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TIME COURSE OF CHANGES IN RENAL TISSUE AND URINARY COMPOSITION AFTER CESSATION OF CONSTANT INFUSION OF LYSINE VASOPRESSIN IN THE CONSCIOUS, HYDRATED RAT

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SUMMARY

1. The changes in urinary and renal tissue composition in conscious rats were determined for up to 2 hr following the cessation of intravenous infusion of lysine vasopressin, LVP (at 60 μu./min. 100 g body wt. for 4½ hr). A constant water load (4% body wt.) was maintained during and after lysine vasopressin infusion, by quantitative replacement of excreted water. In these circumstances, any changes in urinary and renal tissue composition are presumed to represent direct consequences of the rapid plasma and tissue clearance of lysine vasopressin.

2. Urinary flow increased and osmolality decreased, rapidly, reaching stable values characteristic of sustained water diuresis after about 60 min.

3. The steepness of the corticomedullary solute concentration gradients also decreased rapidly. Papillary Na and urea concentrations fell to values characteristic of sustained water diuresis in about 45 min.

4. The changes in medullary composition were compounded of a moderate significant increase in water content, a moderate, significant decrease in Na content, and a profound decrease in urea content.

5. In the eventual steady-state water diuresis, urinary outputs of Na and K were significantly lower, and of NH_4 significantly higher, than those observed in control experiments where LVP infusion was continued for the corresponding 2 hr.

6. It is concluded that the diuresis following the cessation of LVP infusion is due not merely to reduced nephron permeability to water but also to a rapid reduction in the osmotic force responsible for water reabsorption from the collecting duct.

INTRODUCTION

The urinary concentrating ability of the mammalian kidney depends on the water permeability characteristics of the nephron; and on the osmotic force driving water reabsorption, particularly from the collecting duct into the surrounding hypertonic medullary interstitium (see review by Berliner & Bennett, 1967).

It is accepted that physiological control of the water permeability of the distal tubule and collecting duct in the rat is effected via circulating antidiuretic hormone (ADH). It is less certain, however, whether physiological changes in medullary composition are directly and specifically controlled by ADH; e.g. it has been suggested that the changes in medullary Na content induced by differences in hydration are attributable to the associated changes in body fluid volume rather than to those in ADH levels (Valtin, 1966).

In an attempt to demonstrate that neurohypophysial hormones exert a specific influence on medullary composition, Hai & Thomas (1969) used a protocol in which lysine vasopressin (LVP) was continuously infused into conscious, diuretic rats, the water load being kept approximately constant by quantitative replacement of excreted water. Subsequently, this approach was extended in an examination of the LVP dose-dependence of the changes in urinary and tissue composition (Atherton, Green & Thomas, 1971). In this latter paper, the main conclusion was that the time-dependent and dose-dependent changes in urinary and renal tissue composition were most reasonably explained on the basis that whereas the water permeability characteristics of the nephron were rapidly sensitive to low doses of LVP, the formation of more concentrated urine at higher doses required medullary accumulation of solute (urea and Na) and exclusion of water.

The present experiments extend this protocol further. LVP infusion was stopped at a time when maximal and stable urinary and medullary osmolalities had been achieved, but the water load was still maintained essentially constant with hypotonic dextrose. Under these circumstances, it may be assumed that endogenous ADH levels are minimal, and that any subsequent changes in urinary and renal tissue composition are entirely ascribable to removal of exogenous LVP. As in the previous experiments (Hai & Thomas, 1969; Atherton, Green & Thomas, 1971), particular emphasis is placed on the time course of the changes in individual renal tissue constituents, and on the changing relation between urinary and renal papillary osmolality. A preliminary account of these experiments has been given previously (Atherton, Evans, Green & Thomas, 1971).

METHODS

Experiments were performed on conscious male albino rats (Wistar strain, weighing 250–360 g) previously maintained on a normal (21·7 % protein) diet and free access to water.

The methods of water loading (Atherton, Hai & Thomas, 1968) and LVP infusion (Atherton, Green & Thomas, 1971) have been described in detail previously. In brief:

(*a*) only rats with overnight urinary osmolalities in the range 800–1400 μosmole/g H_2O were used:

(*b*) diuresis was induced by I.V. administration of hypotonic (2·5 %) dextrose over 2 hr; subsequently, the positive water load was maintained approximately constant (at 4 % body wt.) by infusing a volume equal to the urinary water output;

(*c*) 3 hr from the start of water loading, LVP infusion was commenced (60 μu./min. 100 g body wt.) and continued (for 4½ hr) until a steady state existed in respect to the antidiuresis (Atherton, Green & Thomas, 1971; present experiments). This time is subsequently designated as 0 hr.

