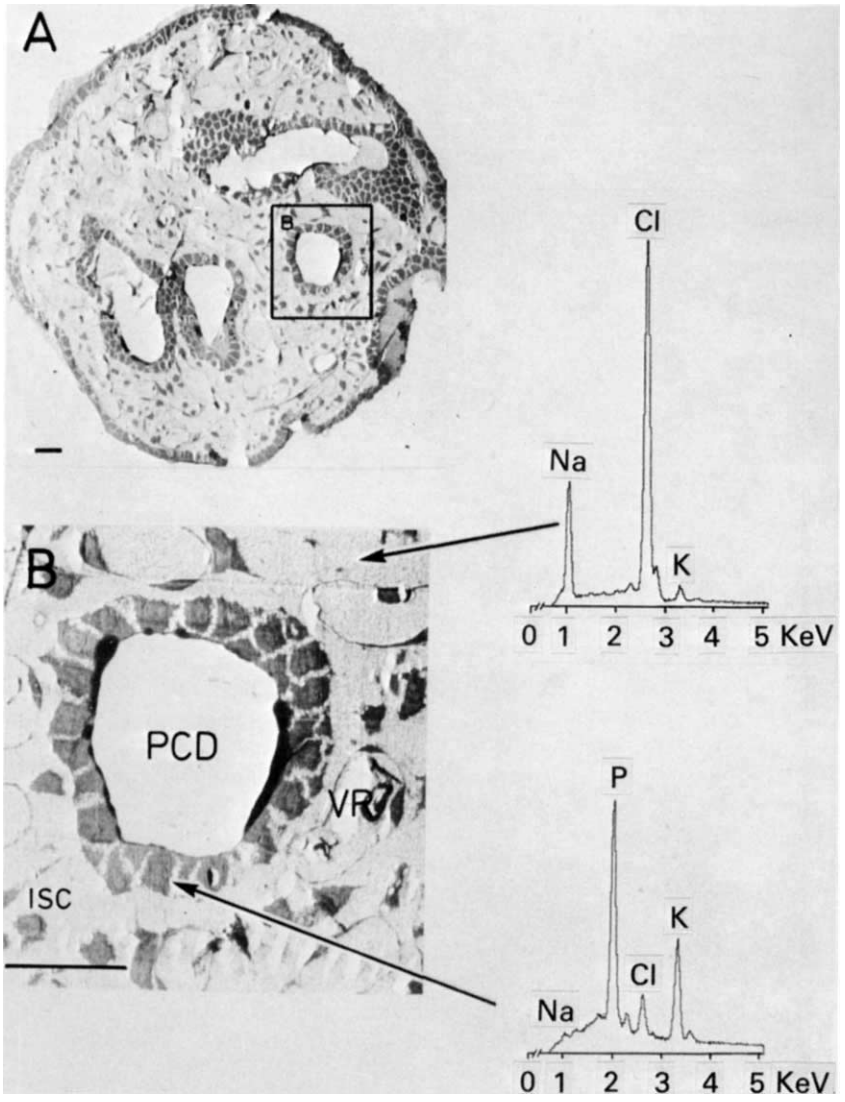

Fig. 1. *Scanning transmission electron micrographs of a freeze-dried cryosection of rat renal papilla at low* **(A)** *and higher* **(B)** *magnification.* Abbreviations are: PCD, papillary collecting duct; ISC, interstitial cell; VR, vas rectum. The x-ray spectra were obtained in the interstitium (*upper spectrum*) and the nucleus of a papillary collecting duct cell (*lower spectrum*). The *bar* represents 20 μm.

potassium and the accompanying anions for one fourth of the urinary osmolality.

Figure 1 shows scanning transmission electron micrographs of a freeze-dried cryosection of a renal papilla at a distance of about 150 μm from the papillary tip and two energy-dispersive x-ray spectra. Even at low magnification **(A)** the different compartments (papillary collecting ducts, interstitium, vasa recta, thin loops of Henle, and papillary epithelium) can be easily recognized. At a higher magnification **(B)** the nuclei of cells and wide intercellular spaces between papillary collecting duct cells are apparent. The upper spectrum, obtained in the interstitial space, is characterized by high sodium and chlorine peaks, whereas the lower spectrum, obtained in the nucleus of a collecting duct cell, exhibits high phosphorus and potassium peaks.

