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COLLECTING DUCT FLOW RATE AS A DETERMINANT OF EQUILIBRATION BETWEEN URINE AND RENAL PAPILLA IN THE RAT IN THE PRESENCE OF A MAXIMAL ANTIDIURETIC HORMONE CONCENTRATION

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SUMMARY

1. Antidiuretic hormone (ADH) was infused into normal male rats at a rate of 60 μu./min. 100 g body wt., to maintain an effectively constant maximal circulating level. Four groups of rats were used; they were water-loaded by receiving together with the ADH, I.V. infusions of hypotonic dextrose (2·5 g/100 ml.) at different rates (1·0, 4·5, 9·0 and 12 ml./hr, respectively), over an infusion period of 4 hr.

2. Urine flow rate increased in all groups, the rate and extent of the increase being related to the volume rate of infusion. The differences in urine flow rates between the four groups were due almost entirely to increases in free water clearance, with no consistent differences in osmolal clearance between the groups. At the end of the 4 hr infusion period, osmolal clearances were closely similar in the four groups.

3. Papillary and medullary tissue solute concentrations were progressively reduced at the higher rates of infusion. The changes were due to small increases in the water content, together with a profound decrease in urea concentration and a smaller decrease in sodium concentration. However, papillary osmolality was consistently higher than urine osmolality at the three highest rates of dextrose infusion.

4. As urine flow rate increased, there was a progressive reduction in the degree of osmotic equilibration between the final urine and the papillary tip. For urea, however, the degree of equilibration remained high.

5. It is concluded that, in the rat, the rate of flow *per se*, along the collecting duct, is an important determinant of final urine concentration; even if there is an osmotic driving force for water re-absorption in the renal medulla, and the collecting duct walls are permeable to water, osmotic equilibration is restricted by tubular flow rate.

INTRODUCTION

Three major determinants of urinary osmolality, which influence the distal segments of the mammalian nephron, have been recognized. They are: (*a*) the permeability of the tubule wall, which is regulated by antidiuretic hormone (Ullrich, Rumrich & Fuchs, 1964; Morgan, Sakai & Berliner, 1968; Berliner & Bennett, 1967); (*b*) the effective osmotic reabsorptive force in the renal medulla (rapid changes in medullary composition can contribute to the physiological regulation of urinary osmolality; Thomas, 1971); and (*c*) the rate of excretion of certain solutes; these influence renal concentrating ability, as has been demonstrated in experimental osmotic diuresis, such as that induced by mannitol (Koike & Kellogg, 1957).

However, there is another possible factor influencing final urine osmolality which has not been systematically investigated, namely the rate of flow of fluid along the collecting ducts. The diuresis induced by ingestion of water (or infusion of hypotonic dextrose) is generally considered to be caused by the suppression of endogenous antidiuretic hormone (ADH) release, leading to impaired water absorption in the distal tubule and collecting ducts. The concurrent reduction of the osmotic gradient in the renal medulla facilitates the production of dilute urine (Saikia, 1965; Valtin, 1966; Zain-ul-Abedin, 1967), and has been attributed to effects of altered ADH levels on medullary blood flow (Thurau, Deetjen & Kramer, 1960; Atherton, Green, Thomas & Wood, 1972). In the present study, variable water loading was accompanied by a sustained high level of circulating ADH (by continuous infusion of Pitressin) so that differences in the extent of the diuresis, or of changes in medullary composition, cannot be attributable directly to altered ADH levels. In these circumstances, it was possible to determine the effects of urine flow rate *per se* on the degree of equilibration between medullary interstitium and urine, in the conscious, water-loaded rat.

METHODS

Male Wistar rats (225–365 g) were used, which had been maintained on a rat-cake diet (21 % protein), with free access to water. The osmolality of an overnight urine sample was determined before experimentation in order to ensure that the animals were normally hydrated.