Seven groups of rats were killed immediately after collecting a terminal urine sample:

Group 1, at 0 hr ($n = 7$)

Group 2, at 2 hr ($n = 4$), after continued infusion of LVP; urine at 1, 2 hr.

Groups 3–7 were killed at intervals of time after stopping LVP infusion at 0 hr:

Group 3, ¼ hr ($n = 4$); urine at ¼ hr.

Group 4, ½ hr ($n = 4$); urine at ½ hr.

Group 5, ¾ hr ($n = 4$); urine at ½, ¾ hr.

Group 6, 1 hr ($n = 5$); urine at ½, ¾, 1 hr.

Group 7, 2 hr ($n = 6$); urine every ½ hr.

In most experiments, urines were collected by inducing voiding without catheterization (Atherton *et al.* 1968). The adequacy of collection was checked by the absence of residual bladder urine, immediately after death, in all experiments where collection periods were ½ hr or longer. In the ¼ hr periods in Group 3 experiments, urine was collected by aspiration from the bladder immediately after death. Any residual urine in the final ¼ hr period of Group 5 experiments was similarly aspirated and added to the corresponding voided sample.

In all experiments after stopping LVP infusion, the positive water load was maintained constant at 4 % body wt. by I.V. replacement (with 2·5 % dextrose) of the volume voided in the preceding urine collection period (Atherton, Evans, Green & Thomas, 1971).

Full details of the preparation and analysis of blood, urine and renal tissue samples, and of the calculation of tissue solute concentrations and plasma clearances, have been given in previous publications from this laboratory. The appropriate references were summarized, recently (Atherton, Green & Thomas, 1971).

Values are presented as means ± S.E. of mean. The statistical significance of differences between means was assessed by Student's *t* test; the probability that a mean difference was zero was assessed by the paired *t* test.

RESULTS

Urinary flows, osmolalities and solute outputs during the water loading and during the 4½ hr period of LVP infusion up to time 0 hr were all very similar to values obtained in similar experiments previously (Atherton, Green & Thomas, 1971). Data for 0 hr are included in Table 1.

TABLE 1. Urinary flow, osmolality and solute outputs; and plasma clearances of urea (C_{urea}), osmoles (C_{osm}) and osmotically free water (C_{H_2O}). For each group, 0 hr represents the time of achieving steady, maximal urinary and renal papillary osmolalities during LVP infusion (60 μu./min. 100 g body wt.) in water-loaded rats (i.e. 4½ hr from the start of LVP infusion) as described in Methods. For comparison of the 0 hr values, data obtained in previous experiments (Atherton, Green & Thomas, 1971) are included.

Values represent mean ± S.E. of mean.

P values represent the probability that the differences between the means at 2 hr are zero (*t* test)

		Urine									
				Output					Plasma clearance		
Time (hr)		Flow (μl./min)	Osmolality (μ-osmole/ml.)	Osmoles (μ-osmole/min)	Na (μequiv/min)	K (μequiv/min)	NH_4 (μequiv/min)	Urea (μmole/min)	C_{urea} (μl./min)	C_{osm} (μl./min)	C_{H_2O} (μl./min)
Atherton *et al.* (1971)	0	9·8 ± 2·5	1402 ± 170	11·8 ± 2·0	2·17 ± 0·57	1·02 ± 0·21	0·74 ± 0·07	4·09 ± 0·33	1256 ± 149	41·9 ± 7·3	−32·3 ± 5·1
Group 2 (n = 4)	0 LVP	12·2 ± 4·0	1426 ± 293	14·1 ± 1·1	3·51 ± 0·80	1·38 ± 0·10	0·61 ± 0·07	3·56 ± 0·37	—	—	—
	2 continued for 2 hr	11·3 ± 5·0	1550 ± 322	13·5 ± 2·4	2·70 ± 0·64	1·26 ± 0·11	0·64 ± 0·07	3·64 ± 0·60	1147 ± 268	48·9 ± 12·9	−35·9 ± 7·0
Group 7 (n = 6)	0 LVP	9·2 ± 1·3	1438 ± 173	12·2 ± 0·9	2·84 ± 0·41	0·97 ± 0·08	0·60 ± 0·10	4·13 ± 0·61	—	—	—
	2 stopped at 0 hr	171 ± 18	71 ± 5	11·9 ± 0·8	0·50 ± 0·11	0·55 ± 0·08	1·60 ± 0·12	5·20 ± 0·56	2009 ± 142	40·1 ± 2·1	+121·8 ± 18·0
	P	< 0·001	< 0·001	—	< 0·01	< 0·001	< 0·001	—	< 0·02	—	< 0·001