Table 2 lists the element concentrations obtained from the various extra- and intracellular compartments in the terminal 200 μm of the papilla. Intracellular measurements were performed in the nuclei of the collecting duct, the papillary epithelial and the interstitial cells, extracellular determinations in the interstitial space and in the vasa recta plasma. Since the data obtained from exposed and unexposed papillae (see **Methods**) exhibited no systematic differences, the values obtained from these two groups were pooled. No significant differences were found in the cellular element composition between collecting duct and papillary epithelial cells. The interstitial cells, however, exhibited higher sodium and chloride concentrations whereas phosphorus and potassium concentrations were similar to those in collecting duct and epithelial cells. The element composition of the vasa recta plasma and the interstitium did not significantly differ from each other. In the plasma of the vasa recta electrolyte concentrations were: sodium, 445; chloride, 458; and potassium, 32 mmoles $\cdot$ kg^{-1}. In the interstitial space, the values were: sodium, 437; chloride, 438; and potassium, 35 mmoles $\cdot$ kg^{-1} wet wt. In a few collecting duct cells data were obtained from the cytoplasm in the apical region of the cells close to the nucleus in addition to nuclear measurements. There was no difference in the element concentrations between the two cellular compartments (Table 3).

Discussion

To quantify the microprobe data obtained in various extra- and intracellular compartments, an internal standard was introduced by applying a thin layer of albumin solution of known

Table 2. Element concentrations and urea free dry weights in the papillary tip of antidiuretic rats[a]

	N	Na	P	Cl	K	Na + K	Urea free dry weight
		mmoles · kg wet wt^{-1}					*g · 100 g*$^{-1}$
Papillary collecting duct cells	154	27.5 ± 1.4	316.1 ± 7.2	76.3 ± 2.5	135.2 ± 2.5	162.7 ± 3.0	19.7 ± 0.5
Papillary epithelial cells	55	22.5 ± 2.3	333.0 ± 12.3	79.9 ± 3.6	135.9 ± 4.8	158.4 ± 5.6	20.9 ± 0.9
Interstitial cells	56	50.2 ± 3.0[b]	309.5 ± 11.8	107.2 ± 4.3[b]	138.9 ± 4.2	189.1 ± 5.7[b]	21.0 ± 0.9
Interstitium	56	437.4 ± 18.8	14.9 ± 1.2	437.5 ± 19.9	34.9 ± 2.2	472.3 ± 18.8	11.8 ± 0.5
Plasma	56	445.0 ± 18.7	12.9 ± 0.9	458.2 ± 20.8	32.1 ± 2.0	477.1 ± 18.9	12.0 ± 0.9

[a] Data are presented as mean ± SEM; *N* represents the number of measurements.
[b] The value is statistically different from the corresponding value of papillary collecting duct cells.

Table 3. Nuclear and cytoplasmic element concentrations and urea free dry weight in papillary collecting duct cells[a]

	N[b]	Na	P	Cl	K	Na + K	Urea free dry weight
		mmoles · kg^{-1} *wet wt*					*g · 100 g*$^{-1}$
Nucleus	18	24.2 ± 3.6	300.7 ± 22.4	67.3 ± 5.9	135.7 ± 7.9	159.9 ± 9.3	19.1 ± 1.1
Cytoplasm	18	22.6 ± 3.0	279.2 ± 22.1	76.3 ± 8.4	129.0 ± 8.9	151.6 ± 10.0	18.7 ± 1.0

[a] Data are presented as mean ± SEM.
[b] *N* equals the number of paired measurements.

electrolyte composition to the papillary surface. This was achieved either by dipping the excised papilla in the albumin solution or by opening the renal pelvis and dripping the standard onto the papilla. Exposure of the papilla in antidiuresis is known to result in a reduction in urinary osmolality [11] and therefore, presumably, to changes in papillary tissue element concentrations. However, in view of the fact that the defect in concentrating ability requires at least 15 min to develop fully [12], the exposure time of less than 5 min in the present experiments should lead to only minor changes in urinary composition. In any case, since no significant differences in the element concentrations between exposed and unexposed papillae were seen, it is unlikely that the present microprobe data were influenced by such a concentrating defect.

