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TECHNICAL NOTE

Measurement of osmolality in kidney slices using vapor pressure osmometry

MARK A. KNEPPER

Laboratory of Kidney and Electrolyte Metabolism, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, Maryland

Estimation of the osmolality of renal tissue fluid has been carried out in many investigations of the renal concentrating and diluting mechanism. The method applied has usually been a variation of the technique of Appelboom et al [1]. This involves equilibration of the tissue with some volume of diluent after boiling to prevent generation of osmotically active particles due to metabolism. The osmolality of the tissue fluid is calculated from the freezing point depression of the diluted fluid and the dilution factor. Corrections are applied for the change in activity coefficients due to dilution, loss of carbon dioxide due to boiling, and incomplete equilibration between tissue and dilution fluid. These techniques have proven practical for many applications but are necessarily limited in accuracy and precision due to the complexity of the procedure and the corrections involved. In this paper a simple, direct method is described for determination of tissue slice osmolality using vapor pressure osmometry. The method has proven to be more accurate and reproducible than previous ones and yields better spatial resolution. Vapor pressure osmometry has been applied previously to the determination of osmolality in the rat brain by Tornheim [2].

Rabbits weighing 1500 to 2500 g were used. The rabbits were thirsted for 48 hr and fasted for 18 hr prior to sacrifice. They also received Pitressin tannate in oil (1 U/kg of body wt i.m.) 18 hr prior to sacrifice. The rabbits were sacrificed by decapitation. After a small amount of blood was collected into a beaker containing heparin and water-equilibrated mineral oil, the left kidney was rapidly removed and frozen in liquid nitrogen. The kidney was wrapped in aluminum foil and kept frozen in a beaker above a saline-ice slurry before slice preparation. Urine samples were collected from the bladder.

Slices were prepared in a 2° C cold room. A cork borer (7.5 mm diameter), chilled on dry ice, was used to prepare a tissue core along the cortico-papillary axis. The core was wrapped in aluminum foil and kept frozen on dry ice prior to sectioning. Disc-shaped slices of thickness 0.5 to 1.0 mm were prepared along the entire length of the cortico-papillary axis with a chilled straight razor. The slices were sealed in polyethylene film (Glad® wrap) and aluminum foil, and kept frozen on dry ice until the measurement of tissue osmolality was made. Because the renal medulla is cone-shaped and because the boundaries between zones of the kidney are curved, the slices from the inner stripe of the outer medulla and from the inner medulla were not homogeneous. Consequently, only the center portion of these slices was used, trimming them with smaller cork borers (6 mm diameter for the inner stripe, and 4 mm diameter for the inner medulla).

Measurements of tissue slice, plasma, and urine osmolality were made with a vapor pressure osmometer (Wescor, model 5100CXR). The instrument was calibrated using 290 and 1000 mOsm/kg H_2O with sodium chloride standards. The procedure used for standards, plasma, and urine was that recommended by the manufacturer. Specifically, a 6.5 mm diameter filter paper disc was placed in the instrument and was saturated with 7 μl of the solution to be measured. For measurement of tissue fluid osmolality, the tissue slices were placed directly into the instrument. When a tissue slice was approximately the same size as the filter paper discs (that is, cut with a 7.5 mm diameter cork borer), only the slice was used. When tissue slices were smaller (medullary slices), they were placed on the filter paper discs allowing the tissue fluid to wet the filter paper. This procedure had the effect of equalizing the effective surface area of all samples. This was necessary because preliminary studies showed that small slices placed in the instrument alone gave artificially high values. This finding is consistent with the observation by Tornheim [2] that measurements of water activity in brain slices are highly sensitive to tissue geometry.

From the technical data provided with the instrument and from the studies of Tornheim [2], it is clear that vapor pressure osmometry yields accurate, reproducible results when applied to single-phase aqueous samples. However, the accuracy and reproducibility of the method applied to renal tissue samples requires some documentation. To assure that the presence of the tissue in the equilibration chamber of the instrument did not result in a systematic error, osmolality measurements were done with tissue slices that were osmotically equilibrated with a buffered saline solution. Disc-shaped slices were prepared from

