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EFFECTS OF 0·9% SALINE INFUSION ON URINARY AND RENAL TISSUE COMPOSITION IN THE HYDROPAENIC, NORMAL AND HYDRATED CONSCIOUS RAT

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SUMMARY

1. Changes in water and solute outputs of hydropaenic, normal and hydrated conscious rats were determined during intravenous infusion (0·2 ml./min) of isotonic (0·9%) saline for 4 hr; renal tissue composition was determined before, and after 1 or 2 hr, infusion.

2. In normal and hydrated rats increased excretion of water and sodium was such that urinary output matched intravenous input from about 2 hr. In hydropaenic rats, the diuretic and natriuretic response was much reduced; a retention of infused saline, equivalent to 15% body weight, occurred over 4 hr.

3. A considerable increase in urea output and clearance, and a smaller increase in potassium and ammonium outputs, occurred in all groups.

4. The corticomedullary osmolal gradients characteristic of non-diuretic rats were largely dissipated during saline infusion: by 1 hr in normal and hydrated rats, and by 2 hr in the hydropaenic group.

5. These changes were ascribable mainly to an increase in tissue water content in all segments, particularly in the hydropaenic group; and to a profound decrease in urea content in all groups.

6. Changes in tissue sodium content were smaller, and differed between segments and between the differently hydrated groups. A decrease in papillary content occurred in hydropaenic and normal groups and an increase in cortical and outer medullary content occurred in all groups.

7. After 2 hr saline infusion, incomplete papillary–urinary osmotic equilibration was evident in all groups.

8. These changes in medullary osmolality and in papillary–urinary osmotic equilibration preceded the maximal diuresis, and must contribute to the diuresis induced by saline infusion, as in water and osmotic diureses.

INTRODUCTION

An increased flow of urine may be produced by several procedures. Administration of water or hypotonic solutions causes an increased flow of hypotonic urine, with little change in solute excretion (water diuresis). Administration of osmotically active solute, e.g. mannitol, leads to an increased flow of slightly hypertonic urine containing large quantities of the solute (osmotic diuresis). In several species, including the dog (see Cushny, 1917; Wesson, 1957), administration of isotonic saline usually provokes increased excretion of both water and salt, i.e. a saline diuresis. The response to saline loading in man is more variable; orally ingested saline is usually excreted slowly (Adolph, 1923), but depending on a variety of circumstances, e.g. prehydration (Ladd, 1951), posture and time of day (Blomhert, Gerbrandy, Molhuysen, de Vries & Borst, 1951), saline administration in man may provoke a water diuresis or an increased excretion of both water and salt (see review by Smith, 1957). In the rat, the fewer observations indicate that, as in the dog, saline loading normally induces a rapid saline diuresis with water and salt excretion matching the rate of administration (e.g. Kellogg, Burack & Isselbacher, 1954).

The mechanisms responsible for this saline diuresis have previously been considered, almost exclusively, in terms of the concomitant increase in filtered sodium load (Wesson, 1957), and of changes in the flow and composition of intratubular fluid in various nephron segments due to changes in sodium transport. In this respect, it has been clearly demonstrated (de Wardener, Mills, Clapham & Hayter, 1961) that during saline infusion, decreased tubular reabsorption of sodium may occur independent of any changes in the release of adrenal corticosteroids and vasopressin (A.D.H.) and despite renal denervation.

However, little attention has been directed at the possible participation of changes in renal medullary function during saline diuresis. According to currently accepted views, the ultimate step in the formation of concentrated urine by the mammalian kidney is the passive abstraction of water from the collecting duct into the hypertonic medullary interstitium; medullary hypertonicity is generated and sustained by countercurrent mechanisms (see recent reviews by Morel, 1967; and Berliner & Bennett, 1967). The quantity of water reabsorbed from the collecting duct is influenced by the osmotic difference between the medulla and collecting duct; and by factors affecting the extent of medullary–urinary osmotic equilibration. Conversely, increased urine flow would be expected to result both from dissipation of the corticomedullary osmolal gradient and from incomplete medullary–urinary osmotic equilibration. The sequential changes in medullary composition observed during the generation of water diuresis

and osmotic diuresis in the rat have been described previously (Atherton, Hai & Thomas, 1968*b*, *c*).

The main purpose of the present work was to determine whether changes in renal medullary composition and in the extent of medullary–urinary osmotic equilibration occur during isotonic saline loading in the rat. Since the changes in renal tissue composition during water diuresis are influenced by the antecedent hydration status (Hai & Thomas, 1969*b*) the existence of a similar effect during saline loading was also investigated.

METHODS

Male albino rats (Wistar strain) weighing 192–343 g were maintained on a rat cake diet (21·7 % protein) and allowed free access to water until required. Some rats were then deprived of food and water for up to 48 hr. In others, food was withheld for 24 hr, but free access to water was continued. For these final 24–48 hr, each rat was kept in a fine-meshed wire cage placed over a funnel, allowing collection under toluene of an overnight urine sample uncontaminated with faeces. On the basis of the measured osmolality of the overnight urine, the rats were classified in three groups: over 2300, 800–1600 and below 800 μ-osmole/g H_2O. For convenience, these groups are subsequently referred to as hydropaenic, normal and hydrated, respectively. Two series of experiments were performed.

In Series 1, the conscious rats were restrained, comfortably, in a Perspex cage after inserting a nylon cannula into a tail-vein under light ether anaesthesia. Saline (0·9 % w/v) was then infused (0·2 ml./min) for 4 hr. In normal (four rats) and hydrated (four rats) groups, the rats were induced to void urine at 1 hr (as described by Atherton, Hai & Thomas, 1968*a*), and in the hydropaenic (four rats) group at 1½ hr, from the start; thereafter, urine collections were made every 30 min in all groups. Immediately after the final urine sample, the rat was anaesthetized with ether, and a blood sample obtained by cardiac puncture.

In Series 2, the rats were also used for renal tissue analyses. Of each group, some were sacrificed at once (five hydropaenic, five normal and five hydrated rats). The others were prepared for infusion as for Series 1; 0·9 % saline was infused (0·2 ml./min) via the tail-vein cannula for either 1 hr (five hydropaenic, five normal and five hydrated rats) or for 2 hr (five hydropaenic, five normal and five hydrated rats). Serial urine samples were collected every 30 or 60 min as in Series 1. Immediately after collecting the terminal urine sample, each rat was rapidly anaesthetized with ether. The kidneys were removed as rapidly as possible and immediately frozen in a dry ice–acetone mixture. A blood sample was obtained by cardiac puncture.

A detailed description of the preparation of plasma and of kidney slices has been given previously (Atherton *et al.* 1968*b*). The kidney slices (two each from the cortex, medulla and papilla of both kidneys) were weighed in gold-foil containers on a Cahn Gram Electrobalance (Atherton, Green & Hai, 1969).

Analyses. Sodium and potassium were determined by flame photometry (EEL, Model 150); urea and ammonium by a modification of the method of Fawcett & Scott (1960); creatinine by the alkaline picrate method of Bonsnes & Taussky (1945); plasma and urine osmolality by cryoscopy (Advanced Osmometer, Model 31 LAS); and tissue osmolality by calculation as 2 $(Na^+ + K^+ + NH_4^+)$ + urea. The validity of this calculation has been discussed, briefly, previously (Atherton *et al.* 1968*c*); to the extent that it does not include the small contribution made by other osmotically active solutes (e.g. amino acids) the method may slightly underestimate tissue

osmolality as determined more directly (Robinson, Owen & Schmidt-Nielsen, 1966). The method will be further discussed later.

Calculations. Collection of terminal blood samples in both series (at 0, 1 and 2 hr in Series 2 and at 4 hr in Series 1) allows calculation of osmolal and urea clearances in hydropaenic, normal and hydrated rats. Plasma clearances were calculated as UV/P, where

U = urinary concentration,
V = urine flow rate, and
P = concentration in terminal plasma sample.

Tissue water and solute contents are expressed with reference to the weight of urea-free dry solid, UFDS (Saikia, 1965; Atherton *et al.* 1968*b*).

RESULTS

The overnight urinary flows and solute outputs in the hydropaenic, normal and hydrated rats in Series 1 were closely similar to those in the corresponding groups in Series 2; accordingly, the values presented in Table 1 represent the pooled data for both series. The values for normal rats are similar to those of a large series presented previously (Atherton *et al.* 1968*a*).

In the non-infused animals, osmolal outputs (Table 1) and clearances (Table 2) were related to urinary flow, the lowest values occurring in hydropaenia. These differences between the three groups were attributable, mainly, to corresponding differences in urea outputs (Table 1) and clearances (Table 2). Since these results are similar to those presented and discussed elsewhere (Hai & Thomas, 1969*b*), they will not be considered further.

The changes in urinary flow and composition induced by saline administration in the rats used for urine collections only (Series 1) are summarized in Figs. 1 to 3. The corresponding urinary data for the Series 2 experiments were closely similar and so are not given in detail; osmolal and urea urine/plasma concentration ratios and clearances for this latter series are presented in Table 2.

