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Renal Tubular Effects of Hydrocortisone and Aldosterone in Normal Hydropenic Man: Comment on Sites of Action *

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It is generally accepted that glucocorticoid and mineralocorticoid adrenal hormones influence the urinary excretion of sodium and potassium, but the mechanism(s) and tubular site(s) of action have not been clearly defined. Aldosterone has been reported to decrease sodium and chloride and to increase potassium and hydrogen ion excretion (1–3). With the stop-flow technique, this increment in sodium and chloride reabsorption was localized to the distal tubule (4, 5). With the observation that aldosterone increased urine solute concentration in hydropenic subjects as a basis, it was proposed that this agent enhanced sodium reabsorption at the ascending limb of the loop of Henle (6). However, in these studies the influence of changes in urine flow rate and composition of the urine solute was not considered. When aldosterone was administered to hydrated normal subjects, an increase in the excretion of solute free water (C_{H_2O}) was noted (7). The authors concluded that the primary action of this hormone was to increase sodium reabsorption in the distal tubule. In similar experiments, reported by others, C_{H_2O} was not elevated by aldosterone (8).

It is well established that glucocorticoids, in contrast to aldosterone and desoxycorticosterone acetate (DOCA), correct the water clearing defect in Addisonian patients and adrenalectomized dogs (9–18). The correction of this defect by hydrocortisone could not be attributed solely to the rise in glomerular filtration rate (GFR) frequently produced by this agent (16–18). In addition, others have recently reported that a glucocorticoid (methyl-prednisolone) increased solute free water reabsorption ($T^c_{H_2O}$) in salt-depleted cirrhotics without altering GFR (19).

Under hydropenic conditions, solute concentrations in the medullary interstitium and collecting duct fluid have been shown to be the same in any plane cut perpendicular to the axis of the medulla (20). Others have demonstrated that urea is highly diffusible across the collecting duct membrane, producing similar concentrations of this solute within the collecting duct and surrounding medullary interstitial fluid (21, 22). It therefore follows that urine nonurea solute concentration (total urine solute concentration minus urine urea concentration) will reflect changes in the medullary nonurea-solute (primarily sodium and chloride) concentration (23). In addition, considerable evidence is available to suggest that the quantity of salt deposited within the medulla depends upon ascending limb sodium transport (24). With these assumptions, changes in total urine solute and urine urea concentrations, electrolyte excretion, and renal hemodynamics produced by hydrocortisone and aldosterone were analyzed in normal hydropenic subjects to determine the renal tubular site(s) of action of these agents.

Methods

The acute effects of the intravenous administration of 200 mg hydrocortisone (8 experiments) or 1.0 mg *d*-aldosterone[1] (8 experiments) were studied in 14 maximally

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TABLE I

*Summary of mean changes in hydropenia—low urine flow studies (group I)**

ΔV†	ΔC_{osm}†	Initial U_{osm}	Final U_{osm}	ΔU_{osm}†	ΔU_{Urea}†	ΔU_{NUS}†	$\Delta U_{Na}V$†	$\Delta U_{K}V$†	$\Delta U_{Cl}V$†	ΔGFR†
ml/min	*ml/min*	*mOsm/kg H_2O*	*mOsm/kg H_2O*	*mOsm/kg H_2O*	*mmoles/L*	*mOsm/kg H_2O*	*μEq/min*	*μEq/min*	*μEq/min*	*ml/min*
Placebo										
0	+0.15	901	957	+56	+12	+44	+28	+5	−5	−2
SE ±0.056	±0.15	±29	±27	±15	±11	±10	±17	±9	±19	±2.9
Aldosterone										
−0.25	−0.59	923	1031	+107	+70	+37	−56	−14	−89	+4
SE ±0.029	±0.10	±36	±32	±10	±8	±8	±12	±8	±10	±2.9
Hydrocortisone										
−0.03	+0.14	911	1051	+140	+25	+115	−14	+52	−13	+4
SE ±0.055	±0.17	±36	±54	±17	±9	±18	±16	±8	±14	±2.8

* V = urine volume; C_{osm} = osmolar clearance; U_{osm} = urine osmolality; U_{NUS} = urine nonurea solute; GFR = glomerular filtration rate.