In the control experiments where LVP infusion was continued for a further 2 hr (Group 2), no significant changes in urinary flow and composition occurred over this period; and the plasma clearances of osmoles (C_{osm}), urea (C_{urea}) and osmotically free-water (C_{H_2O}) at 2 hr were very similar to those observed in the control animals at 0 hr (Group 1). It is concluded that an essentially steady state prevailed over this period during continued LVP infusion.

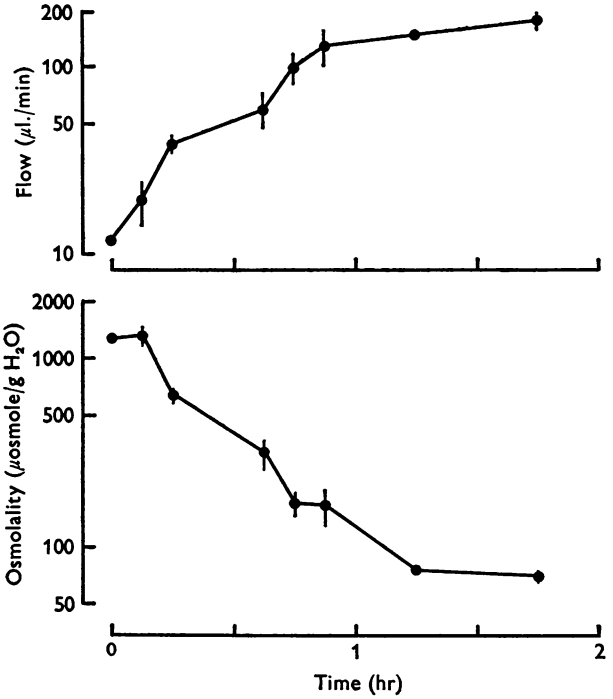

Fig. 1. Mean (± S.E.) urinary flow and osmolality after discontinuing LVP infusion at 0 hr (i.e. after 4½ hr infusion of LVP at 60 μu./min. 100 g body wt.) in water-loaded rats. Values are plotted at the mid-point of each urinary collection period. Values up to 1 hr represent pooled data from the corresponding periods in the various groups. The data are plotted on a log scale in order to make evident changes at low values.

As would be expected, rapid changes in urinary composition occurred after LVP infusion was stopped. Fig. 1 shows the patterns of change in flow and osmolality. An increase in flow was always evident in the first ¼ hr. It is presumed that the absence of a significant decrease in urinary osmolality in this period was due to the excretion of previously formed concentrated urine from the renal tract dead space. Subsequently, urinary flow continued to rise and osmolality decreased for about 1 hr (Fig. 1), even-

tually reaching values approximating those previously observed during sustained water diuresis in conscious rats (Atherton, Green & Thomas, 1971). Only small changes in urinary flow and osmolality occurred during the second hr.

Because of the renal tract dead space effect mentioned above, and because of the changing urinary flow over the first hr after stopping LVP infusion, calculations of urinary solute outputs over this period would have dubious validity. However, it is to be expected that if the only event involved in urinary concentration during this non-steady state phase is a change in the abstraction of water from the nephron, then changes in the