Saline infusion caused a decrease in urinary osmolality and an increase in flow in each group (Fig. 1). Since the increment in flow was greater, relatively, than the decrease in osmolality, a considerable increase in urinary osmolal output occurred (Fig. 1), due mainly to a marked increase in sodium output (Fig. 2). Smaller, but statistically significant, increases in the outputs of potassium (Fig. 2), ammonium (Fig. 2), urea (Fig. 3) and creatinine (Fig. 3) also occurred. For some urinary constituents, the changes in concentration and output were influenced by the antecedent hydration status.

TABLE 1. Mean (± S.D.) overnight urinary solute concentrations and outputs in hydropaenic, normal and hydrated rats. The values in parentheses are the ranges. For each group, the values are pooled data from experiments used for urine sampling only (Series 1), and from experiments used for collection of plasma and renal tissue samples (Series 2)

	Concentration		Output				
	Osmolal (μ-osmole/g H_2O)	Urea (μ-mole/ml.)	Osmolal (μ-osmole/min)	Urea (μ-mole/min)	Sodium (μ-equiv/min)	Potassium (μ-equiv/min)	Ammonium (μ-equiv/min)
Hydropaenic	2701 ± 300	1580 ± 66	4·8 ± 1·3	2·8 ± 0·8	0·20 ± 0·11	0·40 ± 0·12	0·33 ± 0·14
n = 21	(2316–3240)	(1277–1898)	(2·4–7·0)	(1·3–4·0)	(0·07–0·45)	(0·22–0·73)	(0·14–0·71)
Normal	1156 ± 194	591 ± 138	8·9 ± 1·8	4·5 ± 1·0	0·41 ± 0·18	1·38 ± 0·38	0·47 ± 0·14
n = 21	(877–1542)	(361–987)	(5·9–13·2)	(3·3–7·2)	(0·19–0·72)	(0·87–2·23)	(0·27–0·93)
Hydrated	592 ± 117	292 ± 87	10·0 ± 3·0	4·9 ± 1·6	0·35 ± 0·16	1·70 ± 0·67	0·52 ± 0·23
n = 20	(363–756)	(167–496)	(4·2–14·4)	(1·8–7·1)	(0·12–0·63)	(0·56–2·68)	(0·17–0·99)

TABLE 2. Mean (± S.E.) plasma concentrations, urine/plasma (U/P) concentration ratios and clearances (C) of urea and osmoles before and during intravenous infusion of isotonic saline (for 1, 2 and 4 hr) into hydropaenic, normal and hydrated rats (Series 2)

	Plasma concn.			U/P		Plasma clearance	
	Sodium (μ-equiv/ml.)	Osmolal (μ-osmole/g H_2O)	Urea (μ-mole/ml.)	Osmolal	Urea	C_{osm} (μl./min)	C_{urea} (μl./min)
Hydropaenic							
Non-infused ($n = 4$)	146 ± 4	323 ± 2	7·4 ± 0·5	8·12 ± 0·41	203·5 ± 10·7	14·4 ± 2·0	370 ± 63
Saline, 1 hr ($n = 5$)	148 ± 3	328 ± 3	6·8 ± 0·2	4·42 ± 0·05	110·8 ± 13·1	21·7 ± 3·0	549 ± 93
Saline, 2 hr ($n = 5$)	138 ± 3	327 ± 4	5·6 ± 0·3	1·47 ± 0·28	27·9 ± 7·7	78·0 ± 14·5	1349 ± 265
Saline, 4 hr ($n = 4$)	138 ± 1	333 ± 7	4·8 ± 0·6	1·55 ± 0·16	15·4 ± 1·9	157·5 ± 7·5	1560 ± 149
Normal							
Non-infused ($n = 5$)	149 ± 7	323 ± 2	6·2 ± 0·2	4·32 ± 0·22	124·2 ± 12·0	22·5 ± 1·2	638 ± 49
Saline, 1 hr ($n = 5$)	149 ± 1	318 ± 4	5·3 ± 0·6	1·79 ± 0·42	44·3 ± 10·8	29·6 ± 4·3	771 ± 175
Saline, 2 hr ($n = 5$)	146 ± 4	319 ± 3	4·7 ± 0·5	1·10 ± 0·03	15·4 ± 1·4	187·6 ± 26·8	2505 ± 148
Saline, 4 hr ($n = 4$)	139 ± 1	314 ± 2	3·0 ± 0·4	1·10 ± 0·03	11·7 ± 0·8	210·0 ± 14·7	2245 ± 245
Hydrated							
Non-infused ($n = 5$)	142 ± 7	314 ± 5	5·9 ± 0·7	1·81 ± 0·21	52·3 ± 9·9	32·8 ± 2·4	929 ± 145
Saline, 1 hr ($n = 5$)	146 ± 5	318 ± 4	5·3 ± 0·6	0·91 ± 0·14	24·4 ± 3·9	34·0 ± 7·2	944 ± 239
Saline, 2 hr ($n = 5$)	153 ± 2	310 ± 6	4·8 ± 0·3	1·03 ± 0·06	14·8 ± 4·1	153·0 ± 26·9	1856 ± 224
Saline, 4 hr ($n = 4$)	139 ± 1	317 ± 2	2·6 ± 0·3	1·12 ± 0·05	12·1 ± 1·5	195·0 ± 6·5	2078 ± 237

Osmolality

A consistent finding in the normal and hydrated rats was that at some stage during the first 2 hr of saline infusion, the fall in urine osmolality resulted in the production, transiently, of hypotonic urine. The extent and consistency of this hypotonicity is not apparent from inspection of the mean values (Fig. 1; Table 2) because of variability in the time of onset and

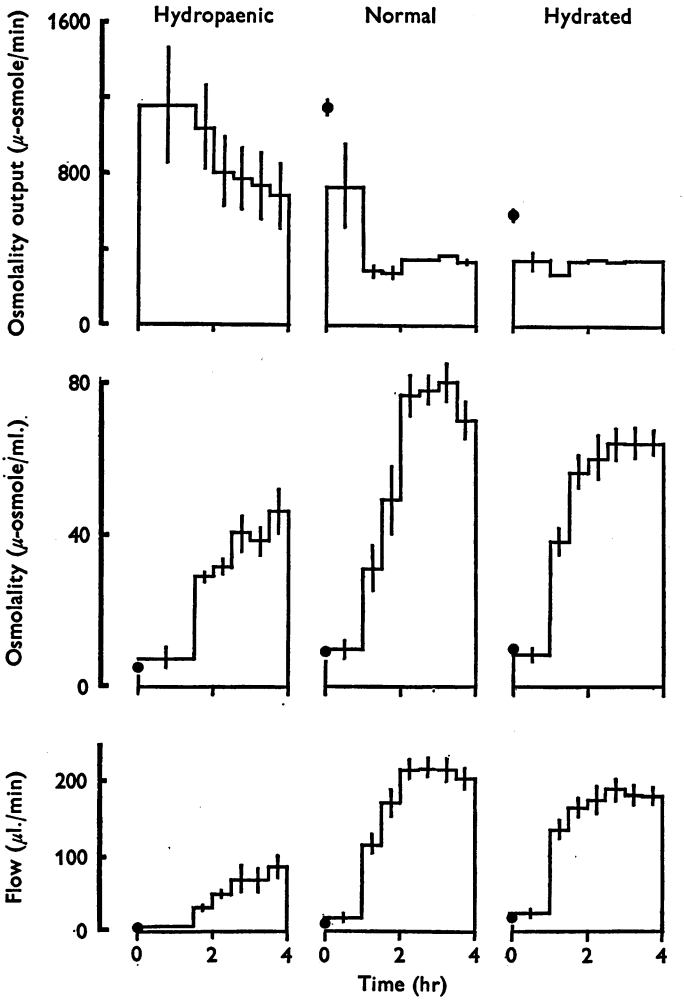

Fig. 1. Mean (± S.E.) urinary osmolality, osmolal output and flow during intravenous infusion (0·2 ml./min) of 0·9 % saline in hydropaenic, normal and hydrated rats. Values before infusion (●) are included, where convenient.

in the duration; in fact, hypotonic urine (range 135–309 μ-osmole/ml.) was formed, transiently, for at least one 30 min period in every experiment of both Series 1 and Series 2. From 2 hr onwards, urinary osmolalities stabilized at slightly hypertonic values in both the normal and hydrated groups. In the hydropaenic group, saline infusion also caused a fall in urinary osmolality, but the urine remained hypertonic even after 4 hr (Fig. 1; Table 2).