Each rat was lightly anaesthetized with ether, and a fine Polythene catheter (Portex PP25) was inserted into a tail vein. The rats were then secured in cylindrical Perspex restraining cages, and allowed to recover. Four groups of rats were used, denoted A–D. They were water-loaded by receiving infusions of dextrose (2·5 g/100 ml.) over a period of 4 hr, at the following rates:

group A:	1·0 ml./hr	(n = 5)	group C:	9·0 ml./hr	(n = 5)
group B:	4·5 ml./hr	(n = 5)	group D:	12·0 ml./hr	(n = 5)

All groups also received over the infusion period, ADH at a rate of 60 μu./min. 100 g body wt. (as Pitressin, Parke-Davis). This rate of ADH infusion has been shown to have a maximal antidiuretic effect (Atherton, Green & Thomas, 1971). Urine samples were collected without bladder catheterization, at 30 min intervals throughout the period of infusion. Voiding of urine was encouraged by brief induction with ether, or by gentle sensory stimulation. The volume of each sample was recorded; urinary sodium and potassium were determined by flame photometry using a Beckman clinical photometer; urinary urea and ammonia were determined by a modification of the method of Fawcett & Scott (1960); urinary osmolality was determined by freezing point depression (Knauer cryostat unit).

Immediately after collection of the terminal urine sample, each rat was anaesthetized with ether, and the kidneys were excised and rapidly frozen in liquid nitrogen. A blood sample was collected by cardiac puncture, centrifuged (MSE 'Mistral' 4L) in a heparinized tube, and the plasma separated. Plasma osmolality, sodium, potassium, ammonia and urea were determined as for urine.

Six serial slices were cut from each kidney; two each from papilla, medulla and cortex. Each section was sealed quickly in a previously weighed aluminium foil envelope, in order to limit evaporation of water (after Atherton, Green & Hai, 1969). Each envelope (and contents) was then weighed using a Beckman LM500 electrobalance.

Slices from one kidney were used for determination of renal tissue water, sodium and potassium. Each (opened) envelope and tissue was dried at 120° C for 18 hr, and then reweighed for determination of tissue water content. The dried tissue was digested in nitric acid (Atherton, Hai & Thomas, 1968*b*), and then tissue sodium and potassium were determined as for urine.

Slices from the second kidney were homogenized by grinding with mortar and pestle, and the homogenates were heated to 100° C for 15 min to destroy tissue urease (Atherton *et al.* 1968*b*). After cooling and centrifugation, the supernatants were used for determination of tissue urea and ammonia as for urine.

Tissue osmolality was not determined directly, but an approx. value was calculated as $2(Na^+ + K^+ + NH_4^+)$ + urea (Levitin, Goodman, Pigeon & Epstein, 1962; Saikia, 1965; Valtin, 1966). Tissue water and solute contents are expressed with reference to the wt. of urea-free dry solid (Saikia, 1965; Gardner, 1966).

Osmolal clearance (C_{osm}) was calculated as $U_{osm}\,V/P_{osm}$, where V = urine flow rate and U_{osm} and P_{osm} = urinary and plasma osmolalities, respectively. Free water clearance, C_{H_2O} was calculated as $V - C_{osm}$.

Results are presented as mean ± S.E. of mean. The significance of differences between means was assessed by Student's t test.

RESULTS

Plasma

Data concerning plasma osmolality and solute concentrations are presented in Table 1. There is a tendency towards dilution of the plasma at the higher rates of infusion, but the changes are statistically non-significant.

Urine

At all rates of infusion, urine flow increased progressively over the first 2–3 hr (Fig. 1). The increments in flow rate were greater in magnitude at the higher rates of infusion; at infusion rates of 9 and 12 ml./hr, the final

flow rates were very similar (*ca.* 200 μl./min) and approx. equal to the rates of fluid infusion. Free water clearance (C_{H_2O}) was negative at the low infusion rate (1 ml./hr), and variable at an infusion rate of 4·5 ml./hr, but showed a progressive increase at the two high rates, in which a steady state was achieved after approx. 3 hr infusion (Fig. 1). The higher final urine flow rates at the higher infusion rates were due almost entirely to increased free water clearance (C_{H_2O}), osmolal clearance being very similar in all groups (Fig. 1), both in the time course of the changes and in the final level achieved.