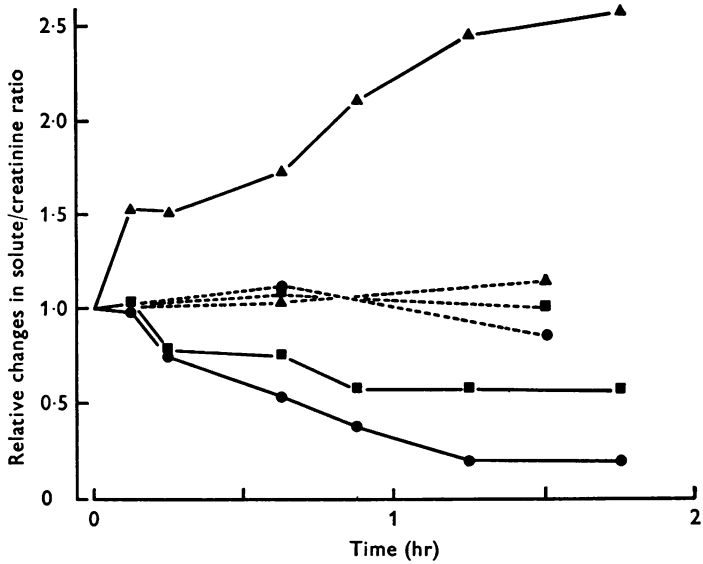

Fig. 2. Mean changes in urinary Na (●), K (■) and NH_4 (▲) concentrations, relative to those in creatinine concentrations, after discontinuing LVP infusion at 0 hr (—), and on continuing LVP infusion for a further 2 hr (----). Values are plotted at the mid-point of each collection period. For each solute, the values are calculated as:

$$\frac{\text{solute: creatinine concentration ratio in post 0 hr period}}{\text{solute: creatinine concentration ratio at 0 hr}}.$$

concentrations of the various urinary solutes, relative to those in the control periods at 0 hr, would be equal for all solutes; and that a change in the concentration of a solute relative to that of an inert, 'glomerular substance' (such as inulin or creatinine) would provide evidence of a change in nephron solute transport (Thomas, 1964). The relative changes in urinary concentrations of Na, K and NH_4 are shown in Fig. 2. Following the cessation of LVP infusion, it may be seen that, relative to creatinine, the con-

centrations of Na and K decreased and the concentration of NH_4 increased; it is inferred that the rate of excretion of Na and K fell, and that of NH_4 rose during this transitional, non-steady state phase.

Effects on solute excretion are also evident during the later periods, when the steady-state conditions prevailing in the control experiments with continued LVP infusion (Group 2, see Table 1) and in those where LVP infusion had been discontinued for 2 hr (Group 7, see Fig. 1) validates calculation of urinary solute outputs. The urinary outputs of Na and K were significantly lower, and that of NH_4 significantly higher, after stopping LVP infusion (Table 1). The mean urea output was also increased, though not significantly so. However, it is probable that this represents a real effect, since C_{urea} was significantly higher 2 hr after stopping LVP infusion (Table 1).

Tissue composition

The urinary values at 0 hr were so similar to those observed in similar circumstances, previously (Atherton, Green & Thomas, 1971), that it is presumed that renal tissue composition would be correspondingly similar. For this reason tissue analyses were not performed at 0 hr in the current series of experiments, and the data plotted at 0 hr (Group 1) in Figs. 3–5 are taken from Atherton, Green & Thomas (1971). The renal tissue composition after a further 2 hr LVP infusion (Group 2) showed only minor differences from that at 0 hr, so that, as with the urinary data, an essentially steady state prevailed over this period. For clarity, the tissue data for this group are not plotted.

After stopping LVP infusion, a rapid reduction in the steepness of the corticomedullary concentration gradient for Na occurred, with an even more profound reduction in that for urea (Fig. 3). These changes, and the consequent reduction in the calculated tissue osmolal gradient, were essentially complete at $\frac{3}{4}$ hr. The effects were most evident in the papillary tip, with only minor changes in the cortical segments. Accordingly, only papillary tip values are plotted for Figs. 4, 5 and for the 1 and 2 hr experiments (Groups 6 and 7) in Fig. 3.

Fig. 4 shows that the changes in papillary solute concentrations were attributable to a significant increase in water content, a significant decrease in Na content, and a profound decrease in urea content. Any changes in K and NH_4 contents were relatively small.