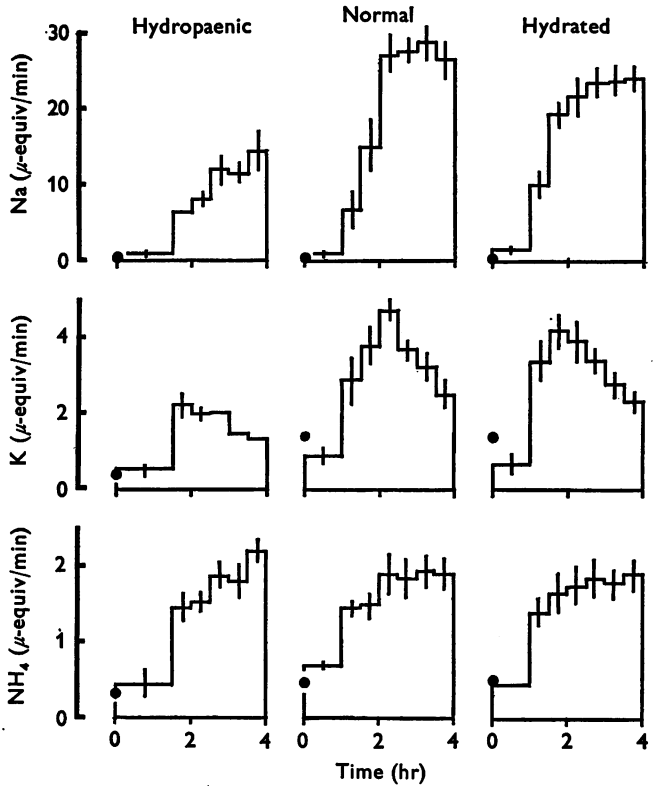

Fig. 2. Mean (± S.E.) urinary sodium, potassium and ammonium outputs during intravenous infusion (0·2 ml./min) of 0·9 % saline in hydropaenic, normal and hydrated rats. Values before infusion (●) are included.

Urine flow: sodium

A considerable increase in urine flow occurred in the normal and hydrated animals (Fig. 1). After about 2 hr from the start of saline infusion, urinary flows were essentially similar to the rate of infusion (200 μl./min), and sodium concentrations in the urine and infusate were also similar; thus, urinary sodium output (Fig. 2) in these two groups matched the intravenous input from this time. In striking contrast, urine flow in-

creased more slowly in the hydropaenic group and outputs of water (Fig. 1) and sodium (Fig. 2) remained lower than inputs even after 4 hr saline infusion. Consequently, of the 48 ml. infused over the 4 hr, hydropaenic rats excreted less than 10 ml. water, representing a fluid retention equivalent to approximately 15% of body weight.

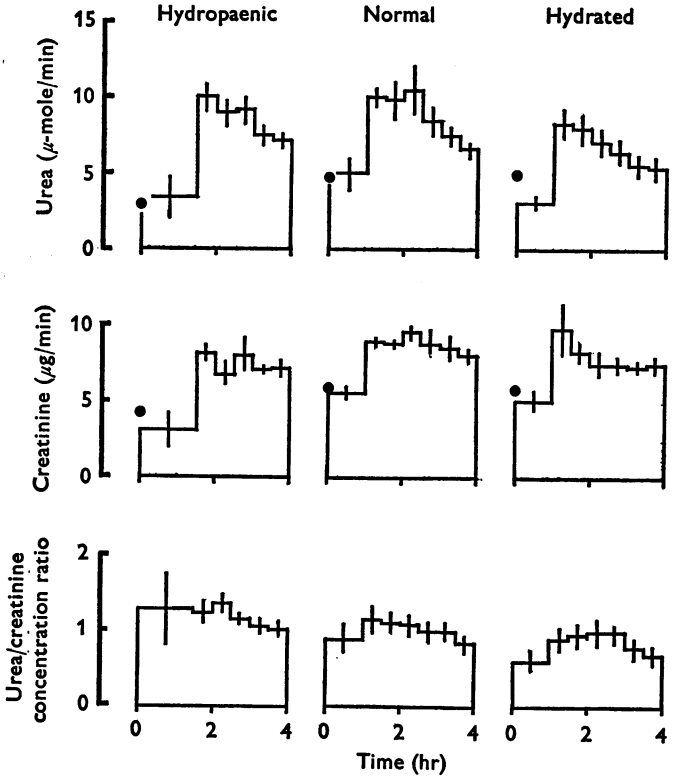

Fig. 3. Mean (± S.E.) urinary urea and creatinine outputs and urinary urea/creatinine concentration ratio during intravenous infusion (0·2 ml./min) of 0·9% saline in hydropaenic, normal and hydrated rats. Where possible, values before infusion (●) are included.

Urea

In all three groups, urinary urea outputs increased abruptly with the onset of diuresis to reach maximal values usually before the achievement of peak flows; subsequently urea outputs tended to decline but remained above pre-infusion values (Fig. 3). Urea excretion in the hydropaenic rats was initially lower than in the other groups (Table 1) but increased more, so that during saline infusion urea outputs became similar in all three groups (Fig. 3).

The initial increase in urea output was accompanied by an increase in urea clearance (Table 2). When, subsequently, urea output tended to decline, plasma concentration also decreased (Table 2) so that urea clearance remained high. Although a decline in plasma urea concentration has been observed in non-infused rats over a similar period (Atherton *et al.* 1968*a*), the greater falls observed here, during saline infusion, are presumed to result from the considerable increases in urea outputs and clearances. Since plasma urea concentrations in the hydropaenic rats were higher than in the other groups, both before and during saline infusion, urea clearances were significantly smaller (Table 2) in this group.

Potassium and ammonium

As mentioned above potassium and ammonium outputs increased, significantly, during saline infusion in all three groups (Fig. 2). The increased outputs of ammonium were sustained, and were similar in all groups. The increment in potassium excretion in the hydropaenic group was smaller than in the other two groups; after about 2 hr potassium outputs decreased but remained well above those before infusion.

Creatinine

Creatinine outputs increased in all three groups (Fig. 3). Plasma creatinine concentration was not measured, but was unlikely to be increased during saline infusion; in view of the retention of some of the infused saline in all groups, plasma creatinine concentration seems more likely to have decreased. Assuming that endogenous creatinine clearance gives an approximate estimate of glomerular filtration rate (G.F.R.), the inferred increase in clearance is compatible with the observations of others (see discussion) that isotonic saline infusion causes an increase in G.F.R.

Renal tissue composition

The extensive data concerning tissue composition are summarized in Figs. 4 to 10. In the three non-infused groups, the tissue composition was very similar to that in each of the corresponding groups in experiments of Hai & Thomas (1969*b*); in brief, the steeper corticomedullary osmolal gradient in hydropaenia (Fig. 4) was attributable, mainly, to higher medullary contents of sodium (Fig. 7) and urea (Fig. 8); tissue water contents in hydropaenia were almost identical with those in the normal group (Fig. 5), but in hydropaenia, the papillary water content was significantly ($P < 0{\cdot}05$) lower than in the hydrated group.

Saline infusion caused marked changes in renal tissue composition. As with the changes in urinary composition, the effect of infusion on tissue composition was influenced by the prehydration status.

Osmolality

In all three groups, the steepness of the corticomedullary osmolal gradient was reduced by saline infusion (Fig. 4) to an extent which resulted in the abolition of any differences between the normal and hydrated groups by 1 hr; no further changes in osmolality occurred in these latter groups. Despite the reduction in osmolal gradient in the hydropaenic group, the medullary values remained significantly above those in the other two groups at 1 hr; but after 2 hr infusion, tissue osmolality in the hydropaenic animals had further declined and the values in all three groups were very similar (Fig. 4).

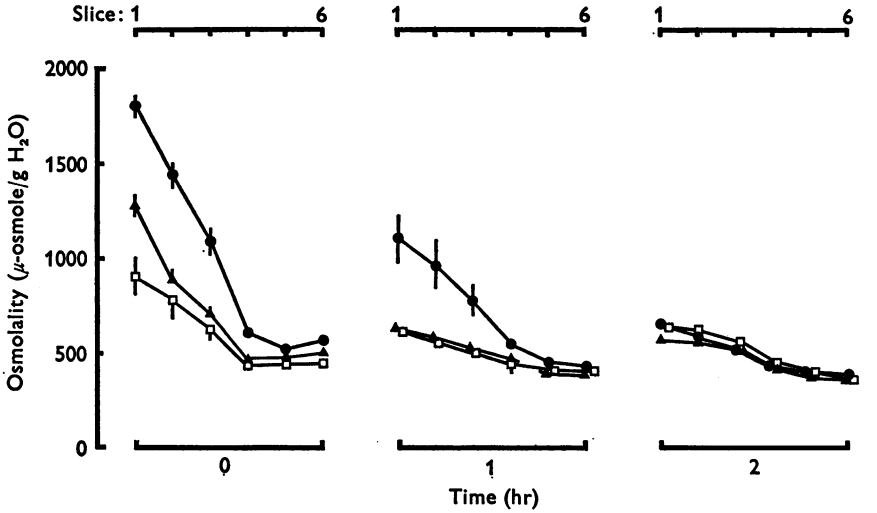

Fig. 4. Osmolal concentration in renal tissue sections from hydropaenic (●), normal (▲) and hydrated (□) rats, before (0 hr) and after intravenous infusion (0·2 ml./min) of 0·9 % saline for 1 hr and 2 hr. Values represent means; vertical bars, ± S.E. In this and subsequent Figures, the tissue slice numbers refer to the level of section 1, papilla tip; 2, papilla base; 3, inner medulla; 4, outer medulla; 5, inner cortex; 6, outer cortex.