† The data are expressed as the change (Δ) between the control period (initial) and final experimental period 3½ hours after the administration of placebo, aldosterone, and hydrocortisone.

hydropenic subjects free of cardiovascular and renal disease. All studies were performed after a 14-hour fast and 11 hours after the intramuscular injection of 5 U of vasopressin tannate in oil. Urine was collected by an indwelling catheter in female subjects and by spontaneous voiding in males. Subjects remained recumbent throughout the experiments except for males who stood while voiding. Two studies were performed on 12 of the 14 subjects. In one experiment either hydrocortisone or aldosterone was administered, and in the other a placebo injection was given. Two subjects received hydrocortisone, aldosterone, and placebo on separate occasions.

Group I. Hydropenia. At 8:00 a.m. on the day of the experiment the bladder was emptied (double air washouts were employed in all catheterized subjects), and priming doses of inulin and para-aminohippurate (PAH) were administered intravenously. Thereafter, an infusion was started containing sufficient quantities of inulin and PAH to produce satisfactory plasma levels and aqueous vasopressin in a concentration adequate to deliver 50 mμ per kg body weight per hour. This solution was delivered at a rate of 0.34 ml per minute throughout the course of the study by a Bowman infusion pump. After allowing 60 minutes for equilibration of plasma inulin and PAH concentrations, the bladder was emptied. The urine collected in the following 30 to 40 minutes served as the control period. The placebo, hydrocortisone, or aldosterone was then administered. Urine was collected at 40- to 60-minute intervals for the next 3½ hours. Blood samples were drawn at suitable intervals.

Group II. Hydropenia-solute diuresis. A. Five subjects, prepared as outlined above for the group I studies, received an intravenous infusion of 10% mannitol 3½ hours after the administration of placebo, hydrocortisone, or aldosterone. The mannitol solution was administered at a rate of 10 ml per minute until urine flow increased to 12 to 15 ml per minute. Urine was collected at 10- to 15-minute intervals throughout the solute infusion. Each subject was studied on three occasions, at least 1 week apart, receiving placebo, hydrocortisone, and aldosterone, respectively. The rate of osmolar clearance (C_{osm}) and solute free water reabsorption ($T^{c}_{H_2O}$) was ascertained during the solute diuresis.

B. With the protocol outlined in group II A, studies were performed in one Addisonian patient (3 months postbilateral adrenalectomy) and in one patient with panhypopituitarism. Cortisol therapy was discontinued in the Addisonian patient 72 hours before each study. The patient with panhypopituitarism was receiving no hormonal replacement therapy. One week after a placebo

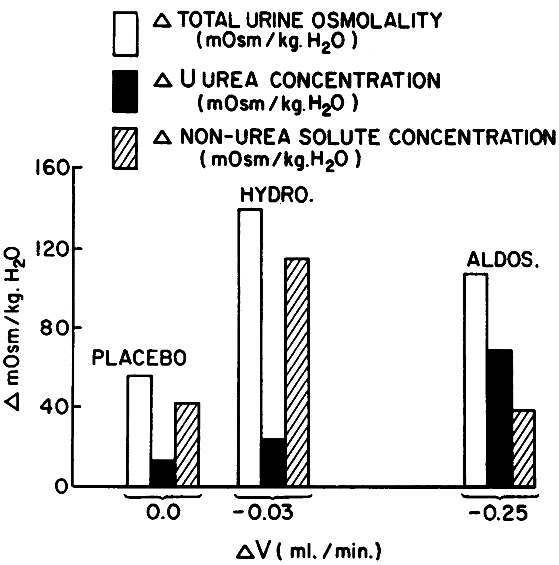

Fig. 1. Mean changes in urine osmolality, urine urea, and nonurea-solute concentrations 3½ hours after the administration of placebo, hydrocortisone, and aldosterone plotted against the change in urine flow rate (V) produced by these agents.

