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Two fluid compartments in the renal inner medulla: a view through the keyhole of the concentrating process

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Approximately four decades ago, the countercurrent theory became influential in studies on the concentrating process in the mammalian kidney. The theory successfully represented the concentrating process in the outer medulla, but the problem of the concentrating mechanism in the inner medulla, as defined by Homer Smith has remained essentially intractable.

In a recent comprehensive review by Knepper and coworkers of various theories and models, attention was refocused on the possible role of hyaluronate (HA) in the inner medullary concentrating process. The authors proposed a hypothesis that HA can convert hydrostatic pressure to concentrating work.

Here, we briefly survey the earlier ideas on the role imputed to HA and present a new hypothesis which is different from that of Knepper and coworkers. We estimate that the hydrostatic pressures available in the inner medulla can account only for a very small fraction of the concentrating work. We hypothesize that the role of HA is tied up with extravasated plasma albumin and suggest that owing to the property of HA solutions to exclude other macromolecules, extravasated plasma albumin and HA constitute two fluid compartments in the interstitium in the inner medulla. In this proposed two-compartment model, the Gibbs-Donnan distribution influences the movement of ions and water between the HA and the extravasated albumin compartment.

To relate the hypothetical role of HA to the concentrating process, we briefly describe new results obtained by other investigators on the accumulation of urea in the inner medulla. This subject has been critically reviewed recently by Yang & Bankir.

Many processes have been identified as contributing to the concentrating process in the mammalian inner medulla. We speculate that among these many processes, the primary responsibility for the final concentration of the excreted urine may be portioned out differently in different mammalian species.

Keywords: hyaluronan; extravasated plasma albumin; Gibbs-Donnan distribution

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1. Introduction

It is generally held that in the outer medulla of the mammalian kidney, the countercurrent multiplier and countercurrent exchanger models working in tandem provide a valid representation of the urine concentrating process (Kuhn & Ryffel 1942; Hargitay & Kuhn 1951; Wirz *et al.* 1951; Gottschalk & Mylle 1959; Kuhn & Ramel 1959; Smith 1959). After our early, though faulty attempt to represent the countercurrent processes with a computer model (Pinter & Shohet 1963; Shohet & Pinter 1964), other investigators proposed various hypotheses and cogent computer models of the inner medullary concentrating process. The latter models attributed operational roles to various anatomical features, tubules and vascular loops, yet the mechanism of urine concentration in the inner medulla and papilla remained unclear. These models were reviewed recently by Knepper *et al.* (2003).

In this communication, we often refer to the review article by Knepper and associates. In particular, we focus on the role of hyaluronan (HA) and the compartmental structure it generates with extravasated plasma albumin (EVPA) in the inner medullary interstitium of the mammalian kidney and on the possible consequences of such a structure. Further, we discuss recent experimental results, which contributed to the emerging new picture of the inner medullary concentrating process.

2. Hyaluronan in the inner medulla

Histochemical studies have demonstrated the presence of large amounts of glycosaminoglycans (GAG) in the inner medulla and papilla of the mammalian kidney (see reviews by Castor & Green (1968) and Pitcock *et al.* (1988)). This substance is mostly HA, a non-sulphated, highly negatively charged polysaccharide, produced locally by interstitial cells. Pitcock *et al.* (1988) found that in tissue culture, early passages of reno-medullary interstitial cells synthesized mostly hyaluronic acid. Morard (1967) noted that this macromolecule is abundant in the interstitium of the inner medulla and the papilla, whereas it is barely found in other parts of the kidney and speculated about its possible role in concentrating the urine. MacPhee (1998) found an increase in HA concentration during the first three weeks of life in neonate rats which ran parallel with their concentrating ability. This finding was suggestive of a role of HA in the concentrating process, although large changes in permeability and transport properties of various tubular segments that take place in the same time frame, as observed by Liu *et al.* (2001), might also have had contributory roles. MacPhee (1998) found that in the inner medullary tissue, the HA concentration increased gradually towards the papilla and that in the papillary tissue, the HA concentration was 0.56 $\mu g\ mg^{-1}$ tissue wet weight. This figure is somewhat lower than that obtained by Castor & Green (1968) who found a total GAG concentration to be 1.27 $mg\ g^{-1}$ wet weight tissue, of which 68.4%, i.e. 0.87 $mg\ g^{-1}$ wet weight, was HA.