Changes in the osmolality of cortical segments were much smaller, relatively; but by 2 hr cortical osmolal concentrations were also reduced in each group (Fig. 4). The possible significance of these changes in cortical osmolality and of the observation that the cortex appeared to be hypertonic to plasma will be discussed below.

Osmolal and water contents

In all three groups, the changes in tissue osmolality were compounded of decreases in osmolal content and increases in water content (Fig. 5). As

with the changes in osmolality, differences in osmolal content between the normal and hydrated groups were abolished by 1 hr, with little further change; and the significantly higher medullary values in the hydropaenic group at 1 hr were further reduced on continued infusion so that any differences between the three groups at 2 hr were small and usually non-significant. Cortical osmolal contents were almost unchanged throughout.

In general, the changes in tissue water content were the converse of those in osmolal content (Fig. 5). In all three groups a significant increase in water content occurred in all segments, including the cortex, during the

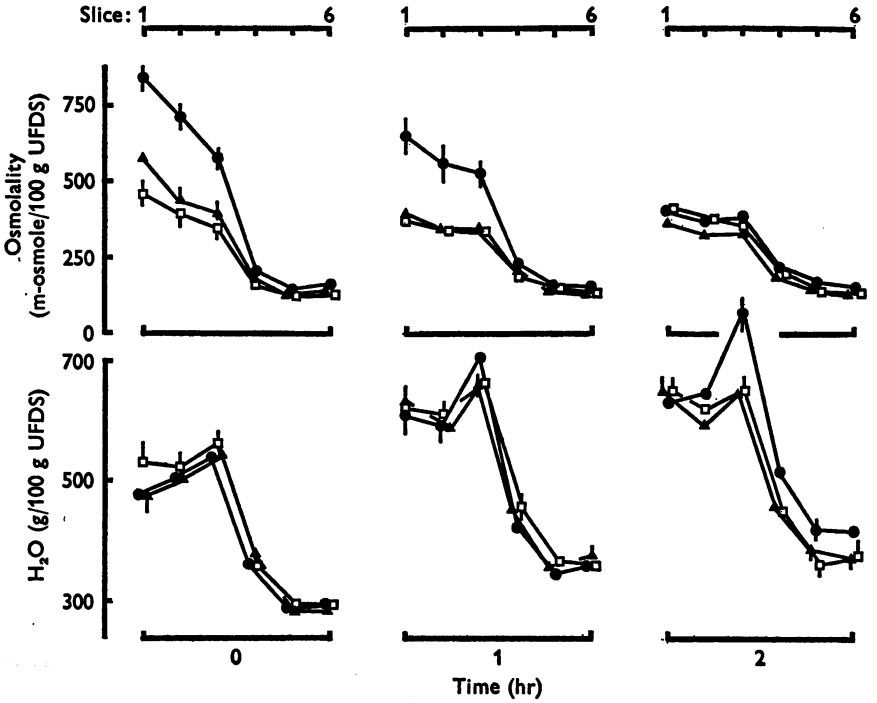

Fig. 5. Osmolal and water contents in renal tissue sections from hydropaenic (●), normal (▲) and hydrated (□) rats, before (0 hr) and after intravenous infusion (0·2 ml./min) of 0·9% saline for 1 hr and 2 hr. UFDS = urea-free dry solid. Further details as for Fig. 4.

first hour of infusion such that pre-infusion differences were largely obliterated. However, whilst there was little further change in the normal and hydrated groups, in hydropaenic rats there occurred a further substantial increase in the water content of all segments other than the papillary tip. This pattern is also evident on consideration of changes in percentage water composition (Fig. 6). In the hydropaenic group, these

increases in water content were particularly large in the inner medulla (Figs. 5 and 6); whether expressed with reference to dry solid content (Fig. 5) or as percentage composition (Fig. 6), inner medullary water content at 2 hr was far higher than has been found in any other condition in this laboratory.

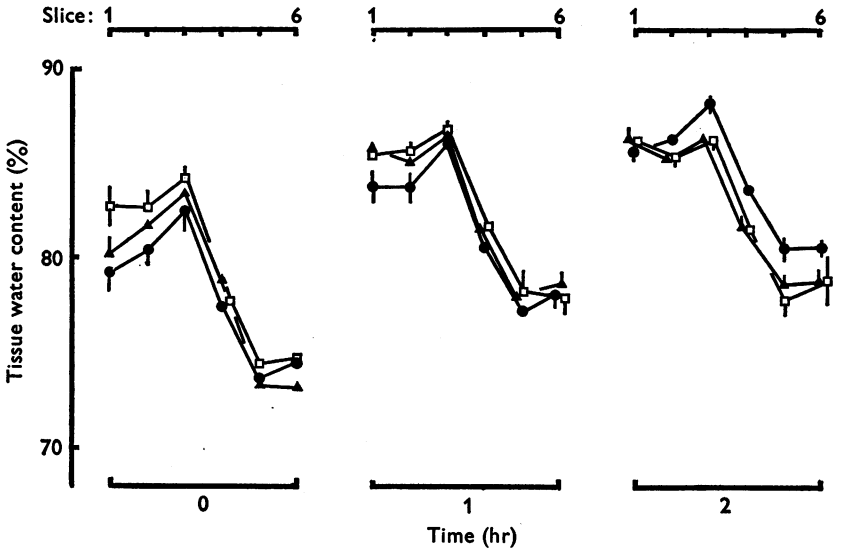

Fig. 6. Percentage water content in renal tissue sections from hydropaenic (●), normal (▲) and hydrated (□) rats, before (0 hr) and after intravenous infusion (0·2 ml./min) of 0·9 % saline for 1 hr and 2 hr. Further details as for Fig. 4.

Sodium

Changes in tissue sodium concentration and content are presented in Fig. 7. In the hydropaenic group, a progressive decrease in sodium concentration and content occurred in the papillary segments. Much of the decrease in concentration in other segments, however, was attributable to the increased water content described above, since apart from the progressive reduction in the papilla, changes in sodium content were small and often non-significant. Similarly, apart from initial decrease in papillary values, changes in sodium concentration and content in the normal and hydrated groups were usually small throughout saline infusion. In the hydrated group, a small increase in sodium concentration and content in the papillary and inner medullary segments occurred during the second hour of saline infusion (Fig. 4). Small, but usually significant, increases in the sodium content of cortical and outer medullary segments occurred in all groups.

Urea

The reductions in medullary urea concentrations and contents (Fig. 8) were relatively much greater than for any other solute. As with sodium and total osmoles, differences between the normal and hydrated groups were largely abolished by 1 hr with little further change. However, in the hydropaenic group, decreases in concentration and content continued up to 2 hr.

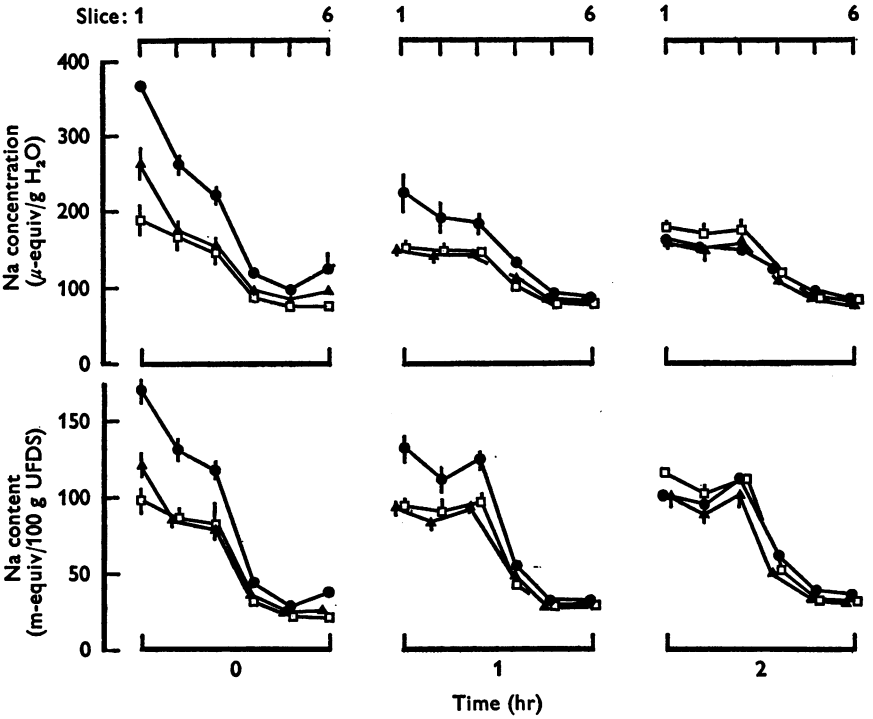

Fig. 7. Sodium concentration and content in renal tissue sections from hydropaenic (●), normal (▲) and hydrated (□) rats, before (0 hr) and after intravenous infusion (0·2 ml./min) of 0·9 % saline for 1 hr and 2 hr. Further details as for Figs. 4 and 5.