TABLE II

Two typical experiments—group I

Time	V	C_{osm}	U_{osm}	U_{Urea}	U_{NUS}	U_{Na}	$U_{Na}V$	U_K	U_KV	U_{Cl}	$U_{Cl}V$	C_{In}*
min	*ml/min*	*ml/min*	*mOsm/kg H_2O*	*mmoles/L*	*mOsm/kg H_2O*	*mEq/L*	*μEq/min*	*mEq/L*	*μEq/min*	*mEq/L*	*μEq/min*	*ml/min*
Subject G. S. Placebo study												
Control	0.40	1.40	1,035	290	745	184	74	204	82	249	100	87
0					Placebo injection							
58	0.45	1.56	1,029	274	755	190	86	198	89	240	108	89
124	0.42	1.52	1,074	288	786	200	84	192	81	255	107	93
180	0.44	1.59	1,068	276	792	213	94	204	90	267	117	92
208	0.41	1.49	1,073	281	792	230	94	195	80	257	105	90
Subject G. S. Hydrocortisone study												
Control	0.28	1.01	1,100	369	731	131	37	197	55	237	66	96
0					Hydrocortisone, 200 mg iv							
60	0.31	1.18	1,150	362	788	148	46	229	71	263	82	100
126	0.27	1.06	1,177	368	809	129	35	256	69	229	62	98
188	0.32	1.29	1,210	372	838	135	43	258	83	247	79	99
217	0.28	1.15	1,231	379	852	125	35	274	77	206	58	100
Subject J. F. Placebo study												
Control	0.82	2.12	737	156	581	182	149	133	109	148	121	108
0					Placebo injection							
60	0.73	1.97	764	174	590	174	127	173	126	135	99	105
118	0.73	2.03	785	175	610	209	153	143	104	142	104	108
182	0.67	1.91	801	193	608	209	140	152	102	137	92	97
214	0.68	1.97	810	193	617	215	146	152	103	134	91	101
Subject J. F. Aldosterone study												
Control	0.70	1.87	762	234	528	122	85	187	131	145	102	89
0					Aldosterone 1.0 mg iv							
64	0.62	1.75	801	263	538	99	61	229	142	140	87	91
125	0.54	1.61	847	288	559	104	56	235	127	153	83	94
180	0.50	1.51	859	305	554	110	55	237	109	140	70	90
209	0.47	1.44	870	319	551	120	56	233	110	129	61	96

* C_{In} = inulin clearance. Other abbreviations, as in Table I.

study, a repeat study was performed with hydrocortisone.

All urine and blood specimens were analyzed for osmolality and for sodium, potassium, chloride, urea, inulin, and PAH concentrations. Osmolalities were determined with a Fiske osmometer. Other determinations were performed by methods previously described from this laboratory (25). GFR and effective renal plasma flow (RPF) were measured as the clearance of inulin and PAH, respectively. Osmolar clearance and solute free water reabsorption were calculated from the following formulas: osmolar clearance (C_{osm}) = [urine osmolality (U_{osm}) × urine volume (V)]/plasma osmolality (P_{osm}), and solute free water reabsorption ($T^c_{H_2O}$) = C_{osm} − V.

Results

Group I. Hydropenia (Tables I and II; Figures 1 and 2). The effects of placebo, hydrocortisone, and aldosterone are expressed as the change (Δ) between the control and the final experimental period, approximately 3½ hours after the injection.

Total urine solute concentration (U_{osm}), urine flow rate (V), and solute clearance (C_{osm}) (Table I; Figure 1). After the placebo injection (14 experiments), U_{osm} rose an average of 56 mOsm per kg H_2O without a mean change in V. In 8 hydrocortisone studies U_{osm} increased a mean of 140 mOsm per kg H_2O associated with a mean decrease in V of 0.03 ml per minute. C_{osm} rose 0.15 ml per minute after placebo and 0.14 ml per minute after hydrocortisone. Although there was no significant alteration in V or C_{osm} between the placebo and hydrocortisone groups, the difference in the increment in U_{osm} was significant ($p <$

0.01). After the administration of aldosterone (8 experiments) U_{osm} rose a mean of 107 mOsm per kg H_2O, which was significantly greater than that noted in the placebo studies ($p < 0.05$). This increase in U_{osm} was associated with a mean fall in V of 0.25 ml per minute and a mean fall in C_{osm} of 0.59 per minute. The decrements in urine flow and solute clearance were also significant when compared to the placebo group ($p < 0.01$).