Urinary-papillary concentration differences

The changes in urea concentrations in the urine were essentially similar to those observed in the papillary tip (Fig. 3); and in the eventually steady state there was no significant difference between urinary and papillary

concentrations. The decline in Na concentration in the urine was more profound and more prolonged than that in the papillary tip, so that 2 hr after stopping LVP infusion the concentration in the urine ($2{\cdot}9 \pm 0{\cdot}5$ μ-equiv/ml.) was far lower than that in the papilla ($125{\cdot}4 \pm 4{\cdot}5$ μequiv/ml.). For the first $\frac{1}{2}$–$\frac{3}{4}$ hr after stopping LVP infusion, the steep fall in urinary osmolality was accompanied by an almost equally steep decrease in calculated papillary osmolality (Fig. 5). However, whereas the reduction in

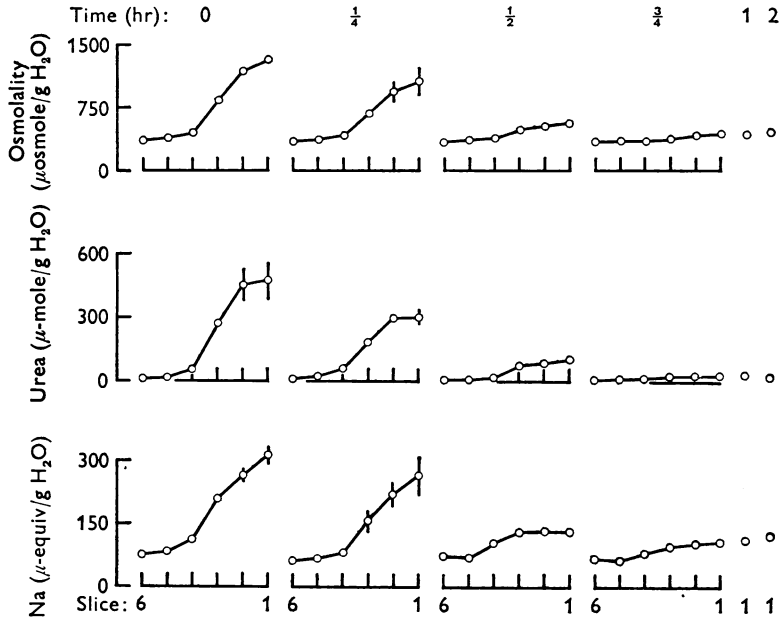

Fig. 3. Mean ($\pm$ S.E.) renal tissue osmolality (calculated) and urea and Na concentrations after discontinuing LVP infusion at 0 hr in water-loaded rats. The tissue slice numbers refer to the level of section 1, papillary tip; 2, papillary base; 3, inner medulla; 4, outer medulla; 5, inner cortex; 6, outer cortex. Since there were only minor changes in tissue composition after $\frac{3}{4}$ hr, only the papillary tip data for 1 and 2 hr are plotted.

Note. The calculated values for tissue osmolality are regarded as approximations only (see Atherton, Green & Thomas, 1971) and are presented only to indicate the probable pattern of change.

tissue osmolality was essentially complete at $\frac{3}{4}$ hr, the decline in urinary osmolality continued for a longer period. Inclusion of earlier data (from Atherton, Green & Thomas, 1971) in Fig. 5 shows that the restitution of a high papillary osmolality during infusion of LVP is much slower than the reduction in osmolality which follows cessation of LVP infusion.

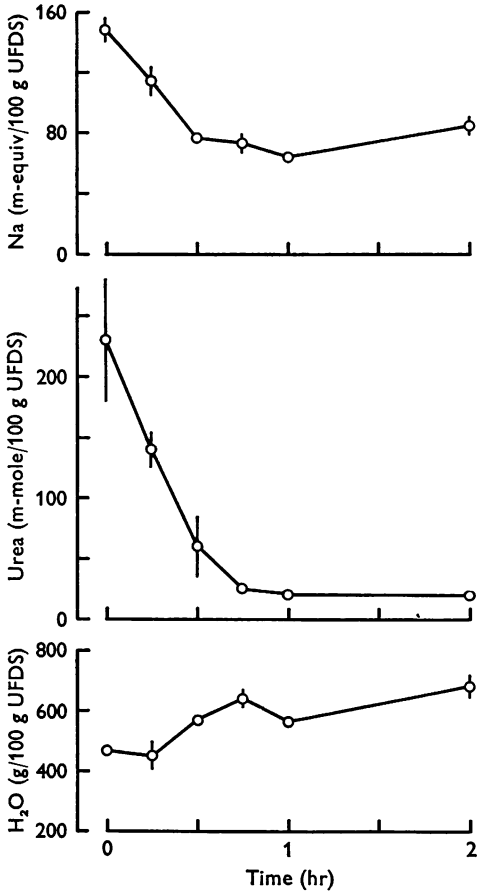

Fig. 4. Mean (± S.E.) papillary tip Na, urea and water contents after discontinuing LVP infusion at 0 hr in water-loaded rats (UFDS = urea free dry solid).