TABLE 1. Plasma osmolality and solute concentrations after a 4 hr infusion of dextrose (2·5 g/100 ml.) containing ADH 60 μu./min. 100 g body wt. Infusion rate: A, 1 ml./hr; B, 4·5 ml./hr; C, 9 ml./hr; D, 12 ml./hr. (Nos. quoted are mean ± S.E. of mean.)

	Plasma osmolality (μosmole/g H_2O)	[Na^+] μmole/g H_2O	[K^+] μmole/g H_2O
A	302 ± 7	141 ± 3	4·4 ± 0·2
B	307 ± 5	142 ± 11	4·6 ± 0·4
C	295 ± 4	143 ± 3	4·0 ± 0·2
D	288 ± 14	136 ± 5	4·0 ± 0·3

The time courses of changes in urinary solute outputs were closely similar at all rates of infusion. After the attainment of a steady state, the urinary outputs of osmoles and sodium were not significantly different at the different rates of infusion, but the output of urea was significantly ($P < 0·01$) lower at the lowest infusion rate (Fig. 2).

As urinary flow rate increased up to 100 μl./min, there was a sharp decline in urinary osmolality, with a more gradual fall as flow increased further (Fig. 2). This pattern is reflected in the flow/concentration relationships of the four component urinary solutes.

Renal tissue

The renal tissue osmolal gradient was dissipated to an increasing extent at higher infusion rates (Fig. 3). Marked reduction occurred in papillary and inner medullary solute and osmolal concentrations, while outer medullary and cortical concentrations remained largely unchanged. The changes in the renal tissue concentrations of ammonia and potassium show no consistent trends; however, since only small, statistically non-significant changes in the papillary contents of these solutes were observed, any differences merely appear to reflect changes in renal tissue water content (Fig. 3), as reported by others (Hai & Thomas, 1969; Atherton *et al.* 1971). The most profound changes in tissue concentration were those for urea. At the two highest infusion rates, the urea gradient was almost totally abol-

ished, with concentrations in the papilla and medulla only marginally higher than in the cortex, and the urea content of the papilla dramatically reduced (Fig. 3).