There have been many attempts to clarify the mechanism by which HA contributes to the urine-concentrating process. Koefoed & Knudsen (1961) proposed that enzymatic degradation of HA would add solute to the inner medulla. Pinter (1967*a*) implied that locally synthesized HA would contribute to

the concentrating mechanism by its ability to entangle water. These authors supposed removal of HA together with the reabsorbed water. This supposition has not been confirmed. Knepper *et al.* (2003) suggested the role of a mechano-osmotic transducer for HA, which would accomplish the conversion of energy derived from the mechanical work of the contractions of the renal pelvic muscles to osmotic work. The mechano-osmotic transducer model relies on the studies of Schmidt-Nielsen (1990) and Dwyer & Schmidt-Nielsen (2003) describing rhythmic contractions of the pelvic wall in the mammalian kidney and on the important fact that in the absence of pelvic contractions, the urine-concentrating process is less effective.

3. Extravasated plasma albumin

To clarify the role of HA in the inner medullary concentrating process, we hypothesize that the mechanism is coupled to plasma albumin, another macromolecule present in the inner medullary interstitium. The renal interstitium contains a substantial amount of extravasated plasma proteins, of which the main component is albumin (Pappenheimer & Kinter 1956; Weaver *et al.* 1956; Lilienfield *et al.* 1958; Pinter 1967*b*). More recently, several cooperative studies on this topic have been published from the laboratories of Pallone and of Jamison (Pallone *et al.* 1990, 2003*a*,*b*; Pallone 1992). Pallone (1994) found that the interstitial protein concentration of albumin was 3.4 g dl^{-1}. MacPhee & Michel (1995) determined the average concentration of native serum albumin in the papillary interstitium to be 25% of the plasma level. A significant positive correlation between the quantity of EVPA in the renal medulla of dogs and the increase in urine osmolality suggested a connection between the concentrating process and the interstitial albumin (Pinter 1967*b*), but the mechanism of this connection remained obscure. Wilde & Vorburger (1967) proposed a mechanism for a nonlinear increase of the intravascular plasma albumin concentration and a parallel increase in sodium concentration in the descending *vasa recta*. MacPhee (1998) suggested that both HA and plasma albumin play joint roles in the concentrating process in the inner medulla, but again the specific mechanism remained unclear.

4. The two-compartment hypothesis

The following hypothesis attempts to specify this joint role of HA and extravasated albumin. We refer to the physical–chemical studies of Ogston and associates (Ogston & Phelps 1960; Laurent & Ogston 1963). These authors demonstrated that the osmotic pressure of a solution containing both HA and another macromolecular solute, in particular plasma albumin, is in excess of the sum of the osmotic pressures exerted by each of the two solutes individually at identical concentrations. They attributed this finding to the exclusion of albumin from the part of the solution that is occupied by hyaluronic acid. They estimated the magnitude of the excluded volume by extrapolating the HA concentration to zero and found that in the presence of plasma albumin, the excluded volume amounts to approximately 25 ml water g^{-1} of hyaluronic acid at low polysaccharide concentration. The phenomenon of molecular exclusion by HA has been repeatedly confirmed (Maroudas 1975; Hardingham 2003).

Based on these findings, we hypothesize that the interstitium of the inner medulla and papilla contains two functionally distinguishable fluid compartments. (The term compartment is used as in tracer studies, e.g. Sheppard 1962.) Each compartment is characterized by the main macromolecule present: one compartment contains HA and no—or very little—plasma albumin that is excluded from it to a high degree. The other compartment contains primarily EVPA. Although HA is not confined to its own compartment by a membrane, the restricted mobility of HA molecules is due to their very large size and the entangled large amount of water. Even though no membrane separates them, the movements of ions and water between the two such compartments are subject to the Gibbs-Donnan distribution (Gregor 1951; Maroudas 1975), and the compartment which contains the highly negatively charged HA should also contain the major portion of sodium, whereas in the EVPA compartment the concentration of sodium should be lower. In considering the distributions of both sodium and chloride ions between these two compartments, it should be noted that these distributions should be influenced by the fact that the excluded albumin molecules also carry negative charges. The concentration of urea should not be different between the two fluid compartments (Maroudas 1975). By assuming that the interstitial volume is approximately 10% of the wet weight of the tissue and that all HA is in the interstitium, using the above quoted figures of 5.6–8.7 mg ml^{-1} by Macphee (1998) and Castor & Green (1968), the excluded volume constituting the EVPA compartment in the rat papillary interstitium may be 15–20% or an even somewhat-higher fraction of the interstitial volume, given that the 25 ml g^{-1} excluded volume was estimated by Ogston & Phelps (1960) for an extrapolated zero HA concentration. The existence of more than one compartment in the interstitium of various tissues has been suggested also by Bell *et al.* (1978) and Powers *et al.* (1988).