Potassium and ammonium

As in water diuresis (Atherton *et al.* 1968*c*) and mannitol diuresis (Atherton *et al.* 1968*b*), the profile of tissue concentration and content for both potassium and ammonium is largely attributable to the differences in water content between adjacent segments. Similarly, the changes induced by saline infusion were mainly due to the changes in water content described above. Examples of the differences between adjacent segments (for 2 hr saline infusion) are shown in Fig. 9.

Papillary–urinary concentration differences

The magnitude and direction of any concentration difference between the papilla and urine are of significance in several respects: first, in determining whether the direction of any concentration difference is compatible with the assumptions of passive transport of water and urea from collecting duct into medullary tissue; and secondly, in determining the

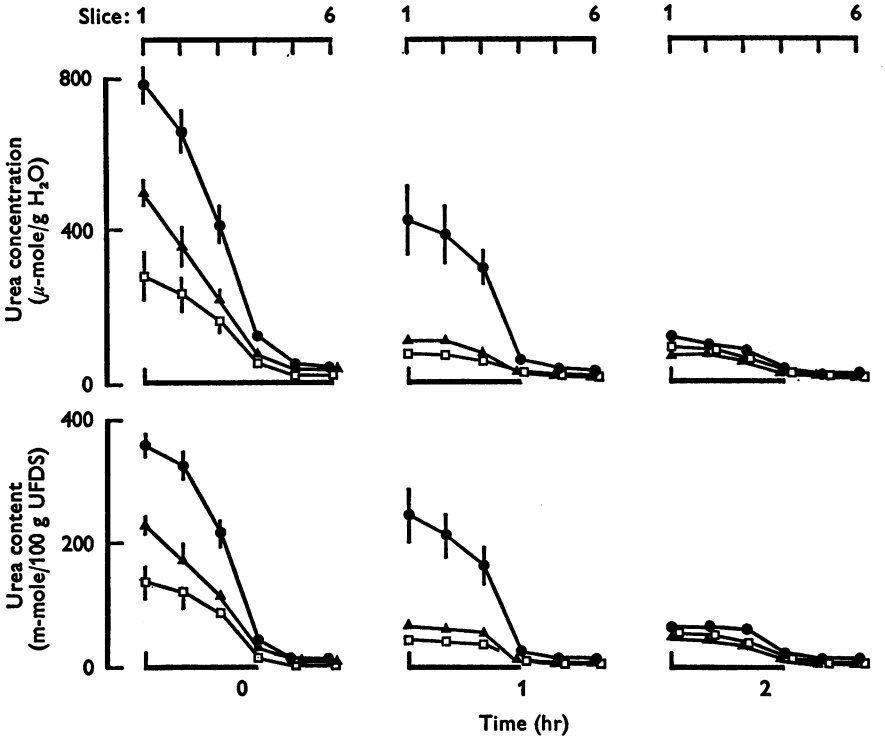

Fig. 8. Urea concentration and content in renal tissue sections from hydropaenic (●), normal (▲) and hydrated (□) rats before (0 hr) and after intravenous infusion (0·2 ml./min) of 0·9 % saline for 1 hr and 2 hr. Further details as for Figs. 4 and 5.

extent of transtubular water and urea equilibration. Accordingly, osmolal and urea concentrations in papillary tip and urine are presented in Fig. 10, together with a statistical evaluation of the significance of any mean differences. Interpretation of the data is subject to the reservation that the urinary data are derived from samples collected over a period of time; this is considered to explain the observations that the osmolality in the overnight urine in the hydropaenic group was considerably higher, and in

the normal group was slightly higher, than papillary osmolality. In the hydrated group, however, urinary osmolality was significantly lower than papillary osmolality. In all groups, the fall in osmolality in the urine was greater than that in the papillary tip, so that after 2 hr saline infusion, osmolality of the papillary tip consistently exceeded that in the urine, significantly so in the normal and hydrated groups.

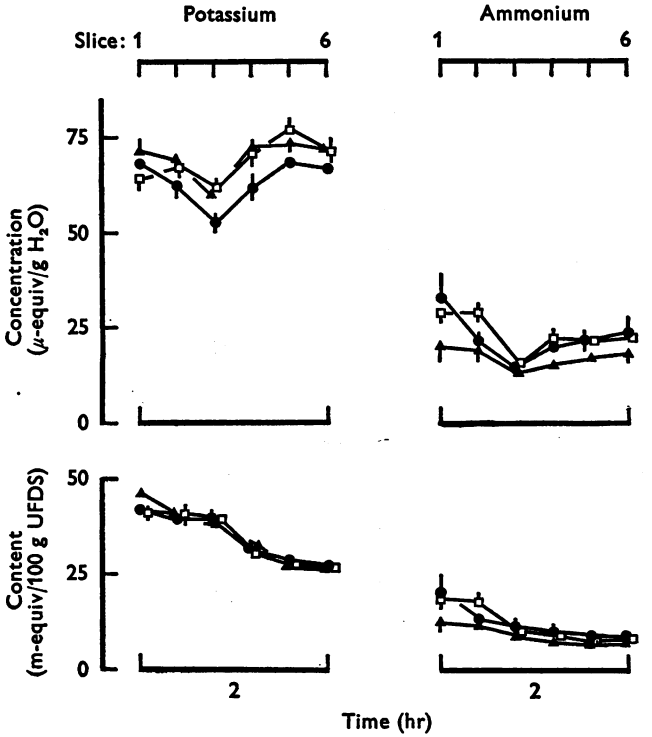

Fig. 9. Potassium and ammonium concentrations and contents in renal tissue sections from hydropaenic (●), normal (▲) and hydrated (□) rats after intravenous infusion (0·2 ml./min) of 0·9% saline for 2 hr. Further details as for Figs. 4 and 5.

Of the non-infused groups, urea concentrations in the urine significantly exceeded those in the papillary tip in the hydropaenic and normal groups but were similar in the hydrated group. In all three groups, saline infusion largely abolished these differences (Fig. 10)· in no group did a reversal of the urinary–papillary concentration difference occur. These data are compatible, therefore, with passive transport of urea out of the collecting duct.

DISCUSSION

Estimation of the main osmotically active solutes has the advantage that, in addition to providing information concerning changes in the concentrations of individual solutes, it enables calculation (see Methods) of an approximate value for tissue osmolality. In this respect, previous data showing incomplete papillary–urinary osmotic equilibration during water

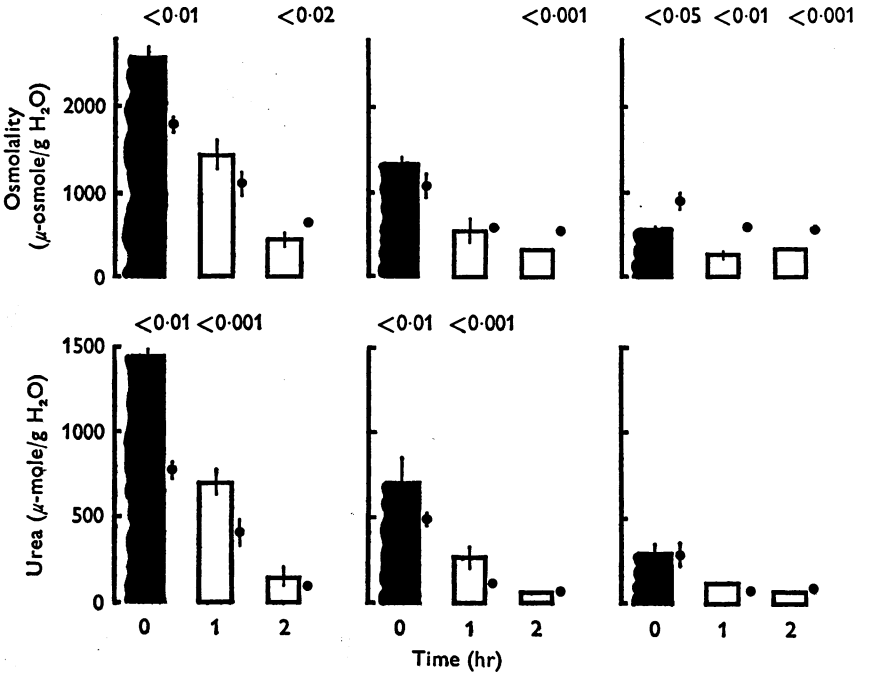

Fig. 10. *Above*, osmolal concentration and *below*, urea concentration in papillary tip and urine from hydropaenic, normal and hydrated rats, before (papillary tip, ●; urine, ■) and after intravenous infusion (0·2 ml./min) of 0·9 % saline for 1 hr and 2 hr (papillary tip ●; urine □). Values represent means: vertical bars, ± S.E. *P* values represent the probability of the mean urine–papilla concentration difference being zero.

diuresis (Atherton *et al.* 1968*c*) and complete equilibration during lysine-vasopressin infusion (Hai & Thomas, 1969*a*) are completely compatible with micropuncture data (see review by Berliner & Bennett, 1967). If the method is also valid for the renal cortex, the present data show apparent cortical osmolalities during saline infusion (Fig. 4) significantly hyperosmolal to systemic plasma (Table 2) as was evident, previously, during water diuresis (Atherton *et al.* 1968*c*), lysine-vasopressin infusion (Hai & Thomas, 1969*a*) and in hydropaenia (Hai & Thomas, 1969*b*).