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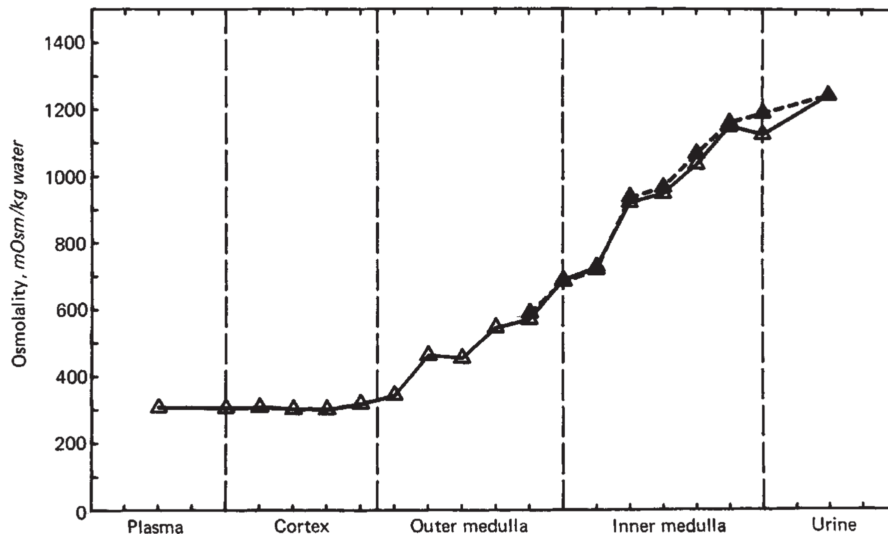

Fig. 1. *Measured plasma, urine, and tissue fluid osmolalities in an antidiuretic rabbit. Open triangles* and *solid lines* indicate data obtained when kidney tissue was placed directly into the equilibration chamber. *Closed triangles* and *dashed lines* indicate data obtained with only filter paper wetted with tissue fluid in the device.

Table 1. Osmolality of renal cortical slices equilibrated with a buffered saline solution[a]

Experiment no.	Osmolality of tissue fluid, *mOsm/kg water*	Osmolality of saline solution, *mOsm/kg water*
1	302 ± 3 (3)	300 ± 2 (3)
2	299 ± 4 (4)	302 ± 5 (4)
3	300 ± 5 (4)	295 ± 2 (6)
4	301 ± 3 (3)	303 ± 7 (4)
Mean	300 ± 2	300 ± 1

[a] Values are mean ± SD; number in parentheses refers to the number of determinations. Overall mean calculated from experiment means.

rabbit renal cortex as described previously. In preliminary experiments, it was found that the osmolality of the unfrozen tissue increased with time during the equilibration procedure presumably because of catabolism. To prevent this increase, the kidneys used in the equilibration experiments were briefly boiled in 0.16 M sodium chloride to inactivate enzymes prior to slice preparation. Three or four slices were placed in a small beaker containing 5 ml of the buffered saline solution (initial osmolality, 298 mOsm/kg water). This preparation was agitated on an orbital shaker at 2° C for 30 min to allow osmotic equilibration. The results of these experiments are shown in Table 1. The mean osmolality of the slices was not found to be different from that of the equilibration solution. Thus, no systematic error appears to result from the presence of the tissue in the instrument. These data also provide evidence that measurements of tissue osmolality with vapor pressure osmometry are highly reproducible. The coefficient of variation for equilibrated cortical slices was 1 to 2% (Table 1).

Figure 1 shows the measured tissue fluid osmolality along the cortico-medullary axis of a kidney from an antidiuretic rabbit using the technique described previously (*open triangles, solid lines*). Because relatively thin slices suffice, measurements were possible at 17 levels, providing excellent spatial resolution. The profile is consistent with the results of previous studies in antidiuretic rabbits [3–5]. The osmolality of cortical slices was consistently equal to or slightly below plasma. The osmolality at the papillary tip was close to urine osmolality. As mentioned above, slices from the medulla were placed on 6.5 mm filter paper discs in order to provide constant geometry. The tissue fluid thoroughly wetted the filter papers. The osmolality was measured both with filter paper plus slice (*open triangles, solid line*) and the same filter paper alone (*closed triangles, dashed line*). The values were in close agreement indicating that the presence of the solid tissue did not result in a value different from tissue fluid alone. Cortico-medullary profiles obtained in two other antidiuretic rabbits were similar to that shown in Figure 1.

Osmolality profiles from both kidneys of the same antidiuretic rabbit are shown in Figure 2. The similarity between the profiles is indicative of the excellent reproducibility of the method. The result was the same in the other pair of kidneys that was tested.

The accuracy and precision of vapor pressure osmometry makes the resolution of small osmolality differences in renal tissue possible. This is illustrated in Figure 3 which shows the osmolality of cortical slices from five antidiuretic rabbits. The osmolalities of the most superficial slices (*C-1*) were not different from plasma. However, a region of slight hypotonicity (13 to 14 mOsm/kg water less than plasma) was detected in the mid-cortex (slices *C-2* and *C-3*; $P < 0.05$, paired t test). This can be explained by the presence of hypotonic tubular fluid in the cortical thick ascending limbs, distal tubules and early collecting ducts. Because lumens of distal segments make up 6 to 7% of the volume of the cortex [6], the mean tubular fluid osmolality in these segments must be about 100 mOsm/kg water to account for a tissue osmolality 13 mOsm/kg water less than plasma. This is consistent with the diluting capacity of the rabbit cortical thick ascending limb [7]. The most superficial slice (*C-1*), which was found to have the same osmolality as plasma, consists primarily of cortex corticis, a region free of thick ascending limbs [8].