In contrast to HA which has a slow turnover, experiments show that extravasated albumin passes quickly through its compartment. Moffat (1969) demonstrated rapid entry of labelled albumin into the medullary interstitium and Tenstad *et al.* (2001) showed rapid removal. Pallone (1992) concluded that albumin is moved across the *vasa recta* by solvent drag. MacPhee & Michel (1995) calculated that differences in hydrostatic and oncotic pressure across the walls of the ascending *vasa recta* are more than sufficient to provide the driving force for the continuous clearance of fluid and plasma albumin from the interstitium. According to Tenstad *et al.* (2001), tracer albumin injected in the papillary interstitium does not have local access to lymph and quickly appears directly in the blood. These results point to the conclusion that fluid and protein are cleared from the renal interstitium by convective flow into the ascending *vasa recta* (MacPhee & Michel 1995). We propose a main role for the two-compartment model: that of facilitating the removal of fluid from the inner medullary interstitium.

5. Fluid extraction

As the calculation here below shows, the mechanical (pressure–volume) work available cannot be responsible for more than only a minuscule portion of the concentrating work in the inner medulla. Therefore, we conclude that

the concentrating work must be done by other processes. One of these, the accumulation of urea, while long considered to be of primary importance, recently received impressive experimental confirmation.

Owing to the interstitial hyperosmolality, fluid is extracted from blood vessels and tubules including the collecting duct, into the albumin compartment. There are two exit routes for fluid from the albumin compartment: (i) it enters the HA compartment or (ii) it sweeps into the ascending *vasa recta* (MacPhee & Michel 1995). The water entering the HA compartment tends to swell and dilute it, but this is counteracted by periodic increases of hydrostatic pressure imparted by contractions of the pelvic muscles, as proposed by Schmidt-Nielsen (1990). The contractions squeeze out fluid from the HA compartment so that, eventually, fluid squeezed out is channelled into the ascending *vasa recta* with the stream of albumin. The sodium lost therewith from the HA compartment is quickly replaced from the vascular and tubular fluids reaching the inner medulla as indicated by the rapid isotopic equilibration of the inner medullary sodium pool with plasma Na activity after intravenous injection of radioactive sodium (Morel *et al.* 1960).

As noted by Knepper *et al.* (2003), the source of energy driving this proposed mechanism is the rhythmic hydrostatic pressure increases created by contraction of the muscles of the renal pelvis. In using approximate figures for estimation, in the human kidney with moderate concentrating ability, the outer medullary concentrating processes raise the tissue osmolality from about 300 to approximately 600 mOsm kg^{-1} and in the inner medulla the osmolality increases further to a high value of approximately 1000–1200 mOsm kg^{-1}. In the inner medulla of the kidneys of many other mammalian species, the urine is concentrated to a much higher osmolality. For dilute solutions, the analogy with the equation of state of an ideal gas (Clark 1952) is commonly applied, and it can be estimated that an increase of 0.4 Osmol kg^{-1} osmotic pressure is counterbalanced by almost 9 atm or approximately 6800 mmHg hydrostatic pressure. The hydrostatic pressure generated by the renal pelvic muscles measured in mmHg is, at most, of the order of the low two digits. Therefore, we conclude that although the mechanism suggested for HA, either as proposed by Knepper *et al.* (2003) or as amended by us here above, appears far from being sufficient to account for the entire process in the inner medulla. However, we hypothesize that the two-compartment structure may play an ancillary role in conjunction with other mechanisms that have more substantial responsibility for urine concentration. Among these, recent evidence points to the importance of the mechanisms of accumulation of urea in the inner medulla.

6. Potential role of urea and the two-compartment model

As we concluded earlier, the mechanism ascribed to the presence of HA in the inner medulla should assist the concentrating process, but the main burden for the urine concentration in the inner medulla should fall on other mechanisms. Recent experiments provided evidence for the existence of special transport proteins responsible for the accumulation of urea in the inner medulla. Here, we briefly survey these studies; an outstanding review by Yang & Bankir (2005) gives a comprehensive picture.