However, it may be objected (*a*) that cortical hypertonicity appears to contravene the general assumption that osmotic equilibrium exists between cells and interstitial fluid, (*b*) that this is at variance with the general view that the renal cortex is isosmolal with systemic blood in all physiological circumstances and (*c*) that the most probable explanation for the apparent hyperosmolality is that a proportion of the chemically determined solute is inactive, osmotically, in the fresh tissue. Since this has considerable importance not only in respect to osmotic water flow in the renal cortex but also to the mechanisms operative in water and solute transport across other tissues, a more detailed analysis and discussion of pertinent data will be presented in a subsequent paper (J. C. Atherton, R. Green & S. Thomas, in preparation). For present discussion we wish to stress the following points:

First, with respect to osmotic water flow in the renal cortex, as in the renal medulla, the pertinent comparison must be between fluid osmolality in the tubules and in the appropriate interstitial or cellular compartment, and not between tubules and systemic blood. As yet, no such comparison has been made, even in micropuncture experiments on renal cortical structures. *Secondly*, interpretation of the extensive literature concerning the osmolality of the renal cortex is controversial (see, for example, the review by Robinson, 1965). The most recent evidence supporting the view that the renal cortex is approximately isosmolal with systemic blood is based on the boiling-water method of Appelboom, Brodsky, Tuttle & Diamond (1958). However, the method requires several corrections (for diffusion equilibrium, change in activity coefficient and loss of CO_2); and no workers have corrected for the loss of ammonia which occurs on boiling (J. C. Atherton, R. Green & S. Thomas, unpublished experiments). Furthermore, the method assumes that extraction of solutes from the tissue by boiling in distilled water is complete; in fact, approximately 10% of the sodium and potassium initially present may remain in the boiled tissue (Robinson, 1962; J. C. Atherton, R. Green & S. Thomas, unpublished experiments). 'Unless a similar quantity of ions is bound so that it does not contribute to intracellular osmolality during life, the agreement of osmolalities determined on boiled extracts with the osmolality of plasma does not establish that living cells are in osmotic equilibrium with their immediate surroundings' (Robinson, 1962). *Thirdly*, the standing-gradient osmotic flow hypothesis of Diamond (e.g. Diamond & Bossert, 1967)—which may apply to water transport in the renal cortex (e.g. de Wardener, 1969) and in a wide variety of tissues (Keynes, 1969)—requires the existence of hyperosmolal compartments even in tissues transporting isosmolal or hypo-osmolal fluids.

We conclude that current methods do not exclude the existence of renal

cortical hyperosmolality; and that the present data are compatible with current concepts concerning water transport. However, to the extent that it is considered that the calculation method provides only a rough approximation to, or even an over-estimate of, tissue osmolality, any subsequent discussion relating to the extent of tissue–urinary osmotic equilibration should be treated with appropriate reserve.

Most discussions of the mechanisms involved in saline diuresis have been presented in terms of changes in the sodium load delivered to, and in the reabsorptive characteristics of, the various nephron segments; the increased excretion of water is generally regarded as an osmotic consequence of the increased sodium excretion. However, the present results show first, that in addition to the expected increase in water and sodium outputs during saline loading, increased excretion of urea, potassium and ammonium also occurs; secondly, that the pattern of diuresis and the extent of fluid retention may be modified by the antecedent hydration status; and thirdly, that saline loading is accompanied by a reduction in medullary osmolality, mainly attributable to a decrease in sodium and urea contents and an increase in water content, and by incomplete medullary–urinary osmotic equilibration. A reduction in medullary osmolality is thus common to saline, osmotic (Atherton *et al.* 1968*b*) and water (Atherton *et al.* 1968*c*) diureses.

Interpretation of the changes in medullary composition in terms of component changes in transtubular active and passive driving forces, in membrane permeability and in the flows of intratubular fluid and medullary blood is subject to the restriction that tissue composition at any instant is the resultant of the volume and composition of several component compartments. In the present experiments, the water content in most renal segments in hydropaenic rats after 2 hr saline infusion was higher than in the normal and hydrated groups, despite lower urine flows in the former. It is likely, therefore, that as in mannitol diuresis (Goldberg & Ramirez, 1967; Atherton *et al.* 1968*b*), the tissue data reflect, primarily, the composition of extratubular fluid, although histological examination of the renal cortex after saline infusion shows expansion of tubular lumina as well as of interstitial space (Hayslett, Kashgarian & Epstein, 1967).

Sodium

The expansion of extracellular fluid volume induced by saline infusion in the rat causes increased renal blood flow and G.F.R. (Cortney, Mylle, Lassiter & Gottschalk, 1965; Rector, Sellman, Martinez-Maldonado & Seldin, 1967; Landwehr, Klose & Giebisch, 1967), and so an increase in the filtered load of sodium. Micropuncture experiments in the dog (Dirks, Cirksena & Berliner, 1965) and rat (Rector *et al.* 1967; Landwehr *et al.*

1967; Hayslett *et al.* 1967) have amply confirmed the suggestion (de Wardener *et al.* 1961) that reduced proximal tubular reabsorption of sodium also occurs; the mechanisms responsible for these changes in the tubular handling of sodium during saline infusion are uncertain (see Berliner, 1968).

Since the large increment in fluid leaving the proximal tubule far exceeds the increment in water and salt excretion during saline loading, a net increase in fluid reabsorption in more distal segments must occur. There is some disagreement concerning the occurrence of increased fluid reabsorption in the distal convoluted tubule (Cortney *et al.* 1965; Landwehr *et al.* 1967; Hayslett *et al.* 1967); but any such change must be small compared with the considerable increase in sodium and water reabsorption in the loop of Henle (Cortney *et al.* 1965; Landwehr *et al.* 1967) during saline loading.

Despite increased delivery of sodium into the medulla, however, the present results show that only in the hydrated group did even a modest increase in content occur; on the contrary, a substantial decrease in medullary sodium content occurred in the hydropaenic rats (Fig. 7). This can only be due to increased loss of sodium into the medullary circulation. Increased medullary blood flow (which would reduce the efficiency of the countercurrent exchanger action of the medullary vasa recta) has been inferred during isotonic (Earley & Friedler, 1965) and hypotonic (Eknoyan, Suki, Rector & Seldin, 1967) saline infusion in the dog. Because papillary sodium content increased with urine flow during higher rates of infusion of hypotonic saline, Eknoyan *et al.* (1967) suggested that increased medullary blood flow did not effectively accelerate net medullary washout of sodium. However, the relative significance of changes in medullary blood flow and in increased medullary delivery of sodium may have altered with progressive increase in flow through the loop of Henle. Such a progressive increase in sodium transport in the loop of Henle has been demonstrated by micropuncture in the rat (Landwehr *et al.* 1967; Landwehr, Schnermann, Klose & Giebisch, 1968). The fact that in the present study, papillary sodium contents tended to be highest in the hydrated group after 2 hr saline infusion (Fig. 7) may result from higher flow rate and sodium load in the loop of Henle in this group.

Both mannitol and saline administration increase the sodium load leaving the proximal tubule. However, the limitation on osmotic reabsorption of water imposed by the presence of non-reabsorbable mannitol results in a lowered intraluminal sodium concentration (Windhager & Giebisch, 1961). We agree with the suggestion (Goldberg, McCurdy & Ramirez, 1965; Goldberg & Ramirez, 1967) that the consequent restraint on sodium reabsorption in the loop (Windhager & Giebisch, 1961) accounts for the fact that sodium contents in the papillary and inner medullary segments in

mannitol diuresis (Atherton *et al.* 1968*b*) were significantly lower than those observed in the present experiments with saline loading.

Urea

There seems to have been no previous study of the effects of isotonic saline loading on renal urea handling. The marked reduction in medullary content of urea during saline diuresis (Fig. 8) is very similar to that which occurs in both mannitol (Atherton *et al.* 1968*b*) and water (Atherton *et al.* 1968*c*) diureses; the present data are reasonably interpreted in terms of passive handling of urea.

In antidiuresis, the steep corticomedullary concentration gradient for urea is presumed to result from passive diffusion through the collecting duct wall, the urea-permeability of which is enhanced by A.D.H. (Morgan, Sakai & Berliner, 1968; Morgan & Berliner, 1968). Loss of medullary urea into the loop of Henle is compensated by subsequent reabsorption from the collecting duct (Lassiter, Gottschalk & Mylle, 1961; de Rouffignac & Morel, 1969). Hypertonic saline infusion in the rat causes a considerable increase in urea excretion; fractional urea reabsorption in the proximal tubule is unchanged, but there is little subsequent movement out of the nephron, and little or no recycling of urea from the collecting duct to the loop of Henle (Lassiter, Mylle & Gottschalk, 1964). Essentially similar results have been reported for osmotic diuresis (Ullrich, Schmidt-Nielsen, O'Dell, Pehling, Gottschalk, Lassiter & Mylle, 1963; Jones, Mylle & Gottschalk, 1965). The profound reduction in medullary urea content in all three groups during isotonic saline loading can be similarly explained. In addition, increased medullary blood flow will enhance loss of medullary urea into the circulation.

