25 Karin, M., Andersen, R.D., Slater, E., Smith, K., and Herschman, H.R., Nature *286* (1980) 295.
26 Karin, M., Herschman, H.R., and Weinstein, D., Biochem. biophys. Res. Commun. *92* (1980) 1052.
27 Sobocinski, P.Z., Canterbury, W.J., Knutsen, G.L., and Hauer, E.C., Inflammation *5* (1981) 153.
28 Garvey, J.S., in: Nephrotoxic Mechanisms of Drugs and Environmental Toxins, chap. 39, pp. 437–439. Ed. G.A. Porter. Plenum, New York 1982.
29 Garvey, J.S., Envir. Hlth Persp. *54* (1984) 117.
30 Vander Mallie, R.J., and Garvey, J.S., Immunochemistry *15* (1978) 857.
31 Krieger, D.T., Endocrinology *95* (1974) 1195.
32 Armario, A., Castellanos, J.M., and Balasch, J., Neuroendocrinology *39* (1984) 330.
33 Al-Dujaili, E.A.S., Williams, B.C., and Edwards, C.R.W., Steroids *35* (1981) 157.
34 Riordan, J.R., and Richards, V., J. biol. Chem. *255* (1980) 5380.
35 Suzuki, K.T., and Yamamura, M., Biochem. Pharmac. *29* (1980) 689.
36 Marotta, S.F., Lanuza, D.M., and Hiles, L.G., Horm. Metab. Res. *6* (1974) 329.
37 Garvey, J.S., and Chang, C.C., Science *214* (1981) 805.
38 Armario, A., Restrepo, C., Castellanos, J.M., and Balasch, J., Life Sci. *36* (1985) 2085.
39 Keller-Wood, M.E., and Dallman, M.F., Endocr. Rev. *5* (1984) 1.
40 Levine, R.L., McIntosh, D.A., Lothrop, B.T., and Jackson, B.T., Hormone Res. *13* (1980) 385.
41 DiSilvestro, R.A., and Cousins, R.J., Life Sci. *35* (1984) 2113.
42 Durnam, D.M., Hoffman, J.S., Quaife, C.J., Benditt, E.P., Chen, H.Y., Brinster, R.L., and Palmiter, R.D., Proc. natn. Acad. Sci. USA *81* (1984) 1053.

0014-4754/86/091006-05$1.50 + 0.20/0

On the renal inner medullary concentration of sodium

G.G. Pinter and P.D. Wilson

Departments of Physiology and Preventive Medicine, University of Maryland School of Medicine, Baltimore (Maryland 21201, USA),
19 November 1985

Summary. The principle of 'central volume' is applied to the sodium and water contents of the inner medulla of the mammalian kidney. The analysis raises questions about the possibility of concentrating sodium ions in the inner medulla by a mechanism that postulates, in the same tissue segment, water withdrawal from the descending thin limb of the loop of Henle and sodium entry from the ascending thin limb.
Key words. Central volume principle; kinetics of water and sodium; transit times of water and sodium; transport of water; water withdrawal.

The purpose of this paper is to re-examine the inner medullary concentrating process in the mammalian kidney, from a point of view heretofore not considered, in order to point out some problem which seem as yet unresolved.

Solute concentration in the medullary interstitium is higher than that in the systemic blood plasma: e.g. the concentration of Na is approximately twice as high in the inner medulla as in plasma. Hypotheses have been proposed postulating mechanisms of countercurrent solute recycling either in an active[1–3] or in a passive[4–6] mode, and of water extraction from descending channels[7]. Nevertheless, the mechanisms which generate high solute concentrations in the inner medulla are as yet not fully understood. To evaluate the potential mechanisms involved in the generation of high Na concentration increasing towards the papilla, it is helpful to look at the kinetics of solute and water movements across the medullary tissue in steady state conditions. For this purpose, divide the inner medulla into segments with imaginary surfaces parallel with the corticomedullary (C–M) border (i.e. perpedicular to the direction of the tubular and vascular loops and collecting ducts). Number the segments serially, from 1 to n, with the first nearest to the C–M border. Denote the amounts of extracellular Na and water present in the ith segment (including those in vascular and tubular channels) as $m_{i,s}$ and $m_{i,w}$, respectively, $i = 1,2,\ldots n$. Thus the average extracellular concentration of Na in the ith segment is $\frac{m_{i,s}}{m_{i,w}}$.