The renal handling of urea in many species has been thoroughly studied and its potential importance in the concentrating process emphasized among others by Bonventre *et al.* (1980) and Schmidt-Nielsen (1987). It has long been recognized that in the mammalian kidney, urea is utilized in the process of water conservation (Gamble *et al.* 1934). It has also been pointed out that simple diffusional transport of urea in the kidney tubules and between red cells and plasma within the renal circulation, would not be sufficient to allow urea to fulfil this important role (Pinter & Zilversmit 1960; Chinard *et al* 1965; Pinter *et al.* 1972). In the past two decades, several urea-transporting proteins have been discovered, and recent reviews present admirably clear descriptions of their molecular biology and physiological roles (Sands 2003*a*,*b*). The urea transport proteins found in the mammalian kidney provide facilitated diffusion. In particular, molecular approaches resulted in cloning of several cDNA isoforms derived from two gene families: UT-A and UT-B. The former type, present in the inner medullary portion of the collecting duct, is inhibited by phloretin and stimulated by vasopressin. The latter transport protein is found in red blood cells, some other organs and also in the walls of the descending *vasa recta*. Experiments with transgenic mice have indicated that each of these transporters plays significant roles in the accumulation of urea in the inner medullary interstitium. A review by Yang & Bankir (2005) presents an excellent description of the studies with the UT-B gene family.

Recent studies with transgenic mice that lacked UT-B urea transporter (Yang *et al.* 2002; Bankir *et al.* 2004) have shown a severe concentrating defect in these animals. Under conditions of controlled food and unlimited water intake, the average urine osmolality in mice lacking the urea transporter UT-B was 1780 mOsm kg^{-1}. In contrast, the osmolality in the wild-type controls was 2650 mOsm kg^{-1}. Furthermore, while control mice responded to urea loading with increasing urine osmolality, such response did not occur in the transgenic mice lacking the UT-B transporter. The authors concluded that the UT-B transporter facilitated countercurrent exchange of urea in the renal-medullary vessels in the normal animals, and that this accounts for a major part of the kidney's concentrating ability and the adaptation of renal urea handling during high and low protein intake.

The roles of UT-A1 and UT-A3 transporters found in the inner medullary collecting duct (IMCD) have also been studied. In a recent series of experiments (Fenton *et al.* 2004), normal control, i.e. wild-type mice, were compared with transgenic counterparts in which the phloretin-sensitive urea transporters UT-A1 and UT-A3 were deleted (referred to in the following as knockout mice). These authors determined that urea diffusion (P_{urea}) from isolated perfused IMCD of normal mice was rapid, and was further accelerated by addition of vasopressin; whereas P_{urea} from the IMCD isolated from knockout mice was significantly slower and not different from that expected from simple lipid phase diffusion. Moreover, addition of vasopressin in these experiments almost quadrupled P_{urea} in the IMCD of the wild type mice, and had no effect in the knockout group. Furthermore, experiments on whole animals showed that on a 20% protein diet and 24 h restricted water intake, wild-type mice increased urine osmolality to nearly 4000 mOsm kg^{-1}, whereas the osmolality of the urine produced in the knockout mice was around 1000 mOsm kg^{-1}. Apparently, the knockout mice were unable to respond to water restrictions and increased protein

intake. Providing that the general conditions of the mice in the two groups were comparable, this finding also offers evidence for the role of UT-A transporters in the concentrating process.

A further finding of the study by Fenton *et al.* (2004) indicated that in the inner medullary tissue of the knockout mice, urea concentration was significantly lower than in the wild-type group (less than 100 mM in knockout mice versus more than 300 mM in the wild type), whereas sodium, chloride and potassium concentrations were not different between these groups. The authors concluded that this observation did not support the models of Stephenson (1972) and Kokko & Rector (1972) in which deposition and accumulation of sodium in the inner medulla required a prior presence of a high concentration of urea already in the interstitium. These models made use of the countercurrent mechanism originally described by Kuhn & Ryffel (1942) in which concentrating was accomplished by selective permeabilities to water and two different solutes.