The electrolyte concentrations in the extracellular compartments of the renal papilla, that is, vasa recta plasma and interstitium, obtained in this investigation are consistent with published data, although the latter vary widely. High values for sodium and potassium, similar to the present data, have been found in centrifuged, papillary interstitial fluid [3, 4], in micropuncture samples from vasa recta [13–19] the loop of Henle [13–17, 20, 21] and by microprobe analysis of the papillary interstitium [5]. The relatively large variations in the reported data might be explained by species differences, differences in the diuretic state and dietary treatment, and analysis at different levels in the papilla.

On the basis of micropuncture experiments it is assumed that, at the same level within the papilla, the element composition of the vasa recta plasma fairly closely reflects that of the interstitium [17]. The present finding of almost identical electrolyte concentrations in the vasa recta plasma and interstitium confirms this view and supports the hypothesis that the vasa recta function as countercurrent exchangers in the context of the medullary concentrating mechanism. Data from micropuncture experiments [16, 20] suggested that the concentration of sodium chloride in the descending vasa recta is somewhat lower than in the interstitium and somewhat higher in the ascending vasa recta. This question cannot be addressed in the present study, however, for two reasons: Firstly, it is not yet possible with the present technique to discriminate between descending and ascending vasa recta plasma, and secondly, since the analyzed tissue sections were obtained from the extreme tip of the papilla, the element concentration differences between the vasa recta limbs and interstitium may be too small to be detectable.

Since freeze-drying of cryosections inevitably leads to gross dislocation of the elements within watery compartments containing no matrix material [22], measurements were not performed in the lumina of Henle's loops and the collecting ducts. Hence, a direct comparison between the composition of the tubular fluid of these two compartments and those of the interstitium, vasa recta plasma, and intracellular compartments at the same papillary level is not possible. However, since the present measurements were made not more than 200 μm from the papillary tip, it can be assumed that the osmolality and the composition of the collecting duct fluid at the site of analysis are practically identical with those of the final urine. Compared with the interstitium, the urinary concentration of sodium is several-fold lower, whereas that of potassium is considerably higher. Previous micropuncture studies have demonstrated a correlation between urinary osmolality and vasa recta sodium chloride concentration at urinary osmolalities up to 1400 mOsm · kg^{-1} [19]. No correlation (Fig. 2) was observed in the present study, in which urinary osmolalities ranged from 1800 to 3500 mOsm · kg^{-1} (mean 2548 mOsm · kg^{-1}). Thus, it appears that such correlation is lost at higher ranges of osmolalities. This may be due to the increasing proportion of the total interstitial osmolality contributed by non-electrolytes, probably urea. Electrolytes will account for about 1000 mOsm · kg^{-1} of interstitial osmolality leaving some 1500 mOsm · kg^{-1} of urea assuming osmotic equilibrium between urine and interstitium. Such an interpretation is consistent with the data of Ullrich, Pehling, and Espinar-Lafuente [19], in which the correlation between vasa recta electrolyte concentration and urinary osmolality deviates considerably from linearity at higher osmolalites.