Let $f_{i,s}$ and $f_{i,w}$ be the flows of Na and water through the entire ith segment and let $t_{i,s}$ and $t_{i,w}$ be the mean transit or residence times of Na and water, respectively, in the ith segment. Sodium or water flows represent total flows occurring by convection, active transport, and diffusion, and are the sums of both descending and ascending flows entering the particular segment. The driving force for all these flows is the blood pressure, i.e. the pressure which drives the descending vascular and tubular flows into the medulla. Mean transit times represent weighted average transit times by all means of transport.

Since both sodium and water are in dynamic steady state in each segment, i.e. the inflows of sodium and water into each segment are equal to their respective outflows from that segment, the central volume principle is applicable: $m_{i,s} = f_{i,s} \cdot t_{i,s}$, i.e. the amount of sodium in segment i can be expressed as the product of flow of sodium through the segment and the mean transit time of sodium in that segment.

Thus we write

$$\frac{m_{i,s}}{m_{i,w}} = \frac{f_{i,s} \cdot t_{i,s}}{f_{i,w} \cdot t_{i,w}}.$$

If in the (i + l)st segment (nearer to the papilla than is the ith) the concentration of Na is higher than in the ith, i.e.

$$\frac{m_{i+1,s}}{m_{i+1,w}} > \frac{m_{i,s}}{m_{i,w}},$$

then

$$\frac{f_{i+1,s} \cdot t_{i+1,s}}{f_{i+1,w} \cdot t_{i+1,w}} > \frac{f_{i,s} \cdot t_{i,s}}{f_{i,w} \cdot t_{i,w}}.$$

The following table lists four propositions which, if true, would contribute to increasing Na concentration with depth in the medulla. The table also shows our current state of knowledge of each proposition.

Propositions	Inner medulla
1. $f_{i+1,s} > f_{i,s}$	False
2. $t_{i+1,s} > t_{i,s}$	?
3. $f_{i+1,w} < f_{i,w}$	True
4. $t_{i+1,w} < t_{i,w}$	?

To evaluate the truth of propositions 1 and 3, we have assumed that there is no entry of Na and water from the pelvic urine. Then, in steady state the amount of either Na or water entering

the ith segment from the (i − l)th segment can not be less than that amount which flows from the ith to the (i + l)th segment. This is necessarily true because any substance that has crossed the (i − l)-to-i boundary line has a choice of either recrossing that boundary line without penetrating deeper into the medulla, or crossing the i-to-(i + l) boundary line. Furthermore, as ascending flows pick up additional substance in their paths, the ascending flow crossing the (i + 2)-to-(i + l) boundary line into segment (i + l) cannot be larger than the ascending flow crossing the (i + l)-to-i boundary line into the ith segment. Therefore, proposition 1 is false and proposition 3 is true in the inner medulla, if there is no entry of Na and water from the pelvic urine.

(A brief look at the *outer* medullary functions clarifies the significance of these propositions: In the *outer* medulla proposition 2 is true because of the process of countercurrent multiplication, i.e. active transport of Na from the ascending thick limb of the loops of Henle into the peritubular interstitium. This transport along the ascending thick limb results in the prolongation of transit times of Na ions, and this prolongation increases with increasing depth. This follows since the probability increases with depth that a Na ion carried by convective flow in the ascending tubular channel will be transported into the interstitium, whereby its rapid convective transport is changed to slow diffusion. Moreover, in the *outer* medulla, water is short-circuited between adjacent descending and ascending vascular loops by countercurrent exchange in the vascular bundles. By this mechanism water inflow into successively deeper regions of the medulla is reduced, rendering proposition 3 also true in the *outer* medulla.)