Since maximal urea outputs preceded peak urine flows and occurred when a considerable decrease in medullary urea content was evident (cf. Figs. 3 and 8), it is probable that loss of medullary urea into the nephron contributed to the early increase in urea excretion during saline loading, as in water and mannitol diureses (Atherton *et al.* 1968*a*). However, during saline infusion, urea clearances (Table 2) and outputs (Fig. 3) were much higher than those previously found at comparable urine flows in water (Atherton *et al.* 1968*a*; Hai & Thomas, 1969*b*) and mannitol (Atherton *et al.* 1968*a*) diureses. It is probable that a higher filtered load of urea, due to the increased G.F.R. discussed above, is mainly responsible; this conclusion is supported by the fact that only relatively small changes occurred in the urea/creatinine urinary ratio during saline infusion (Fig. 3).

During water diuresis urea concentrations in the urine fall to values significantly less than those in the papilla (Atherton *et al.* 1968*c*). In mannitol diuresis, however, the rapid decline in medullary urea content is

accompanied by the abolition of urinary–papillary urea concentration differences (Atherton *et al.* 1968*b*). To the extent that saline infusion resulted in the abolition of significant differences in urea concentration between papillary tip and urine, the present results resemble those in mannitol diuresis. However, osmotic equilibration between papilla and urine was incomplete during saline diuresis (Fig. 10), suggesting that collecting duct permeability to water was relatively restricted. If the collecting duct permeability to urea was similarly restricted, it may be that rapid washout of medullary urea into the loop of Henle and into the medullary circulation was primarily responsible for the apparent urea equilibration between papillary tip and urine (Fig. 10).

Potassium and ammonium

An increased potassium excretion as a result of isotonic saline administration in the rat has not been reported previously, although a transient increase is apparent in the data of Kellogg *et al.* (1954). Similar increases have been observed in man (Blomhert *et al.* 1951; Papper, Saxon, Rosenbaum & Cohen, 1956) and in the dog (e.g. Wesson, Anslow, Raisz, Bolomey & Ladd, 1950). Increased ammonium excretion during saline infusion has also not been noted previously. Wesson *et al.* (1950) found no change in ammonium excretion in the dog.

The absence of major changes in the medullary contents of potassium and ammonium during the generation of saline diuresis is similar to results obtained in mannitol diuresis (Atherton *et al.* 1968*b*), in water diuresis (Atherton *et al.* 1968*c*), and during lysine-vasopressin administration (Hai & Thomas, 1969*a*). Thus, it seems likely that the changes in potassium and ammonium excretion during saline loading primarily result from enhanced delivery of sodium to potassium and ammonia secretory sites in cortical portions of the nephron.

Water

The tissue water contents during saline loading are higher than those previously found in either mannitol diuresis (Atherton *et al.* 1968*b*) or water diuresis (Atherton *et al.* 1968*c*) at comparable urine flows. A proportion of the increase in renal tissue water content may be attributable to retention of a proportion of the infused saline, particularly in the hydropaenic group. This may, in part, account for the higher tissue water contents after saline infusion in the hydropaenic rats; A.D.H.-induced differences in water reabsorption may also be involved, since some differences in endogenous A.D.H. release are likely to be associated with the differences in prehydration status.

Changes in water movement in the various nephron segments are to be

expected from the changes in solute transport discussed above. In the proximal tubule, the fluid remains essentially isotonic, despite the reduced fractional sodium reabsorption (Cortney *et al.* 1965). The kinetics of sodium and water reabsorption in the loop of Henle are such that as flow rate increases, the increment in net water reabsorption is less than that in sodium movement; thus the sodium concentration of fluid entering the distal tubule decreases (Landwehr *et al.* 1968). If, as mentioned above, any changes in distal tubular fluid reabsorption are small relative to the considerable alterations in more proximal segments, the net effect will be the presentation of large volumes of isotonic, or even hypotonic, fluid to the collecting duct.

Here, the present findings are of particular significance. Even if osmotic equilibration were completely achieved, the considerable reduction in medullary osmolality during saline loading must lead to a reduction in water reabsorption from the collecting duct. In fact, medullary–urinary osmotic equilibration was found to be incomplete during isotonic saline infusion (Fig. 10), as has been found during water loading (Atherton *et al.* 1968*c*; Hai & Thomas, 1969*b*) in the rat and during hypotonic saline loading (Eknoyan *et al.* 1967) in the dog. Since osmotic equilibration was incomplete during isotonic saline loading, irrespective of the antecedent hydration status, the possible influence of differences in collecting duct permeability to water (due to any differences in the release of endogenous A.D.H.) is presumed to be obscured by the delivery of increased volumes of fluid from the distal tubule.

These observations have important implications in several other respects:

(*a*) The large changes in medullary osmolality during saline loading precede the achievement of maximal diuresis (cf. Figs. 1 and 4) as in mannitol (Atherton *et al.* 1968*b*) and water (Atherton *et al.* 1968*c*) diureses. Reduction in medullary osmolality seems to be a prerequisite for the achievement of maximal urinary flows in all types of diuresis, and must be involved in the progressive change in osmolality of the urine towards that of the plasma as urine flow increases during osmotic and saline diureses.

(*b*) The decreased osmotic force for medullary water reabsorption and the incomplete transtubular osmotic equilibration in the collecting duct and, possibly, in the distal convoluted tubule, are both likely to contribute to the transient hypotonicity observed during saline infusion in the rat (present experiments) and in the dog even in the presence of maximal A.D.H. (Wesson *et al.* 1950; McDonald & de Wardener, 1965). The production of hypotonic urine by the isolated, perfused dog kidney (McDonald & de Wardener, 1965) may depend on similar factors.

(*c*) Tubular reabsorption of sodium is primarily responsible for the

generation of solute-free water (measured as the clearance of osmotically free water, C_{H_2O}) in dilute urine, and for the osmotic reabsorption of solute-free water (measured as the negative clearance of solute-free water $T^C_{H_2O}$) in the elaboration of concentrated urine. Consequently, changes in $T^C_{H_2O}$ and C_{H_2O} have been used by many workers as indices of changes in sodium reabsorptive activity, e.g. during saline loading and during administration of diuretics. However, as pointed out by Berliner & Bennett (1967), interpretation of such changes in urine concentration should also consider the major importance of changes in the volume flow entering the collecting ducts. The present results show that changes in medullary osmolality and in the completeness of medullary–urinary osmotic equilibration may also contribute to the altered urinary osmolality in such circumstances.

Influence of antecedent hydration status

The influence of the antecedent hydration status on the pattern of the diuretic response to saline loading is similar, in some respects, to the influence of prehydration on saline diuresis in man (Ladd, 1951). The response to saline loading in the dog is also influenced by the previous salt diet (Keck, Brechtelsbauer & Kramer, 1969). The delayed, reduced response in the hydropaenic rat has not been reported previously, and may have been obscured by the massive rates of saline infusion employed in many studies.

The factors responsible for the altered diuretic and natriuretic response in hydropaenia are unknown. Although the initial changes in medullary osmolality were influenced by the antecedent hydration status the differences in urinary flow and composition persisted even after those in medullary osmolality had disappeared (cf. Figs. 1 and 4). Differences in extracellular fluid volume, in general and renal haemodynamics, in intrarenal distribution of blood, and in the release of A.D.H. and aldosterone may exist between various states of hydration; any of these might be involved in the renal changes. Whatever factors are involved, it is stressed that the fluid retention at 4 hr from the start of saline loading (equivalent to almost 15 % of body weight) considerably exceeded the decrease (at most 5 %) in body fluid water which occurred as a result of the pre-experimental deprivation of water. To this extent, the results are difficult to reconcile with the existence of precise, rapidly adjusting, regulatory mechanisms with respect to sodium balance or body fluid volume.

A comparison between the changes in urinary and renal tissue composition in saline diuresis and those observed in water and mannitol diureses (Atherton *et al.* 1968*a*, *b*, *c*) shows few features which require a specific regulatory mechanism unique to saline diuresis. In many respects, a

saline diuresis exhibits characteristics of osmotic diuresis, with sodium as the extra, osmotically active, intratubular constituent; in other respects, a saline diuresis may exhibit characteristics of water diuresis, possibly depending on membrane permeability and on changes in intratubular flow rate. In all forms of diuresis, a reduction in medullary osmolality must contribute to the changes in urinary flow and osmolality.

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REFERENCES

ADOLPH, E. F. (1923). The excretion of water by the kidneys. *Am. J. Physiol.* **65**, 419–449.