7. Conclusions

In the past four decades, an impressively large amount of intellectual energy and inventiveness have been invested into attempts to clarify the mechanisms of inner medullary urine concentration. These efforts are continuing as evidenced by the number of significant contributions in the past few years. In this brief review, we have attempted to clarify the role of HA in the inner medulla, and concluded that its quantitative significance is very small in raising the osmolality in the inner medulla. We have proposed that the main contribution of HA in the inner medulla is to give rise to a two-compartment structure, i.e. of HA and EVPA. On the one hand, the HA compartment could play a role in reducing the washout of sodium ions from the HA compartment. On the other hand, the albumin compartment which, as a rough estimate, may comprise some 15–25% of the total interstitial fluid volume, should play a significant role by its rapid fluid turnover. This turnover is evidenced by the rapid entry and exit of plasma albumin as it is carried into the ascending *vasa recta* by flow generated by the hydrostatic and oncotic pressures, as proposed by MacPhee & Michel (1995).

Turning to the significant mechanisms that can carry the major responsibility for the concentrating effects in the inner medulla, we have discussed recent evidence for the role of urea published by other investigators. This evidence has been obtained in mice by using the method of genetic manipulation. Yang & Bankir (2005) pointed out the differences between the concentrating needs and urea metabolism of different mammalian species, specifically mouse, rat and human, and noted that the burden for the mouse kidney is greater than that for the kidney of larger mammals. Yang & Bankir concluded that in mice on normal protein diet, the knockout of the UT-facilitated diffusion transporters lowered urine osmolality approximately to 35–50% of that found in normal mice. They speculated that in other species with larger body size, UT-s would play a lesser role in overall urinary-concentrating ability as compared to that in mice.

The full identification of the processes responsible for the concentrating work in the inner medulla of various mammalian species does not yet seem to be at hand. It is generally recognized that in the collecting ducts of the mammalian inner medulla, the composition of only a relatively small amount of urine (in the

concentrating kidney less than 1% of glomerular filtrate) gets finally adjusted to the physiological requirements of the particular species in the given conditions. Further, the inner medulla is a relatively isolated region of the body: it has low blood flow with reduced haematocrit, and consequently a poor oxygen supply, and is protected from penetration of various blood-plasma constituents by effective countercurrent exchange in the *retia mirabilia*. The same countercurrent exchange process prevents loss of other substances from the inner medulla. The anatomical and, presumably, the functional details of these *retia* show substantial differences in different species (Jamison & Kriz 1982). Also, while the combined volumes of the inner medulla and papilla show a rough positive correlation with urine-concentration ability, as Yang & Bankir pointed out it cannot be safely extrapolated that all sizes fit the same functional details. Thus, it would appear that in contrast to the countercurrent multiplication of the single effect of Na^+ transport which is found in the outer medulla in many mammalian species, nature uses more mechanisms in the inner medulla, and that the contribution of these individual mechanisms might be parcelled out differently in different species.

The review article of Knepper *et al.* (2003) described models of a number of other mechanisms, many of which were works in progress. Since the publication of that review, a number of these models have been developed further. Among them, Layton *et al.* (2004) proposed a three-dimensional computer model which took into account a distribution of the length of the loops of Henle with loop bends at all levels of the inner medulla, and identified two modes that produce a significant axial osmolality gradient. Also, Hervy & Thomas (2002) incorporated a mechanism of production of osmolites, specifically lactate, by anaerobic glycolysis in the inner medulla, into a new mathematical model. Simulations with this model showed that under conditions favouring effective recirculation of lactate, an appreciable NaCl gradient, but no urea accumulation, can build up in the inner medulla.

The number of models comprising the armamentarium of inner medullary concentrating mechanisms has not shown a tendency to diminish in the past few years. One of these mechanisms, namely the role of urea discussed earlier, has gained renewed recognition owing to recent experimental evidence demonstrating the effectiveness of facilitated urea transporters.

In general, progress in research is often accomplished by falsification and elimination of hypotheses, which are shown to be untenable (Popper 1963). However, this pattern does not appear to work in the case of the inner medullary concentrating mechanisms, as older ideas are resurrected or given renewed emphasis in newer models. (Our early, 1963 computer modelling attempt is a privileged exception in this regard, as two implicitly contradictory assumptions that went unnoticed by us were discovered immediately upon publication.)

The inner medullary mechanisms put the finishing touches on the excreted urine, and many of them might contribute to different degrees in different species. Although many possibilities have been taken into account and the anatomical features of the inner medulla with their individual permeabilities and transport potentials and geometric arrangements as well as the interstitial composition of the tissue have been considered, it is possible that nature still keeps hidden some mechanisms, which as yet have not been thought of.

Editors' note

Please see also related communications in this focussed issue by Lu *et al.* (2006) and Ribba *et al.* (2006).

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