Information on proposition 2 is scanty with regard to the *inner* medulla. Here, in the absence of active transport, mechanisms validating proposition 2 for Na ions remain elusive. (It should be noted, however, that for urea proposition 2 is valid because entry of urea from the collecting ducts into the papillary interstitium replaces rapid convective transport by a slow diffusive transport. Recirculation of urea in the countercurrent exchanger also contributes to the prolongation of its transit time in the inner medulla.)

With respect to experimental verification of proposition 3 in the inner medulla, Schmidt-Nielsen and associates[8] showed in rats and hamsters that the water content of the papilla decreases with increasing osmolality. Moreover, Morel et al.[9], and Morel and Guinnebault[10] observed in rabbits and hamsters that, in concentrating kidneys, the delivery of labelled water to the inner regions of the medulla is limited, whereas the equilibration with tracer Na is rapid. White et al.[11] published similar observations in the dog. Thus, the experimental evidence indicates that the flow of water diminishes in the direction of the papilla, and supports the conclusion drawn above that proposition 3 is valid in the inner medulla.

At this time no experimental evidence is available regarding whether proposition 4 is valid. The known requirement that the pathways of salt and water must separate in order to achieve an increasing salt concentration, is consistent with the truth of propositions of 2 and 4 jointly. Proposition 4, a shortening of the mean transit of water in the direction of the papilla, should contribute to increasing of the Na concentration with increasing depth.

In a widely referenced model[6], the hypothesis is put forward that, to achieve the concentrating effect in the inner medulla, water is withdrawn from the descending thin limb of the loop of Henle into the inner medullary interstitium, and this creates a diffusion gradient which brings about a subsequent entry of Na into the inner medullary interstitium. In view of our analysis, unless some special conditions exist for the transport of water in the inner medullary interstitium, it is doubtful that the proposed mechanism can achieve the desired result. This follows because a lengthening of the transit time of water ($t_{i+1,w} > t_{i,w}$ as *opposed* to proposition 4) could offset the effect of prolongation of Na transit times ($t_{i+1,s} > t_{i,s}$, i.e. proposition 2) in achieving

$$\frac{m_{i+1,s}}{m_{i+1,w}} > \frac{m_{i,s}}{m_{i,w}}.$$

The experimental evidence and the considerations described above point to the conclusion that a well substantiated mechanism responsible for the inner medullary concentrating of Na ions is a limitation of water inflow; more specifically, that flow of water in the direction of the papilla should diminish in the inner medulla. Experiments also show[8] that, with increasing papillary concentration, this effect is intensified. However, the mechanism of this effect has not been fully clarified. In the absence of vascular bundles in the inner medulla, direct exchange of water between descending and ascending vessels is limited, and a relatively long diffusive path for water though the interstitium must be postulated.

It is possible, however, that some special conditions do exist for water transport in the inner medulla. One of the possibilities is that extraction of water from the descending thin limbs and release of Na into the interstitium from the ascending thin limbs do not occur at the same level in the inner medulla, and that the former occurs primarily in more superficial layers, nearer to the corticomedullary border, while the latter takes place predominantly in deeper layers, nearer to the papilla. This hypothesis concurs with an earlier suggestion of Pennell et al.[12], who postulated that water extraction from, and urea secretion into, the descending thin limbs are spatially separated, water extraction occurring primarily in the initial half. Another possibility is that diffusion pathways for water molecules in the inner medulla may be limited in the sense that diffusive movement of some of the water in the interstitium could be restricted around macromolecules and membranes. Such characteristics may be seen in vicinal water[13]. Diffusion of water molecules in the interstitium would thus be restricted to preferential channels, and an increase of transit time of water molecules due to interstitial diffusion would not offset the concentration enhancing effect of increased interstitial transit time of sodium ions, providing that the diffusion of these ions is not being restricted.

Restricted diffusion of water in the inner medulla is suggested by the preliminary observations of C. F. Hazlewood (personal communication) who subjected hamster renal papillae to NMR spectroscopy, and found that more than 70% of the water content of that tissue showed properties not compatible with those of bulk water in solutions of comparable solute concentration.