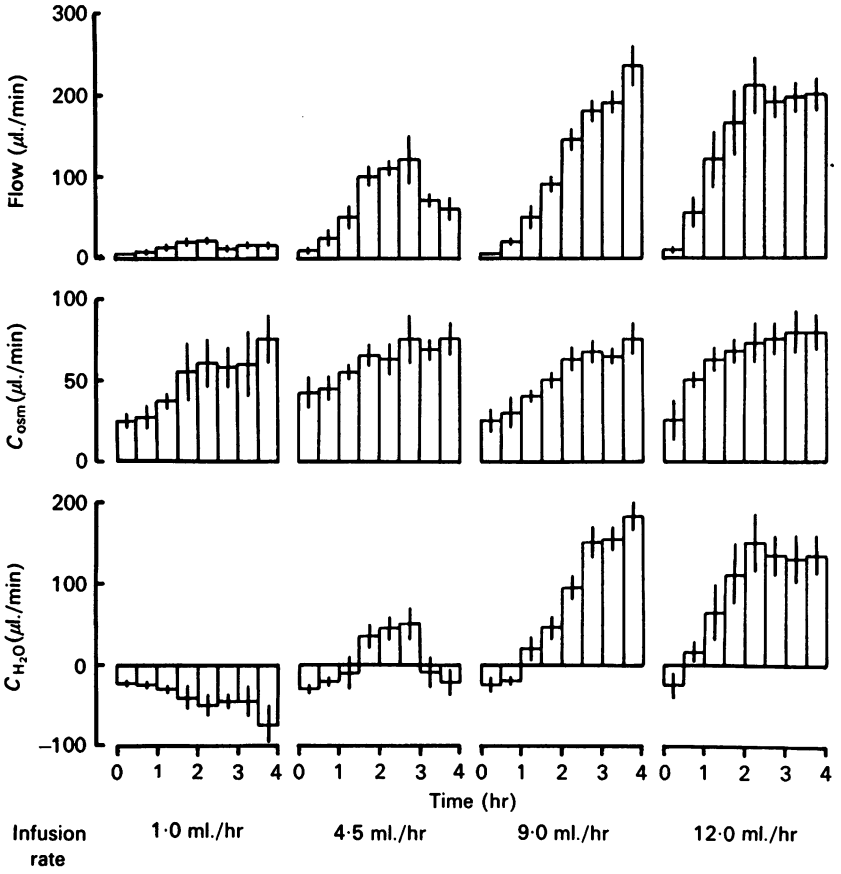

Fig. 1. Urine flow rate (μl./min), osmolal clearance (C_{osm}: μl./min) and free water clearance (C_{H_2O}: μl./min) during a 4 hr infusion of hypotonic dextrose (2·5 g/100 ml.) at the rates shown, together with ADH (60 μu./min. 100 g body wt.).

Urinary–papillary concentration differences

In Fig. 4 is shown the osmolal concentration of the papilla tip (calculated as described in Methods), and the measured osmolality of the terminal urine sample. It is apparent that, during the excretion of large volumes of water, the papilla remained hypertonic to the urine and that, as flow increased, osmotic equilibration across the walls of the collecting duct decreased.