DISCUSSION

As would be predicted, the increase in water content and the decreases in solute (Na and urea) contents in the renal medullary segments after cessation of LVP infusion were the converse of the changes previously observed during continuous LVP infusion (Hai & Thomas, 1969; Atherton, Green & Thomas, 1971). The most important feature of the present results concerns the rapidity with which these changes in tissue composition occurred. Furthermore, the eventual achievement of a steady state in papillary composition actually preceded that in urinary flow and composition (cf. Figs. 1, 3 and 5).

To the extent that changes in over-all slice composition may be

considered as an approximation of changes in medullary interstitial fluid, it can be inferred that the rapid diuresis which develops as LVP is cleared from blood and tissues must depend on an equally rapid and quantitatively important reduction in medullary interstitial osmolality.

LVP-sensitive intrarenal mechanisms

There are two main alternative explanations of the effects of removal of LVP on medullary composition: first, that the changes in medullary solute concentrations are entirely secondary to changes in water-transport as induced by the effects of LVP on nephron permeability to water; secondly, that LVP has direct and specific effects on nephron solute transport, in addition to those on water permeability.

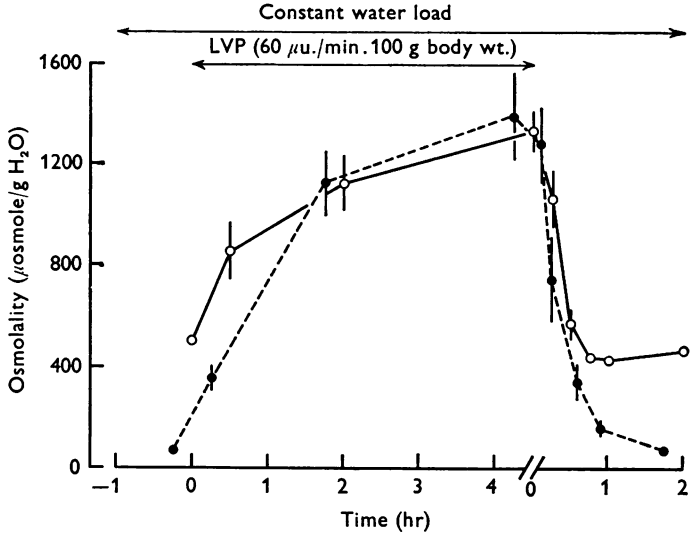

Fig. 5. Mean (± S.E.) urinary osmolality (●---●) and calculated papillary tip osmolality (○—○) in water-loaded rats during LVP infusion; and after discontinuing LVP infusion. Urinary values are plotted at the mid-point of each collection period. The data during LVP infusion are from a previous series (Atherton, Green & Thomas, 1971).

Note. The calculated values for tissue osmolality are regarded as approximations only (see Atherton, Green & Thomas, 1971) and are presented only to indicate the probable pattern of change.

The first possibility arises from the fact that during water diuresis, distal tubular permeability to water is reduced in the rat (Ullrich, Rumrich & Fuchs, 1964; Persson, 1970), though perhaps not in dog (Clapp & Robinson, 1966) or Rhesus monkey (Bennett, Brenner & Berliner, 1968); hence, the volume of water presented to the collecting duct is increased

during water diuresis. Because the volume entering the collecting duct is much larger and the rise in osmolality with water abstraction is much smaller, it has been suggested (Berliner & Bennett, 1967; Morgan, 1970) that, paradoxically, the rate of water abstraction from the collecting duct into the papillary interstitium may be higher during water diuresis despite the reduced collecting duct permeability to water. Any such increase in the influx of water would tend to wash out papillary solute into the medullary circulation.