The analysis attempted in this paper has some disadvantages in comparison with the more customary compartmental analysis: first, it is only semiquantitative, and second, it does not specifically assign functions to various vascular and tubular elements. Yet, this analysis is useful, since it points to the over-all kinetic relationships which favor, or work against, an increase of Na concentration in the direction of the papilla. As our brief parenthetical observations on the outer medullary concentrating processes show, both countercurrent multiplication and countercurrent exchange can be discussed in terms of the type of analysis we have used. It is also apparent that the conditions stated here are broad: they could be fulfilled by other processes besides countercurrent multiplication and exchange. Nevertheless, it appears that, from the point of view of this analysis, explanation of the rising sodium concentration in the inner medulla is incomplete. The above considerations suggest that additional experimental data on water and solute kinetics in both transient and steady states should contribute to a fuller understanding of the inner medullary concentrating mechanism.

1 Kuhn, W., and Ryffel, K., Hoppe-Seilers Z. physiol. Chem. *276* (1942) 145.
2 Hargitay, B., and Kuhn, W., Z. Elektrochem. *55* (1951) 539.
3 Kuhn, W., and Ramel, A., Helv. chim. Acta *42* (1959) 628.
4 Kiil, F., and Aukland, K., J. clin. Lab. Invest. *12* (1960) 290.
5 Stephenson, J. L., Kidney Int. *2* (1972) 85.

6 Kokko, J.P., and Rector, F.C., Kidney Int. *2* (1972) 214.
7 Stephenson, J.K., Rev. Biophys. Bioeng. *7* (1978) 315.
8 Schmidt-Nielsen, B., Graves, B., and Roth, J., Am. J. Physiol. *244* (1983) F472.
9 Morel, F.F., Guinnebault, M., and Amiel, C., Helv. physiol. pharmac. Acta *18* (1960) 183.
10 Morel, F.F., and Guinnebault, M., J. Physiol., Paris *53* (1961) 75.
11 White, H.L., Rolf, D., and Tosteson, D.C., Am. J. Physiol. *200* (1961) 591.
12 Pennell, J.P., Sanjana, V., Frey, N.R., and Jamison, R.L., J. Clin. Invest. *55* (1975) 399.
13 Drost-Hansen, W., The occurrence and extent of vicinal water, in: Biophysics of Water, p. 163. Ed. F. Franks, Wiley & Sons, New York 1982.

0014-4754/86/091010-03$1.50 + 0.20/0

High potassium intake increases the plasma concentration and urinary excretion of vasopressin in the rat

D.P. Brooks, J.T. Crofton, L. Share and A. Nasjletti*

Departments of Physiology and Biophysics, and of Pharmacology, University of Tennessee Center for the Health Sciences, 894 Union Avenue, Memphis (Tennessee 38163, USA), 22 October 1985*

Summary. The effect of alterations of dietary potassium intake on the plasma concentration and the urinary excretion of vasopressin was studied in male rats. Ingestion of a high potassium diet resulted in increases in the plasma concentrations of potassium and vasopressin, systolic blood pressure, urine flow, and urinary vasopressin excretion. Ingestion of a low potassium diet had little effect on the plasma vasopressin concentration and systolic blood pressure but caused decreases in the plasma potassium concentration and urinary vasopressin excretion. The results indicate that physiological changes in the plasma potassium concentration or some other consequence of altered dietary potassium intake can affect vasopressin release and excretion.
Key words. Vasopressin; antidiuretic hormone; potassium.

Sodium and chloride ions are the major ions contributing to the osmotic pressure of plasma and, therefore, changes in their concentration are important in the osmotic control of vasopressin secretion. Changes in the plasma potassium concentration contribute little to plasma osmolality, yet these changes may alter vasopressin secretion. The results of experiments dealing with this issue, however, are inconsistent. For example, potassium depletion has been reported to increase the plasma vasopressin concentration[1] as well as attenuate the vasopressin secretion induced by hypertonicity[2]. The present study, therefore, was designed to delineate further the role of dietary potassium in the control of vasopressin secretion.