The finding that, at the lowest rate of dextrose infusion, the urine appears

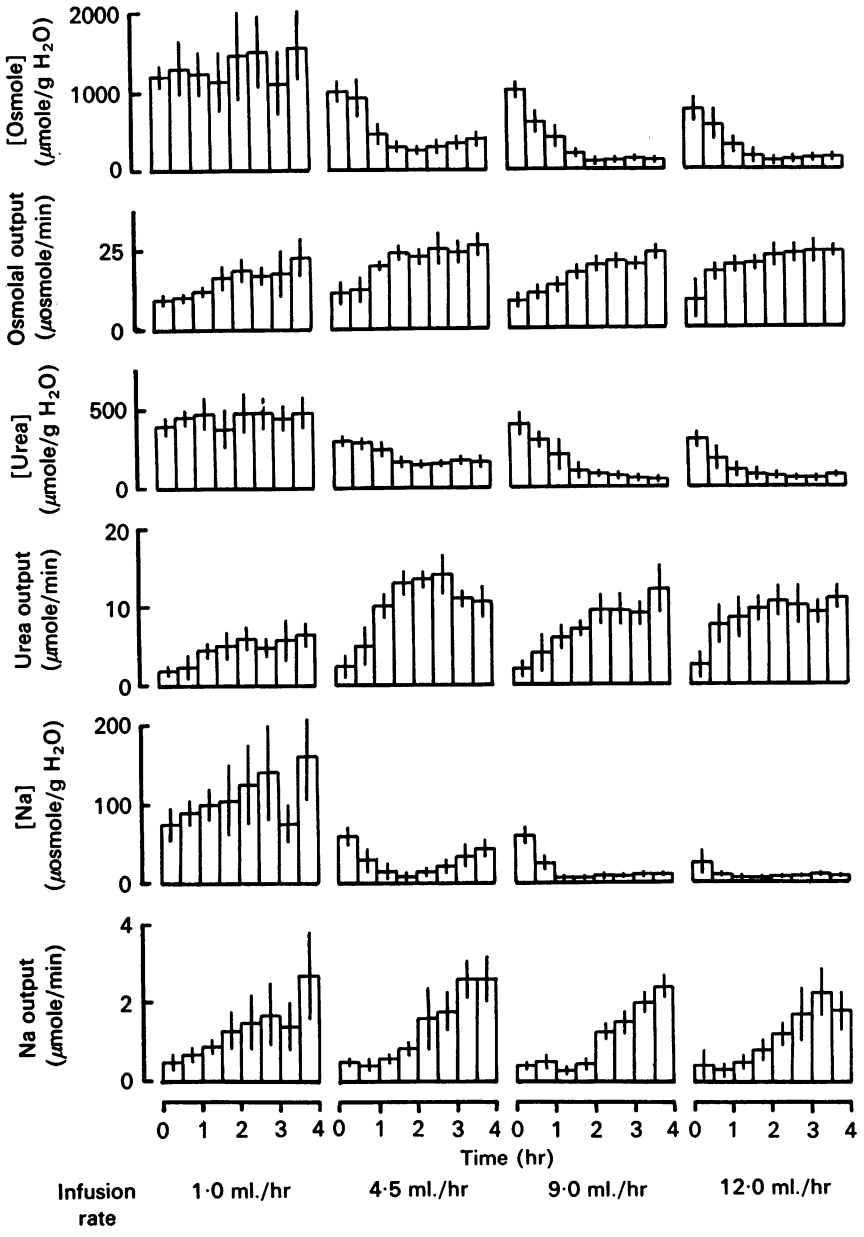

Fig. 2. Urinary concentrations and rates of excretion of osmoles, urea and sodium during a 4 hr infusion of hypotonic dextrose (2·5 g/100 ml.) at the rates shown, together with ADH (60 μu./min. 100 g body wt.).

to be more concentrated than the papilla is due to the limitations of the method – i.e. the papilla tip (slice 1) includes tissue from up to 2 mm below the tip (a region with a steep osmolal gradient) so that, as a consequence, the tissue analyses underestimate the true papillary tip concentrations.

Over the whole range of urinary flow rates, there was no significant difference between urinary and papillary concentrations of urea (Fig. 4).

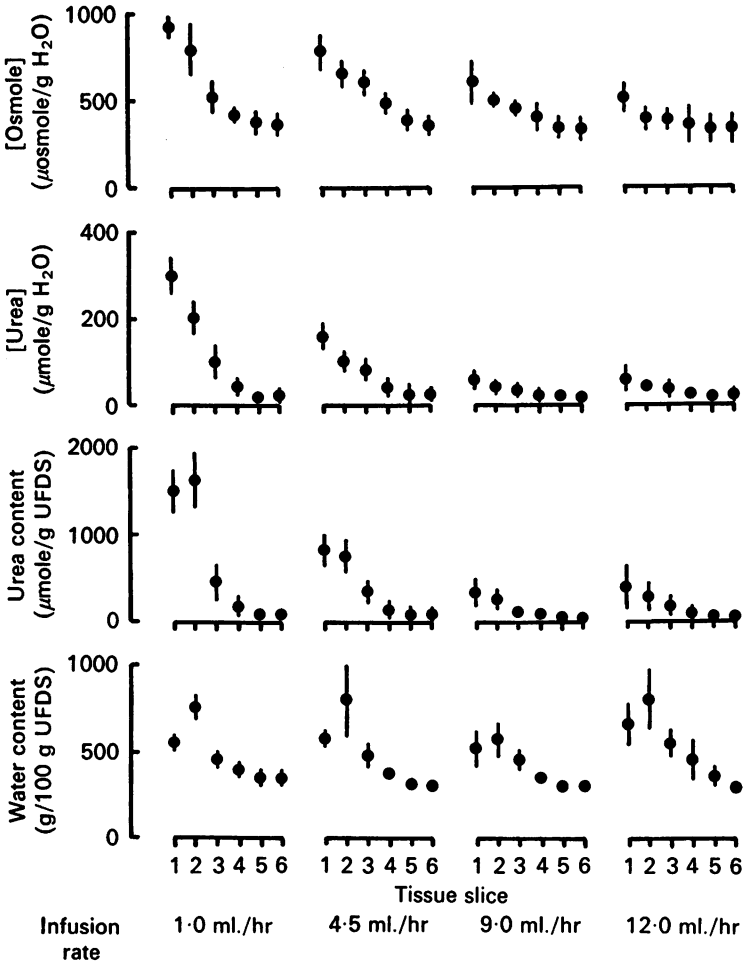

Fig. 3. Mean (± S.E. of mean) renal tissue osmolal concentration (calculated), urea concentration, urea content and water content at the end of a 4 hr infusion of hypotonic dextrose, at the rates shown, together with ADH (details of infusion are in legend to Fig. 1). The tissue slice numbers refer to the level of section: 1, papillary tip; 2, papillary base; 3, inner medulla; 4, outer medulla; 5, inner cortex; 6, outer cortex. Note: UFDS denotes urea-free dry solid.