However, this proposal – that the amount of water entering the medullary interstitium increases during water diuresis – is controversial (see Marsh, 1971). Furthermore, calculations of the amounts of water moving out of the collecting duct during water diuresis and antidiuresis (e.g. Morgan, 1970) have neglected the fact that, as is shown by the present data, a rapid and substantial reduction in medullary osmolality occurs during the generation of a water diuresis. Even if it were accepted that a paradoxical increase does occur in a water diuresis, we consider that the results of the present experiments, together with those observed during continuous LVP infusion (Hai & Thomas, 1969; Atherton, Green & Thomas, 1971), are more reasonably explained on the basis that in addition to effects on nephron permeability to water, LVP exerts direct effects on medullary composition: on solute (Na and urea) transport characteristics of the nephron (see below); perhaps on medullary blood flow (Thurau, 1964) or on the other factors influencing the efficiency of counter-current exchange (Marsh, 1971); or, perhaps, by a shift in the distribution of filtration between cortical and juxtamedullary nephrons (Horster, Schnermann & Thurau, 1969). The evidence, both direct and indirect, for this conclusion is summarized as follows:

(*a*) The relative contribution of changes in medullary water, Na and urea contents during the early, transitional phase of LVP infusion varies with time and with dose (Atherton, Green & Thomas, 1971).

(*b*) In the eventual steady state achieved during continuous LVP infusion, the dose–response characteristics of the various parameters of urinary and renal tissue composition differ (Atherton, Green & Thomas, 1971).

(*c*) During continuous LVP infusion, maximal papillary solute concentrations may be achieved before the time of maximal antidiuresis (Hai & Thomas, 1969; Atherton, Green & Thomas, 1971); and after discontinuing LVP infusion, the achievement of minimal papillary solute concentrations precedes the maximal diuresis (Fig. 5).

(*d*) After discontinuing LVP infusion, rapid and significant reductions in papillary Na and urea contents precede any significant increase in papillary water content (Fig. 4).

Collectively, these observations are difficult to explain simply as consequences of changes in nephron permeability to water. Furthermore:

(*e*) suggestions that ADH influences collecting duct permeability to urea (Jaenike, 1961; Thomas, 1964) have been confirmed, subsequently, by micropuncture (Morgan, Sakai & Berliner, 1968; Morgan & Berliner, 1968), and other (Bowman & Foulkes, 1970) experiments; and

(*f*) LVP infusion causes increased Na and K, and decreased NH_4, excretion (Atherton, Green & Thomas, 1971); conversely, after stopping LVP infusion, decreased Na and K, and increased NH_4, outputs occur (present experiments). If the conventional view is correct, that ADH has little influence on glomerular filtration rate or on the proximal tubular handling of salt and water, then these findings indicate effects of LVP on ion transport in more distal segments of the nephron. We have discussed (Atherton, Hai & Thomas, 1969; Atherton, Green & Thomas, 1971) previously, possible explanations of such effects, and of the paradoxical observation that LVP causes increased Na excretion at a time when Na is accumulating in the renal medulla. Irrespective of the mechanisms involved, these observations support earlier suggestions (e.g. Morel, 1964) that ADH influences the tubular handling of Na. Additional support is provided by recent reports that ADH increases collecting duct permeability to Na in the rat (Ullrich, Baldamus, Uhlich & Rumrich, 1969) and stimulates active Na transport in the isolated rabbit cortical collecting tubule (Helman, Grantham & Burg, 1971).

In summary, the present experiments show that the diuresis associated with plasma and tissue clearance of LVP is attributable not merely to a decrease in nephron permeability to water but also to a rapid decrease in medullary solute concentrations. The results support our previous conclusion (Atherton, Green & Thomas, 1971) that the effects of vasopressin on urinary osmolality may involve rapid adjustments in the medullary osmotic reabsorptive force independent of changes in nephron permeability to water.

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REFERENCES

ATHERTON, J. C., EVANS, JEANNE A., GREEN, R. & THOMAS, S. (1971). The rapid effects of lysine-vasopressin clearance on renal tissue composition in the conscious, water-diuretic rat. *J. Physiol.* **215**, 42–43*P*.

ATHERTON, J. C., GREEN, R. & THOMAS, S. (1971). Influence of lysine-vasopressin dosage on the time course of changes in renal tissue and urinary composition in the conscious rat. *J. Physiol.* **213**, 291–309.

ATHERTON, J. C., HAI, M. A. & THOMAS, S. (1968). Effects of water diuresis and osmotic (mannitol) diuresis on urinary solute excretion by the conscious rat. *J. Physiol.* **197**, 395–410.