The results of the experiments described in this paper demonstrate that vapor pressure osmometry is an accurate, precise

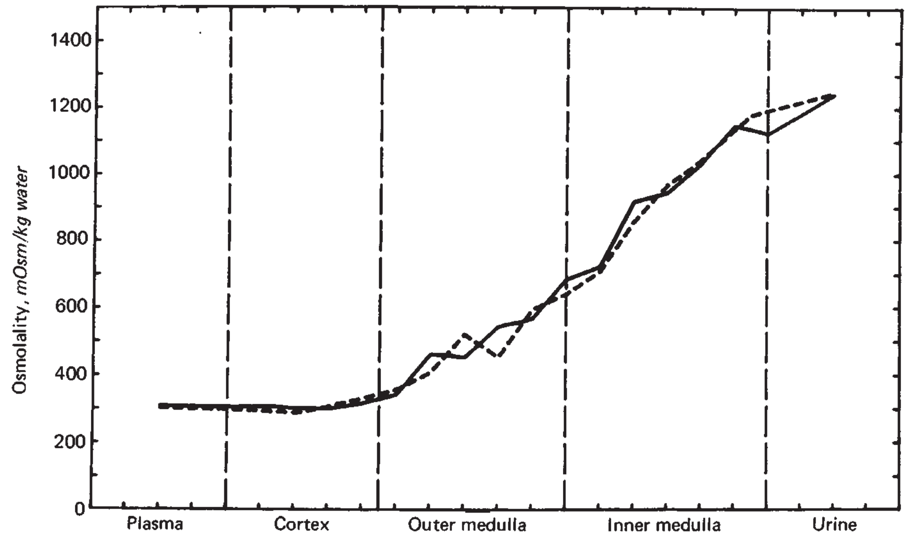

Fig. 2. *Measured osmolalities of plasma, urine, and tissue fluid from both kidneys of an antidiuretic rabbit.* Symbols are *solid line,* left kidney; *dashed line,* right kidney.

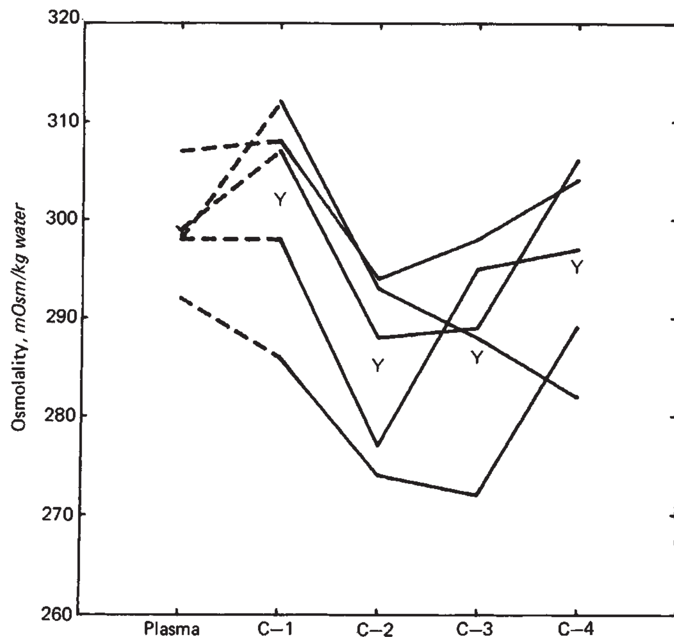

Fig. 3. *Osmolality of plasma and renal cortex in five antidiuretic rabbits.* Slices are designated *C-1* to *C-4* from superficial to deep cortex. Symbols indicate means. Osmolalities in slices *C-2* and *C-3* were significantly lower than plasma (see text).

method for the direct determination of tissue fluid osmolality. An additional advantage compared to the freezing point depression technique is the ease of measurement. Furthermore, better spatial resolution is possible because less tissue is needed. Because freezing and thawing should break down any diffusional barriers that may exist in the tissue in vivo, the measurements yield values representative of the mean tissue osmolality rather than that of any specific tissue compartment.

Summary. A method is described for the measurement of tissue fluid osmolality in intact kidney slices using a vapor pressure osmometer (Wescor). The intact tissue slices were placed directly into the equilibration chamber of the instrument. A high degree of accuracy and reproducibility were obtained for both renal cortical and medullary tissue when care was taken to adhere to specific requirements for tissue slice geometry and size. The measurements proved to be technically easier than using the freezing point depression method, and better spatial resolution was obtained.

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Reprint requests to Dr. M. A. Knepper, Laboratory of Kidney and Electrolyte Metabolism, National Institutes of Health, Building 10, Room 6N307, Bethesda, Maryland 20205, USA

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