Urine urea (U_{Urea}) and nonurea solute (NUS) concentrations (Table I; Figure 1). U_{Urea} increased in all three groups, the change averaging 12, 25, and 70 mmoles per L for placebo, hydrocortisone, and aldosterone, respectively. The increment in U_{Urea} noted with aldosterone was significantly higher than that observed in the placebo group ($p < 0.01$), whereas the change produced by hydrocortisone was not significant. Urinary NUS concentration also rose in all three groups. Hydrocortisone produced an increase in NUS concentration (115 mOsm per kg H_2O), which was significantly greater than the increase noted in the placebo group ($p < 0.01$). The rise in NUS concentration noted with aldosterone (37 mOsm per kg H_2O) did not differ significantly from that seen in the placebo group (44 mOsm per kg H_2O).

Electrolyte excretion (Table I; Figure 2). In the placebo studies, sodium excretion increased progressively over the 3½ hour experimental period (mean Δ, 28 μEq per minute), and potassium excretion increased initially and then returned toward control levels (mean Δ, + 5 μEq per minute). After hydrocortisone administration, potassium excretion rose progressively for approximately 2 hours and then stabilized, the increment averaging 52 μEq per minute after 3½ hours. Sodium excretion fell a mean of 14 μEq per minute during the course of the experiments. In contrast to hydrocortisone, aldosterone produced a prompt and marked fall in sodium excretion that averaged 56 μEq per minute at the end of the experimental period. During this same period, potassium excretion diminished a mean of 14 μEq per minute. Chloride excretion fell 5, 13, and 89 μEq per minute in the placebo, hydrocortisone, and aldosterone studies, respectively.

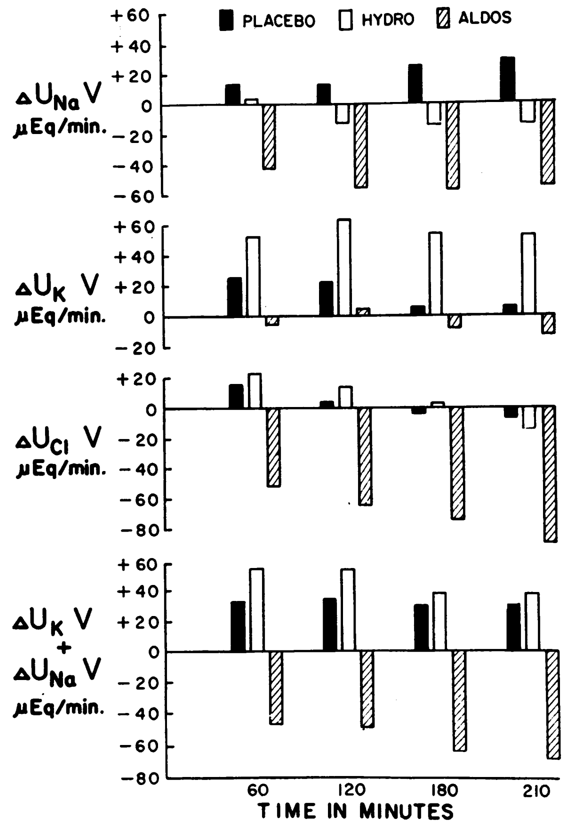

FIG. 2. MEAN CHANGES IN ELECTROLYTE EXCRETION PRODUCED BY PLACEBO, HYDROCORTISONE, AND ALDOSTERONE.

Glomerular filtration rate (GFR) and renal plasma flow (RPF) (Table I). In all three groups the mean change in GFR varied less than 4%. These alterations were not statistically significant and are within the expected experimental error. In the individual experiments there was no correlation between changes in GFR and the magnitude of the alteration in U_{osm} or urinary NUS.

RPF rose 27 ml per minute, 26 ml per minute, and 30 ml per minute in the placebo, hydrocortisone, and aldosterone groups, respectively. There was no significant difference between the groups.

Group II A. Hydropenia-solute diuresis (Figure 3). Over a comparable range of osmolar clearance, the $T^c_{H_2O}/C_{osm}$ curves were almost identical in the placebo and aldosterone groups. After the administration of hydrocortisone, the $T^c_{H_2O}/C_{osm}$ curve was consistently higher than those noted in the placebo and aldosterone groups.