ATHERTON, J. C., HAI, M. A. & THOMAS, S. (1969). Acute effects of lysine-vasopressin injection (single and continuous) on urinary composition in the conscious water diuretic rat. *Pflügers Arch. ges. Physiol.* **310**, 281–296.

BENNETT, C. M., BRENNER, B. M. & BERLINER, R. W. (1968). Micropuncture study of nephron function in the Rhesus monkey. *J. clin. Invest.* **47**, 203–216.

BERLINER, R. W. & BENNETT, C. M. (1967). Concentration of urine in the mammalian kidney. *Am. J. Med.* **42**, 777–789.

BOWMAN, FAYE J. & FOULKES, E. C. (1970). Antidiuretic hormone and urea permeability of collecting ducts. *Am. J. Physiol.* **218**, 231–233.

CLAPP, J. R. & ROBINSON, R. R. (1966). Osmolality of distal tubular fluid in the dog. *J. clin. Invest.* **45**, 1847–1853.

HAI, M. A. & THOMAS, S. (1969). The time course of changes in renal tissue composition during lysine-vasopressin infusion in the rat. *Pflügers Arch. ges. Physiol.* **310**, 297–319.

HORSTER, M., SCHNERMANN, J. & THURAU, K. (1969). Die Funktion der juxtamedullären nephrone in wasserdiurese und ADH-induzierter antidiurese. In *Symp. ges. Nephrol.*, 4th, Vienna, 1968. Vienna: Verlag Wien. Med Akad.

HELMAN, S. I., GRANTHAM, J. J. & BURG, M. B. (1971). Effect of vasopressin on electrical resistance of renal cortical collecting tubules. *Am. J. Physiol.* **220**, 1825–1832.

JAENIKE, J. R. (1961). The influence of vasopressin on the permeability of the mammalian collecting duct to urea. *J. clin. Invest.* **40**, 144–151.

MARSH, D. J. (1971). Osmotic concentration and dilution of the urine. In *The Kidney*, vol. III, pp. 71–126, ed. ROUILLER, C. & MULLER, A. F. New York and London: Academic Press.

MOREL, F. (1964). Action of neurohypophyseal hormones on the active transport of sodium. In *West European Symposia on Clinical Chemistry*, vol. 3, Water and Electrolyte Metabolism II, ed. DE GRAEFF, J. & LEIJNSE, B. Amsterdam: Elsevier.

MORGAN, T. (1970). Movement of solute and water in the counter-current system of the medulla. In *Proceedings of the Fourth International Congress of Nephrology*, 1969, vol. I, ed. ALWALL, N., BERGLUND, F. & JOSEPHSON, B. Basel; Munich; New York: Karger.

MORGAN, T. & BERLINER, R. W. (1968). Permeability of the loop of Henle, vasa recta and collecting duct to water, urea and sodium. *Am. J. Physiol.* **215**, 108–115.

MORGAN, T., SAKAI, F. & BERLINER, R. W. (1968). *In vitro* permeability of medullary collecting ducts to water and urea. *Am. J. Physiol.* **214**, 574–581.

PERSSON, E. (1970). Water permeability in rat distal tubules. *Acta physiol. scand.* **78**, 364–375.

THOMAS, S. (1964). Solute excretion in man during changing urine flow occurring spontaneously and induced by vasopressin injection. *J. clin. Invest.* **43**, 1–10.

THURAU, K. (1964). Renal hemodynamics. *Am. J. Med.* **36**, 698–719.

ULLRICH, K. J., BALDAMUS, C. A., UHLICH, E. & RUMRICH, G. (1969). Einfluss von Calciumionen und antidiuretischen Hormon auf den transtubulären Natriumtransport in der Ratteniere. *Pflügers Arch. ges. Physiol.* **310**, 369–376.

ULLRICH, K. J., RUMRICH, G. & FUCHS, G. (1964). Wasserpermeabilitat und transtubulärer Wasserfluss corticaler Nephronabschnitte bei verschiedenen Diurresezustanden. *Pflügers Arch. ges. Physiol.* **280**, 99–119.

VALTIN, H. (1966). Sequestration of urea and non-urea solutes in renal tissues of rats with hereditary hypothalamic diabetes insipidus: effect of vasopressin and dehydration on the countercurrent mechanism. *J. clin. Invest.* **45**, 337–345.