Methods. Sixty male Sprague-Dawley (Harlan) rats, weighing approximately 240 g, were placed individually in metabolism cages. They were provided with a diet containing 0.16 mmol potassium/g (designated medium-K diet) and water ad libitum. After 5 days (at day 0) they were divided into three groups. One group of 20 rats remained on the medium-K diet, the second group of 20 rats received a low potassium (low-K) diet (0.04 mmol/g), and the third group received a high potassium (high-K) diet (2.7 mmol/g). Water remained available ad libitum. The day before the change of diet (day 0) and 4, 7, and 13 days afterwards, a 24-h urine collection was made. Urine was collected into plastic bottles surrounded by dry ice and enclosed in styrofoam boxes so that the urine froze immediately after it was voided. Urine samples were stored at −40 °C for the later measurement of volume, osmolality, and sodium, potassium, and vasopressin concentrations.

On day 13, ten rats were taken from each group, lightly anesthetized with ether, and decapitated for the collection of trunk blood into chilled plastic tubes containing 0.3 ml sodium heparin (1000 U/ml). A sample of blood was taken for the determination of packed cell volume, and the remainder was centrifuged at 1400 × g at 4 °C. A sample of plasma was taken for the determination of osmolality (freezing point depression; Micro-osmette, Precision Systems) and sodium concentration (flame photometry; IL 343 Flame Photometer, Instrumentation Laboratories). Two or 3 ml of plasma were stored at −40 °C for the subsequent extraction and assay of vasopressin.

The remaining 10 rats from each group were anesthetized with ether, and systolic blood pressure was measured using the tail cuff procedure (Narco). A blood sample was then taken into a heparinized syringe from the abdominal aorta. The blood was centrifuged at 1400 × g at 4 °C, and the plasma concentration of potassium was determined by flame photometry. Aortic blood was used for this purpose because trunk blood is contaminated with potassium from the cellular fluids.

The urine samples from 10 rats in each group were thawed and centrifuged at 1000 × g to remove small traces of food. The volume was determined and an aliquot was taken for the measurement of osmolality and sodium and potassium concentrations. The pH of 5 ml of urine was adjusted to 2.0 and vasopressin was extracted, using octadecysilane cartridges (Sep-Pak C_{18}, Waters). Vasopressin was assayed by radioimmunoassay using equilibrium conditions[3]. The plasma samples were thawed and acidified with 1 N HCl (0.1 ml/ml plasma) and vasopressin was extracted as described above and assayed, using disequilibrium conditions[4]. The USP Posterior Pituitary Reference Standard was used as the standard.

The recoveries of added vasopressin from rat plasma and urine, measured in the same assays in which vasopressin was measured in the experimental samples, were 74 ± 2% (means ± SE) for plasma (n = 6) and 94 ± 3% for urine (n = 12).

Statistical analysis of data was performed using one- and two-way analyses of variance for repeated measures and a subsequent Neuman-Keuls test, when appropriate, to determine differences within and between groups.

Results. The plasma vasopressin concentrations of rats on the medium- and low-K diets were similar (0.67 ± 0.03 versus 0.71 ± 0.06 μU/ml, respectively) at the end of 13 days. Rats fed the high-K diet, however, showed a small but significant ($p < 0.05$) increase in the plasma vasopressin concentration (0.94 ± 0.09 μU/ml; table 1). The urinary excretion of vasopressin (fig.) changed considerably with alteration of dietary potassium intake. Thus, after 4 and 7 days of the low-K diet, rats had a lower ($p < 0.01$) urinary vasopressin excretion (fig.). By day 13, the urinary vasopressin excretion had returned to levels not significantly different from levels observed on day 0 in the same rats or on day 13 in rats on the medium-K diet. Rats that received the high-K diet increased the urinary excretion of vasopressin substantially, such that at days 7 and 13 it was elevated between 4- and 5-fold ($p < 0.01$; fig.).

Alteration of dietary potassium intake resulted in expected changes in the plasma potassium concentration. Thus, rats fed the high-K diet had a significantly ($p < 0.01$) higher plasma potassium concentration than rats fed the medium-K diet at the