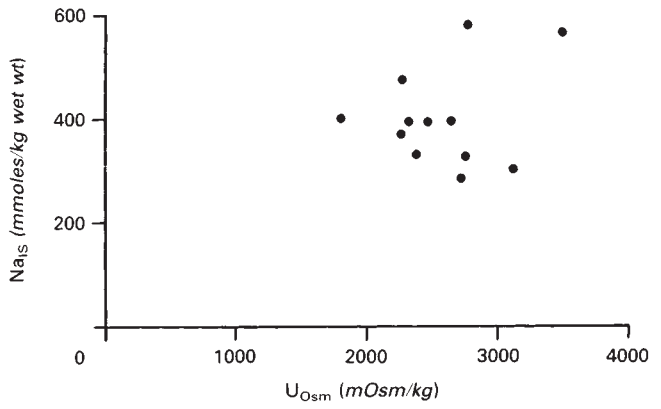

Fig. 2. *Interstitial sodium concentration* (Na_{IS}) *as a function of urinary osmolality* (U_{Osm}). No significant correlation between both parameters was observed ($r = 0.23$; $P > 0.1$). Each *point* represents the data obtained from one kidney.

The most interesting finding in the present experiments is that, despite the elevated interstitial electrolyte concentrations and urinary osmolality, intracellular electrolyte concentrations in the collecting duct and the papillary epithelium are very similar to those of epithelial cells located in normal isotonic environments. They exhibit almost identical low sodium and high potassium concentrations as proximal tubular cells although the chloride concentration is about 50 mmoles · kg^{-1} wet wt higher [23]. The papillary interstitial cells, on the other hand, show sodium and chloride concentrations which are about 30 mmoles · kg^{-1} wet wt higher than in other papillary cell types (Table 2). These raised sodium chloride concentrations, however, may be a consequence of scattered electrons reaching extracellular regions, since these cells are very small. Potassium and phosphorus concentrations are not different between the various papillary cell types. The observation of similar potassium concentrations in papillary cells and other cells has also been made by other investigators. Using chemical methods for tissue analysis even lower potassium concentrations than in the present study were found in in vivo preparations [4] and in isolated collecting duct cells [24]. In one electron microprobe study [5], the potassium concentration of the epithelial cells was similar to those reported here, whereas that of the interstitial cells was lower by about 40%. These differences placed aside, the fact that papillary cells, in extreme antidiuresis or when incubated in solutions of high sodium chloride concentrations [6], do not accumulate potassium excludes potassium from playing a significant role in cellular osmotic adaptation to hypertonic environments.

Studies of papillary composition in antidiuresis uniformly report high cellular sodium concentrations regardless of the analytical method [4–7]. These findings were considered as demonstrating that cellular sodium accumulation, to balance high extracellular osmolality, was the mechanism of cellular osmotic adaptation. This view was further supported by the observation that intracellular sodium concentration in isolated papillae was directly related to the sodium concentration in the bathing medium [6]. The present study provides no evidence in support of such a regulatory mechanism, as the intracellular sodium concentrations are about ten times smaller than those included in previously published data. This marked difference in cellular sodium concentration cannot be accounted for by different functional states and most likely must, therefore, be attributed to analytical artifacts. A major problem in the preparation of tissue for electron microprobe analysis is preventing dislocation of diffusible substances and thus maintaining the in vivo element gradients between compartments in close proximity to one another, in particular between intra- and extracellular compartments. Any possible artifact occurring during the specimen preparation would tend to abolish the physiological gradients between extra- and intracellular compartments. Since the interstitial sodium concentrations are eight to ten times higher than in the intracellular compartment, diffusion of water soluble elements in the course of tissue preparation would necessarily lead to an increase in intracellular sodium concentrations. This interpretation is consistent with our experience according to which low cellular sodium and chloride concentrations were obtained only when the transfer of the specimens from the freeze-drying apparatus to the scanning electron microscope was performed in a dry nitrogen atmosphere. In contrast, when the freeze-dried cryosections were exposed to ambient air during transfer, intracellular sodium and chloride concentrations similar to those reported in the literature were found. The reason for the sodium chloride shift from the extra- to the intracellular compartment during exposure of the sections to air might be re-uptake of water into the freeze-dried tissue which would then allow the diffusion of water soluble substances. Uptake of water is facilitated by the highly hygroscopic nature of the freeze-dried papillary tissue due to its high concentrations of solutes.