APPELBOOM, J. W. T., BRODSKY, W. A., TUTTLE, W. S. & DIAMOND, I. (1958). The freezing point depression of mammalian tissues after sudden heating in boiled distilled water. *J. gen. Physiol.* **41**, 1153–1169.

ATHERTON, J. C., GREEN, R. & HAI, M. A. (1969). Evaluation of a method for weighing small tissue samples. Investigations into freezing and evaporation. *Pflügers Arch. ges. Physiol.* **309**, 203–211.

ATHERTON, J. C., HAI, M. A. & THOMAS, S. (1968*a*). 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. (1968*b*). The time course of changes in renal tissue composition during mannitol diuresis in the rat. *J. Physiol.* **197**, 411–428.

ATHERTON, J. C., HAI, M. A. & THOMAS, S. (1968*c*). The time course of changes in renal tissue composition during water diuresis in the rat. *J. Physiol.* **197**, 429–443.

BERLINER, R. W. (1968). Intrarenal mechanisms in the control of sodium excretion. *Fedn Proc.* **27**, 1127–1131.

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

BLOMHERT, G., GERBRANDY, J., MOLHUYSEN, J. A., DE VRIES, L. A. & BORST, J. G. G. (1951). Diuretic effect of isotonic saline solution compared with that of water. *Lancet* **261**, 1011–1015.

BONSNES, R. W. & TAUSSKY, H. H. (1945). On the colorimetric determination of creatinine by the Jaffe reaction. *J. biol. Chem.* **158**, 581–591.

CORTNEY, M. A., MYLLE, M., LASSITER, W. E. & GOTTSCHALK, W. W. (1965). Renal tubular transport of water, solute and PAH in rats loaded with isotonic saline. *Am. J. Physiol.* **209**, 1199–1205.

CUSHNY, A. R. (1917). *The Secretion of Urine.* London: Longmans, Green.

DE ROUFFIGNAC, C. & MOREL, F. (1969). Micropuncture study of water electrolytes and urea movements along the loops of Henle in Psammomys. *J. clin. Invest.* **48**, 474–486.

DE WARDENER, H. E. (1969). Control of sodium reabsorption. *Br. med. J.* **3**, 611–616.

DE WARDENER, H. E., MILLS, I. H., CLAPHAM, W. F. & HAYTER, C. J. (1961). Studies on the efferent mechanism of the sodium diuresis which follows the administration of intravenous saline in the dog. *Clin. Sci.* **21**, 249–258.

DIAMOND, J. M. & BOSSERT, W. H. (1967). Standing-gradient osmotic flow: a mechanism for coupling of water and solute transport in epithelia. *J. gen. Physiol.* **50**, 2061–2083.

DIRKS, J. H., CIRKSENA, W. J. & BERLINER, R. W. (1965). The effect of saline infusion on sodium reabsorption by the proximal tubule of the dog. *J. clin. Invest.* **44**, 1160–1170.

EARLEY, L. E. & FRIEDLER, R. M. (1965). Changes in renal blood flow and possibly the intrarenal distribution of blood during the natriuresis accompanying saline loading in the dog. *J. clin. Invest.* **44**, 929–941.

EKNOYAN, G., SUKI, W. N., RECTOR, JR., F. C. & SELDIN, D. W. (1967). Functional characteristics of the diluting segment of the dog nephron and the effect of extracellular volume expansion on its reabsorptive capacity. *J. clin. Invest.* **46**, 1178–1188.

FAWCETT, J. K. & SCOTT, J. E. (1960). A rapid and precise method for the determination of urea. *J. clin. Path.* **13**, 156–159.

GOLDBERG, M., MCCURDY, K. & RAMIREZ, M. A. (1965). Differences between saline and mannitol diuresis in hydropaenic man. *J. clin. Invest.* **44**, 182–192.

GOLDBERG, M. & RAMIREZ, M. A. (1967). Effects of saline and mannitol diuresis on the renal concentrating mechanisms in dogs: Alterations in renal tissue solutes and water. *Clin. Sci.* **32**, 475–493.

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

HAI, M. A. & THOMAS, S. (1969*b*). Influence of prehydration on the changes in renal tissue composition induced by water diuresis in the rat. *J. Physiol.* **205**, 599–618.

HAYSLETT, J. P., KASHGARIAN, M. & EPSTEIN, F. H. (1967). Changes in proximal and distal tubular reabsorption produced by rapid expansion of extracellular fluid. *J. clin. Invest.* **46**, 1254–1263.

JONES, N. F., MYLLE, M. & GOTTSCHALK, C. W. (1965). Renal tubular and microinjection studies in normal and potassium depleted rats. *Clin. Sci.* **29**, 261–275.

KECK, W. H., BRECHTELSBAUER, H. & KRAMER, K. (1969). Wasser und Natrium-Ausscheidung nach isotonen Kochsalz-Infusionen bei wachen Hunden mit verschiedenem Natriumbestand. *Pflügers Arch. ges. Physiol.* **311**, 119–130.

KELLOGG, R. H., BURACK, W. R. & ISSELBACHER, K. J. (1954). Comparison of diuresis produced by isotonic saline solutions and by water in rats studied by a 'steady-state' method. *Am. J. Physiol.* **177**, 27–37.

KEYNES, R. D. (1969). From frog skin to sheep rumen: a survey of transport of salts and water across multicellular structures. *Q. Rev. Biophys.* **2**, 177–281.

LADD, M. (1951). Effect of prehydration upon renal excretion of sodium in man. *J. appl. Physiol.* **3**, 603–609.

LANDWEHR, D. M., KLOSE, R. M. & GIEBISCH, G. (1967). Renal tubular sodium and water reabsorption in the isotonic sodium chloride-loaded rat. *Am. J. Physiol.* **212**, 1327–1333.

LANDWEHR, D. M., SCHNERMANN, J., KLOSE, R. M. & GIEBISCH, G. (1968). Effect of reduction in filtration rate on renal tubular sodium and water reabsorption. *Am. J. Physiol.* **215**, 687–695.

LASSITER, W. E., GOTTSCHALK, C. W. & MYLLE, M. (1961). Micropuncture study of net transtubular movement of water and urea in nondiuretic mammalian kidney. *Am. J. Physiol.* **200**, 1139–1146.

LASSITER, W. E., MYLLE, M. & GOTTSCHALK, C. W. (1964). Net transtubular movement of water and urea in saline diuresis. *Am. J. Physiol.* **260**, 669–673.

MCDONALD, S. J. & DE WARDENER, H. E. (1965). Some observations on the production of a hypo-osmotic urine during the administration of 0·9 % saline and vasopressin in the dog. *Clin. Sci.* **28**, 445–459.

MOREL, F. (1967). Current concepts in renal physiology. In *Proceedings of the Third International Congress of Nephrology*, ed. HANDLER, J. S. vol. **1**, Basel: 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.

PAPPER, S., SAXON, L., ROSENBAUM, J. D. & COHEN, H. W. (1956). The effects of isotonic and hypertonic salt solutions on the renal excretion of sodium. *J. Lab. clin. Med.* **47**, 776–782.

RECTOR, JR., F. C., SELLMAN, J. C., MARTINEZ-MALDONADO, M. & SELDIN, D. W. (1967). The mechanism of suppression of proximal tubular reabsorption by saline infusions. *J. clin. Invest.* **46**, 47–56.

ROBINSON, J. R. (1962). Residual sodium and potassium in kidney slices boiled in distilled water. *Proc. Univ. Otago med. Sch.* **40**, 17–18.

ROBINSON, J. R. (1965). Water regulation in mammalian cells. *Symp. Soc. exp. Biol.* **19**, 237–258.

ROBINSON, R. R., OWEN, E. E. & SCHMIDT-NIELSEN, B. (1966). Intra-renal distribution of free amino-acids in antidiuretic ruminants. *Comp. Biochem. Physiol.* **19**, 187–195.

SAIKIA, T. C. (1965). Composition of the renal cortex and medulla of rats during water diuresis and antidiuresis. *Q. Jl exp. Physiol.* **50**, 146–157.

SMITH, H. W. (1957). Salt and water volume receptors. *Am. J. Med.* **23**, 623–652.

ULLRICH, K. J., SCHMIDT-NIELSEN, B., O'DELL, R., PEHLING, G., GOTTSCHALK, C. W., LASSITER, W. E. & MYLLE, M. (1963). Micropuncture study of composition of proximal and distal tubular fluid in rat kidney. *Am. J. Physiol.* **204**, 527–531.

WESSON, JR., L. G. (1957). Glomerular and tubular factors in the renal excretion of sodium chloride. *Medicine, Baltimore* **36**, 281–396.

WESSON, JR., L. G., ANSLOW, JR., W. P., RAISZ, L. G., BOLOMEY, A. A. & LADD, M. (1950). Effect of sustained expansion of extracellular fluid volume upon filtration rate, renal plasma flow and electrolyte and water excretion in the dog. *Am. J. Physiol.* **162**, 677–686.

WINDHAGER, E. E. & GIEBISCH, G. (1961). Micropuncture study of renal tubular transfer of sodium chloride in the rat. *Am. J. Physiol.* **200**, 581–590.