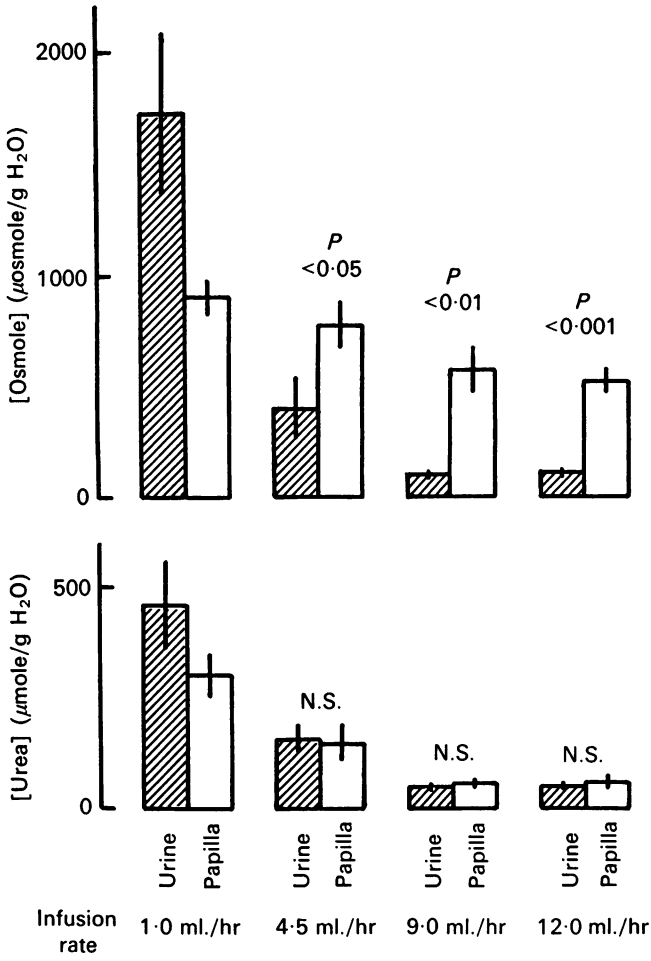

Fig. 4. Comparison of the renal papilla tip osmolality (calculated) and urea concentration with the osmolality and urea concentration of the final urine sample. At the three high rates of infusion, papilla tip osmolality is significantly higher than urine osmolality, but the urea concentration in the papilla tip is not significantly different from the urinary urea concentration. Note: N.S. means not significant.

DISCUSSION

Increased excretion of solutes, such as mannitol and various sodium salts, is known to impair the renal concentrating ability (Koike & Kellogg, 1957; Atherton, Evans, Green & Thomas, 1971). In the present series of experiments, however, the larger urine flow rates at higher infusion rates are almost entirely attributable to increments in free water clearance, with osmolal clearance remaining similar in all groups (Fig. 1); thus, since

osmolal output is similar in the four groups (Fig. 3), this can be discounted as a factor significantly influencing either the final urine flow rate, or the equilibration between final urine and papilla, at the different infusion rates.

Nevertheless, renal tissue solute concentration gradients are progressively reduced at higher rates of infusion, largely owing to reduction in the concentrations of urea (and sodium) in the papillary and medullary segments. The infusion of hypotonic dextrose appears, therefore, to have induced changes similar to those during a normal 'water diuresis' in the rat, despite an essentially constant high level of circulating ADH. With the exception of group A (low infusion rate), in which antidiuretic conditions prevailed, the findings of the present study may be summarized as follows:

(*a*) Urinary osmolality, and the concentrations of the component solutes, tend to decline towards a steady state, the rate and extent of the decline being related to the rate of infusion.