The fact that the present study yielded much lower intracellular sodium concentrations than those obtained by chemical analysis may be explained by an underestimation of the extracellular space in the latter technique, a problem inherent in the use of extracellular marker substances in chemical tissue analysis. If extracellular marker substances do not completely equilibrate throughout the entire extracellular space, a fraction of the extracellular space containing a high sodium concentration is falsely ascribed to the intracellular compartment thus accounting for the apparently high intracellular sodium concentration. Such an underestimation of the extracellular space is also consistent with the relatively low potassium concentrations found with chemical analysis [4, 7, 24]. Such an explanation, however, cannot resolve the discrepancies between the present results and the data obtained by Bulger, Beeuwkes, and Saubermann [5] who also used electron microprobe analysis. These authors reported renal papillary cellular sodium and chloride concentrations several times higher than those found in this study. It should be noted, however, that the methodological approach of those authors [25, 26] differs considerably from the present one and has recently been the subject of detailed criticism by Gupta and Hall [27]. Although we share the main objections raised by Gupta and Hall [27], it is impossible to attribute the discrepancies to one simple methodological artifact.

The relatively low electrolyte concentrations of the papillary cells reported here raise the question of how these cells maintain osmotic equilibrium with their hyperosmotic environment. Assuming that the total osmolality and the urea concentration in all compartments of the papillary tip are similar to those of the final urine and that the contribution of the electro-

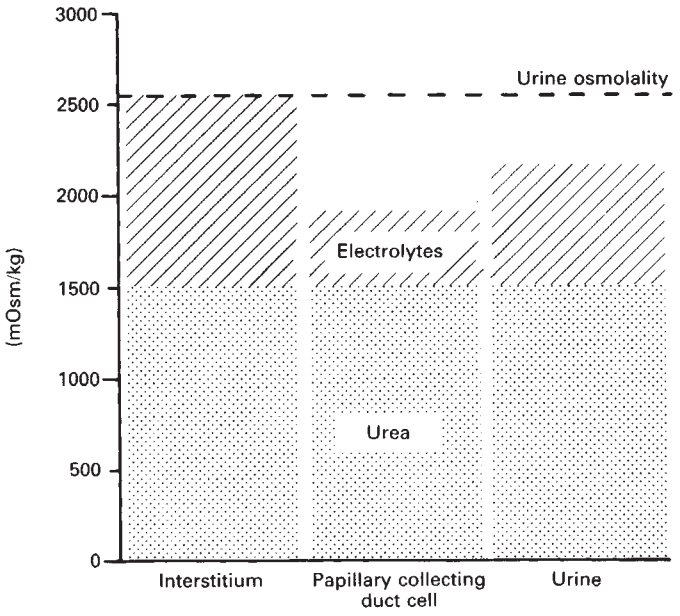

Fig. 3. *Schematic representation of the contribution of various solutes to the total osmolality of the interstitium, papillary collecting duct cells, and final urine.* The *diagram* is based on the assumption that the total osmolality and urea concentrations of all tissue compartments are the same as in urine and that the contribution of the electrolytes to the osmolality is approximately twice the sum of the sodium and potassium concentrations in the different compartments. The data are from Table 1 (urine) and Table 2 (papillary collecting duct cell and interstitium) and are referred to a water space of 80% for the collecting duct cells and 90% for the interstitial space. In papillary collecting duct cells and urine an "osmotic gap" is apparent.