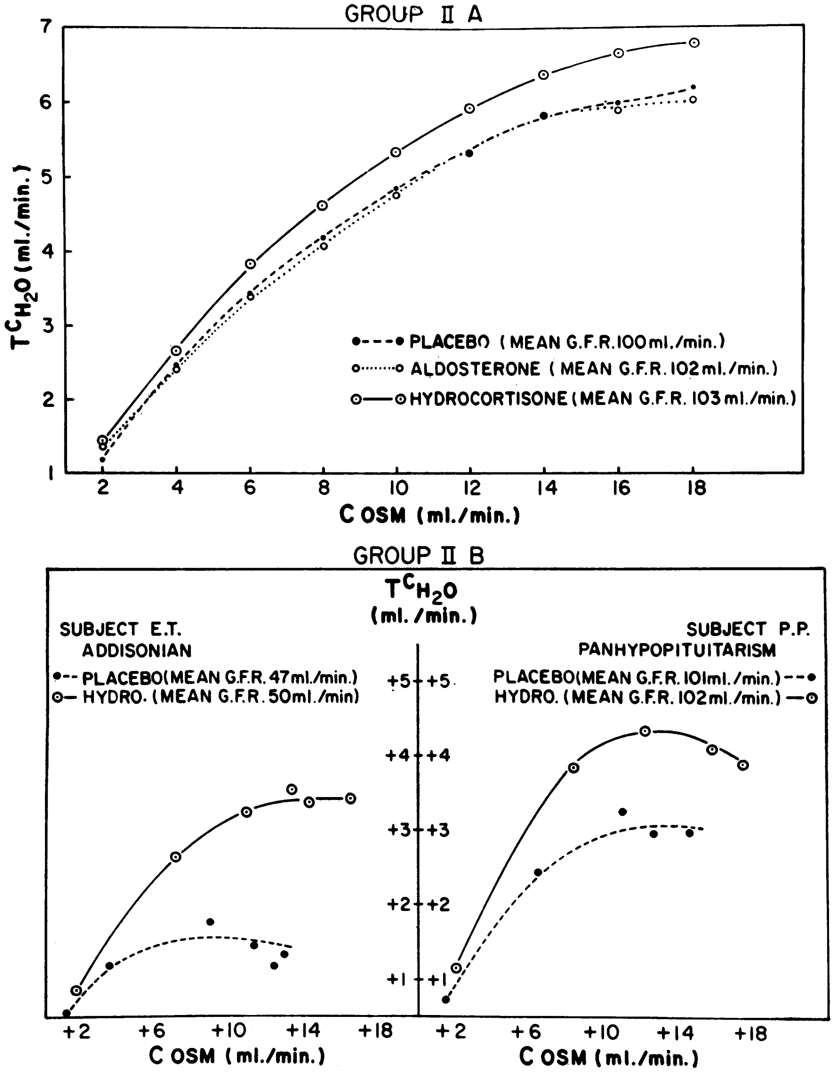

FIG. 3. (Upper) EFFECT OF PLACEBO, HYDROCORTISONE, AND ALDOSTERONE ON FREE WATER REABSORPTION ($T^{c}_{H_2O}$) DURING A MANNITOL DIURESIS IN FIVE NORMAL SUBJECTS. All $T^{c}_{H_2O}$ values are grouped about successive 2-ml increments in C_{osm} and represent the mean values for each group. (Lower) EFFECT OF PLACEBO AND HYDROCORTISONE ON $T^{c}_{H_2O}$ DURING A MANNITOL DIURESIS IN TWO ADRENAL-INSUFFICIENT SUBJECTS.

GFR and RPF were comparable during the solute diuresis in all groups.

Group II B. Adrenal insufficiency-hydropenia-solute diuresis (Figure 3). Hydrocortisone acutely increased $T^{c}_{H_2O}$ in both patients with adrenal insufficiency. The magnitude of the rise (1.3 ml per minute and 1.9 ml per minute) in these subjects with depressed $T^{c}_{H_2O}/C_{osm}$ curves exceeded that produced by hydrocortisone administration in the normal subjects. The change in GFR produced by hydrocortisone in these two subjects was not different from that noted in the group II A studies.