(*b*) The time courses, and extent of the changes in output of osmoles and component solutes, are virtually identical for all groups.

(*c*) Time courses of osmolal clearance are virtually identical in all groups, tending to increase throughout infusion, so that differences in urine flow between groups are due solely to the larger increments in free water clearance at higher rates of infusion.

(*d*) The tissue osmolal concentration gradient declines to a greater extent at higher rates of infusion, largely owing to reductions in the concentrations of urea (accompanied by lesser reductions in sodium concentration and small increases in tissue water content) in the papillary and medullary segments.

(*e*) Changes in tissue concentrations of potassium and ammonia appear merely to reflect changes in tissue water content in all groups.

This pattern of findings is very similar to previous reports of changes during water diuresis in the rat, both in terms of urinary solute excretion, and renal tissue composition (Atherton, Hai & Thomas, 1968*a*, *b*, *c*).

These earlier reports interpreted such results in terms of modifications of tubular re-absorption and counter-current mechanisms, largely as a result of suppression of release of endogenous ADH induced by administration of hypotonic fluid. In the present study, however, a similar effect was obtained, despite an effectively constant high circulating level of ADH resulting from exogenous infusion. Our results imply that some consequence of water-loading, other than ADH-induced modifications of nephron function, is responsible for the diuresis observed here (of course, the diuresis obtained during the present experiment cannot be regarded as a normal physiological response to water-loading).

The present experiments provide no direct evidence as to the mechanism

of this effect. Dicker (1970) speculated that intrarenal distribution of single nephron filtration may vary, even when total glomerular filtration remains unchanged, as during water diuresis. He suggested that the dilution of plasma or the stimulation of volume receptors, during water-loading, might produce an increase in the rate of filtration of the short-looped cortical glomeruli at the expense of juxta-medullary nephrons; this, together with an increase of flow through the vasa recta, might contribute to a reduction of the medullary osmotic gradient. Several workers have suggested that there is an increased medullary blood flow during water diuresis (Thurau *et al.* 1960; Levitin *et al.* 1962; Valtin, 1966), and this may occur as a direct result of water-loading, rather than as a result of diminished ADH release (Valtin, 1966). Our present findings support this hypothesis.

However, we do not wish to emphasize the *cause* of the diuresis, since the experimental situation is an artificial one (normally a water-load induces a reduction in the level of circulating ADH, which did not occur in the present experiments). We wish to consider, primarily, the *effects* of the diuresis: although the concentrating ability of the mammalian nephron is considered generally to depend on the osmotic force for water re-absorption and the permeability characteristics of the nephron, we have shown that increases in urine flow rate in our experiments are accompanied by incomplete osmotic equilibration between papilla and urine. During the excretion of large volumes of water, the medulla remains hypertonic to the urine; as flow increases, osmotic equilibration across the collecting duct walls declines. Since tissue analyses underestimate the true papilla tip concentrations (as explained above), and it seems reasonable to assume that the error is of the same order in all four infusion groups, the disequilibrium between papilla tip and final urine is, in reality, even greater than our results suggest. Thus, it appears that the flow rate along the collecting ducts is an important determinant of equilibration between renal papillary tissue and tubular fluid.

For urea, however, there appears to be effective equilibration between papilla tissue and urine at the higher rates of flow (Fig. 4). In the rat, dog and man, excretion of urea does not require the excretion of water in addition to that obligated for the excretion of other urinary solutes (Berliner & Bennett, 1967; Kellogg & Koike, 1955). In this context, the present findings confirm those of Ullrich, Jarausch & Overbeck (1955), that urea is perfectly equilibrated between papilla and collecting duct, so that its presence in the collecting duct does not necessitate additional excretion of water.

In conclusion: it is apparent that, unless collecting duct flow is small, osmotic equilibration between the duct and the papillary interstitium will

be incomplete, even though the water permeability of the duct walls may be high. The findings emphasize that, in the rat, distal tubular water re-absorption is of great importance in the production of concentrated urine, since there must be only a small delivery of fluid from the distal tubules to the collecting ducts if the concentration process is to be effective.

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