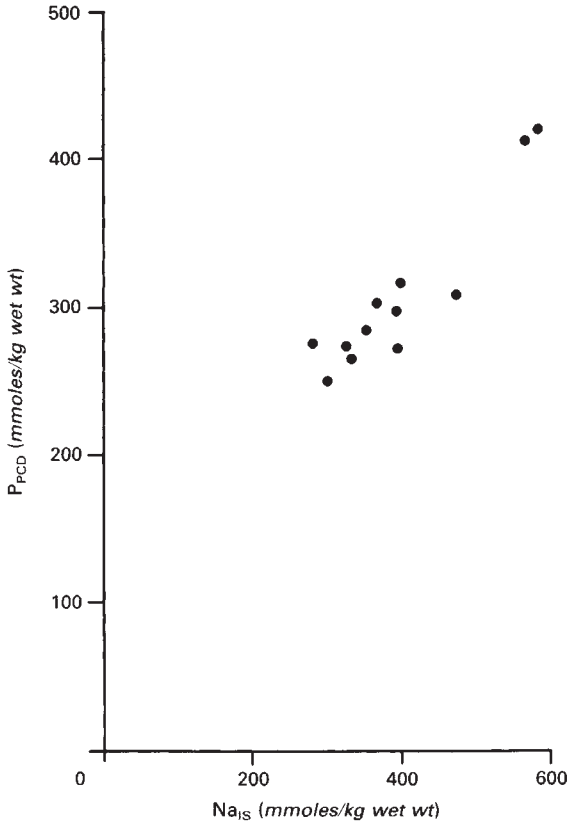

Fig. 4. *Papillary collecting duct cellular phosphorus concentration* (P_{PCD}) *as a function of interstitial sodium concentration* (Na_{IS}). A significant correlation between both parameters was observed ($r = 0.93$; $P < 0.001$). Each *point* represents the data obtained from one kidney.

lytes to the osmolality within the various compartments is reflected by twice the sum of the sodium and potassium concentrations, the contribution of the various components to the total osmolality can be estimated as shown in Figure 3, which was constructed from the mean values in Tables 1 and 2. Whereas the total osmolality of the papillary interstitial fluid can easily be accounted for by sodium and potassium salts and urea, a considerable "osmotic gap" exists for the epithelial and interstitial cells and to a smaller extent for the final urine. The final urine contains considerable amounts of other salts (for example, ammonium) and non-electrolytes (for example, creatinine), which readily account for the apparent osmotic gap of about 380 mOsm · kg^{-1}. The relatively large osmotic gap of about 630 mOsm · kg^{-1} in the papillary cells is more difficult to explain. If it is assumed that the cells are indeed in osmotic equilibrium and that urea is freely permeable, other osmotically active substance(s) must have been accumulated intracellularly to balance the high extracellular electrolyte concentrations. Possible candidates are free amino acids [28], glyceryl phosphorylcholine [29–31] and inositol [29]. While no evidence exists that amino acids play a major role in the osmotic adaptation of mammalian papillary cells [32, 33], the concentration of glycerylphosphorylcholine has been shown to increase gradually in antidiuresis from the outer medullary zone to the papillary tip in the dog [29, 30]. If this substance were mainly intracellularly located, it could account for a large part of the osmotic gap. Consistent with this assumption is the present observation of very high phosphorus concentrations in all cells of the papillary tip and low concentrations in the intercellular spaces (Table 2). Even so, it seems unlikely that these phosphorus compounds can account for the total osmotic gap, considering a cellular phosphorus concentration of 320 mmoles · kg^{-1} wet wt as found in this study. Even if all this phosphorus were osmotically active and distributed in the cellular water space, an osmotic gap of more than 200 mOsm · kg^{-1} remains to be explained. In this context it should be noted that Schimassek, Kohl, and Bücher [29] observed high concentrations of inositol in the papillae of antidiuretic dogs. If this substance were also mainly located within the papillary cells, it would certainly help to close the rest of the gap.

The question as to whether or not cellular volume changes may contribute to the osmotic adaptation of papillary cells to different osmotic situations cannot be answered from the present data. The observation of large intercellular spaces in the present study indicates that the papillary cells shrink in antidiuresis. Using conventional histological techniques, relatively wide intercellular spaces have also been observed in papillary [34] and collecting duct epithelium [35] during antidiuresis. The positive correlation between the phosphorus concentration of the collecting duct cells and the interstitial sodium concentration (Fig. 4) might thus not only reflect increased amounts of intracellular glycerylphosphorylcholine but also some degree of cell shrinkage.

Acknowledgments

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