Discussion

With the assumption that urinary NUS concentration reflects medullary salt concentration, the rise in NUS concentration produced by hydrocortisone suggests that this agent increases salt concentration within the medulla. The augmented salt concentration might result from an increased rate of sodium transport at the ascending limb or from a decrease in effective medullary blood flow (decreased "medullary wash-out"). There is no available evidence to suggest that hydrocortisone diminishes medullary blood flow. In fact, the clearance of PAH, although not necessarily a direct index of medullary blood flow, tended to rise in the studies presented here. It appears, therefore, that the increment in medullary NUS concentration can best be explained as a consequence of increased sodium transport at the ascending limb of the loop of Henle. Since $T^c_{H_2O}$ represents an index of ascending limb sodium transport (26–28), the increment in this parameter produced by hydrocortisone is consistent with the proposed action of this agent.

An increase in sodium transport at the loop could result indirectly from an enhanced sodium supply or from a direct hormonal effect on the active sodium transport mechanism located at this site. If there were an increase in supply, it does not appear to have been mediated by a measurable rise in GFR. No significant alteration in GFR was evident in our studies or in those reported by others (19, 29). In fact, hydrocortisone has been shown to produce a prompt rise in GFR only in glucocorticoid or salt-depleted subjects with reduced filtration rates (16–18, 29). When hydrocortisone was administered to these subjects under hydrated conditions, an abrupt increase in GFR and C_{H_2O} occurred. The increase in C_{H_2O}, however, could not be explained solely by the rise in GFR (16–18). Since C_{H_2O} is formed primarily at the ascending limb, the hydrated studies may also be explained by the proposal that hydrocortisone increases ascending limb sodium transport, apart from its effect on GFR. The increments in $T^c_{H_2O}$ noted in normal and Addisonian subjects after hydrocortisone (Figure 3) are compatible with this view.

The rise in potassium excretion produced by hydrocortisone confirms the conclusion that this agent increases the sodium/potassium exchange process (2, 3, 16, 30) located in the late distal tubule and collecting duct (31, 32). Since the increment in potassium excretion was associated with a fall in sodium excretion, this alteration in potassium excretion apparently was due primarily to a direct hormonal effect. However, the data suggest that potassium excretion may, in part, have been enhanced by an increased supply of isosmotic fluid to the late distal tubule and collecting duct. The failure for urine flow to fall in the face of a more concentrated medulla is consistent with an increased water supply to the collecting duct. In addition, the increment in the combined rate of sodium and potassium excretion (Figure 2) implies that there was a coincident increase in sodium supply to these distal sites.

In summary, it has been suggested that hydrocortisone acutely enhances sodium transport at the ascending limb without a decrease (and possibly a modest increase) in sodium and water supply to more distal sites. This combination of findings is explicable only if the rate of isosmotic fluid escaping proximal tubular reabsorption has been augmented. This increment in loop sodium supply can result only from a hydrocortisone-induced inhibition of proximal tubular sodium reabsorption or an increase in GFR produced by this hormone, or both. If this increase in loop solute supply is due to a rise in GFR rather than to a proximal tubular block, the alteration is too small to be measured by our present techniques. In addition to its proximal effect, hydrocortisone apparently directly enhances sodium/potassium exchange in the late distal tubule and collecting duct. It is tempting to ascribe to hydrocortisone a similar direct hormonal effect on the rate of sodium transport at the ascending limb. Other investigators have suggested that methylprednisolone exerts such a direct hormonal action at this site (19). Although our data do not contradict this interpretation, the increase in ascending limb sodium transport may be explained entirely by an increased sodium supply to the loop of Henle.

The administration of aldosterone did not appear to alter renal hemodynamics. In constrast to hydrocortisone, aldosterone produced a distinct fall in sodium and chloride excretion, solute clearance, and urine flow rate without a significant change in urinary NUS concentration. Apparently aldosterone enhances sodium and chloride reabsorption without effecting medullary salt concentration. Other physiologic stimuli producing a fall in sodium excretion have been shown to reduce urinary NUS concentration in both man and dog (25, 27, 33). It was suggested that these stimuli decreased sodium supply to the loop of Henle. The failure of aldosterone to alter urinary NUS concentration implies that this agent did not exert its action primarily in the proximal tubule or ascending limb. Thus, it is suggested that aldosterone enhances sodium and chloride reabsorption in the distal convoluted tubule. This proposed distal site of action of aldosterone is consistent with the failure of this agent to effect $T^c_{H_2O}$ formation, another parameter reflecting loop sodium transport during a mannitol diuresis. Moreover, the observation that aldosterone reduces U_{osm} in hydrated subjects is also explicable by this proposal (7). Finally, experiments utilizing the stop-flow technique have indicated that aldosterone influences a distal sodium absorptive mechanism (4, 5).

An alternative explanation to a singular tubular site of action is an aldosterone-induced increase in sodium reabsorption throughout the nephron. Such an action would demand a delicate balance between decreased loop sodium supply (increased proximal reabsorption) and an enhanced rate of sodium transport at the ascending limb. This balance of effects would have to be precisely adjustable to progressively changing loop sodium supply and altered tubular fluid sodium concentrations to explain the failure of aldosterone to influence $T^c_{H_2O}$ during increasing mannitol diuresis. For these reasons, an effect of aldosterone throughout the tubule appears unlikely.

The rise in U_{Urea} associated with the almost inversely proportional fall in V noted in the present studies (Table II) indicates that the aldosterone-enhanced absorption of water occurred at a segment relatively impermeable to urea despite the presence of antidiuretic hormone (ADH). Others have suggested previously that the distal tubule possesses such a low order of permeability to urea (24, 34). More recently, in micropuncture studies a concentration gradient for urea was demonstrated in the distal convoluted tubule (35, 36). Thus, the observed changes in U_{Urea} and V are also compatible with the proposal that aldosterone enhances salt and water reabsorption in the distal tubule. This action of aldosterone would decrease flow rate and increase urea concentration of the isosmotic fluid entering the collecting duct. The higher tubular fluid urea concentration would favor the passage of urea from collecting duct into the medulla. Since the collecting duct membrane is highly permeable to urea, this solute would equilibrate between collecting duct fluid and medullary interstitium. When equilibrium has been established, medullary urea and total urine solute concentration will be increased without any alterations in NUS concentration.

It would be anticipated that the aldosterone-induced increment in sodium and chloride reabsorption would decrease sodium available for sodium/potassium exchange. However, after an initial decrease, potassium excretion remained unchanged as sodium excretion continued to fall (Figure 2). Apparently aldosterone increases the rate of sodium/potassium exchange despite a progressive decrease in sodium available for exchange. These data are consistent with the reports of others that aldosterone has a direct hormonal action on the sodium/potassium exchange mechanism in addition to increasing sodium and chloride reabsorption in the distal convoluted tubule (3, 7, 37, 38).

The aldosterone studies revealed that as urine flow rate decreased, total urine solute concentration increased while NUS concentration and presumably medullary salt concentration remained unchanged. Others investigators have also noted this divergence of urine osmolality and NUS concentration (39, 40). This increase in urine concentration without an increment in medullary salt concentration may result from the relative impermeability of the distal convoluted tubule to urea. Thus, an increase in distal abstraction of water will increase urine solute concentration without an effect on the countercurrent multiplier system. The importance of distal tubule impermeability to urea in the determination of urine solute concentration under conditions of low flow demands the separation of urea and nonurea-solute when studying the concentration mechanism under these conditions.

Summary

1) Changes in urine solute concentration, urine urea concentration, electrolyte excretion, and renal hemodynamics produced in normal hydropenic subjects by the intravenous administration

of hydrocortisone and aldosterone were compared to those produced by a placebo injection administered under the same experimental conditions.

2) Hydrocortisone produced a significant increase in urine osmolality without any alteration in urine flow rate, solute clearance, and urine urea concentration. Aldosterone also increased urine solute concentration but with a significant decrease in urine flow rate and solute clearance. In contrast to hydrocortisone, the increment in urine solute concentration produced by aldosterone was entirely accounted for by the increase in urine urea concentration.

3) Neither hydrocortisone nor aldosterone influenced renal hemodynamics. Hydrocortisone increased potassium excretion and decreased sodium excretion to a lesser extent. Aldosterone produced a significant reduction in sodium and chloride excretion with only a minor decrease in potassium excretion.

4) Hydrocortisone increased $T^c_{H_2O}$ formation in both normal and adrenal-insufficient subjects. This parameter was not affected by aldosterone.

5) These data indicate that hydrocortisone enhances sodium supply and transport at the ascending limb. In contrast, aldosterone appears to enhance directly sodium and chloride reabsorption in the distal convoluted tubule. Both agents also directly augment the sodium/potassium exchange mechanism in the late distal tubule and collecting duct.

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