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The Effect of Mineralocorticoid Deficiency on Renal Concentrating and Diluting Capacity (38646)

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(Introduced by G. Eknoyan)

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The localization of action of aldosterone in the nephron has been quite controversial and has not been clearly defined. Previous studies (1–3) have suggested that aldosterone had no effect on sodium reabsorption in the proximal tubule. Recent studies by Gill and coworkers (4) suggest that aldosterone stimulates sodium reabsorption in the proximal tubule. Most of the experimental evidence accumulated thus far suggests that aldosterone acts on the distal tubule (5–6); however, studies evaluating its action on the ascending limb are few and contradictory (7–11).

The present study was designed to evaluate the effect of aldosterone on sodium reabsorption in the ascending limb of Henle's loop in the nephron of the dog by studying the effect of aldosterone deficiency on renal concentrating and diluting capacity. We reasoned that if aldosterone were necessary for sodium transport by the ascending limb of the loop of Henle, its absence should result in impaired concentrating and/or diluting capacity of the kidney.

Methods. Studies measuring solute free water excretion ($^{C}H_2O$) and reabsorption ($^{Tc}H_2O$) were performed on each of six mongrel dogs in both the mineralocorticoid sufficient and aldosterone deficient states. These studies were performed on three healthy dogs receiving 1.0 mg desoxycorticosterone acetate (DOCA) daily, then repeated after bilateral adrenalectomy while receiving only dexamethasone 0.75 mg daily. The same studies were also performed on three bilaterally adrenalectomized dogs receiving parenteral DOCA 1.0 mg, and dexamethasone 0.75 mg daily. Thus, two groups of mineralocorticoid sufficient and aldosterone deficient dogs were studied, each dog being studied on four separate occasions, with a total of 24 experiments being performed. Free water reabsorption was also studied in four normal dogs not given exogenous desoxycorticosterone prior to the study and not given ADH during the study. This group was studied to see if the small dose of exogenous DOCA, given to insure adequate amounts of circulating mineralocorticoid caused some degree of volume expansion and resultant inhibition of sodium reabsorption in the ascending limb. ADH was not used in this group of animals since pharmacologic doses have been shown to cause a natriuresis, possibly by inhibiting sodium reabsorption in the ascending limb (12, 13). Dogs in the aldosterone deficient state were allowed to develop hyperkalemia and mild metabolic acidosis after adrenalectomy to make certain that adrenal insufficiency had been induced. The dogs were treated with DOCA and allowed to recover prior to the study. A minimum of ten days was allowed between studies on the same dog. Daily weights were obtained on all dogs.

All the dogs studied during water diuresis were placed on a "zero" electrolyte diet, identical to that described by Cohen (14) to which 50 mEq NaCl were added daily. Water *ad libitum* was given for 3 days prior to the study. DOCA was withheld for 96 hr prior to those studies done in the aldosterone deficient state. One hour prior to beginning the study, 50 ml of water/kg body wt was administered via a nasogastric tube. Water diuresis was maintained by the continuous intravenous infusion of 0.45% saline at 1.0 ml/kg/min for 20 min and then the rate of infusion was varied from 0.25 to 1.0 ml/kg/min. The urine osmolality was less than 75 mOsm/kg prior to the initiation of the 10-min collection periods.

TABLE I. SOLUTE-FREE WATER EXCRETION IN A MINERALOCORTICOID SUFFICIENT DOG.

Time	GFR	V	U_{Osm}	P_{Osm}	C_{Osm}	C_{H_2O}	$\frac{C_{H_2O}}{GFR} \times 100$	$\frac{V}{GFR} \times 100$	$\frac{C_{Na}}{GFR} \times 100$	$\frac{C_K}{GFR} \times 100$
min	ml/min	ml/min	mOsm/kg	mOsm/kg	ml/min	ml/min	%	%	%	%
Dog IG4, wt 11.7 kg										
0–20	Infuse 0.45% NaCl at 1.0 ml/kg/min									
20–30	Infuse 0.45% NaCl at 0.25 ml/kg/min									
30–40	31.2	2.80	69	299	0.65	2.15	6.89	8.97	0.38	3.20
40–50	33.8	2.85	63	295	0.61	2.24	6.62	8.43	0.27	3.12
50–60	39.5	3.30	57	294	0.64	2.66	6.73	8.35	0.21	3.09
	Infuse 0.45% NaCl at 0.50 ml/kg/min									
60–70	38.2	3.10	57	293	0.60	2.50	6.54	8.11	0.26	3.11
70–80	36.5	3.75	62	298	0.78	2.97	8.13	10.27	0.54	3.95
80–90	39.7	5.00	67	298	1.12	3.88	9.77	12.59	0.67	4.10
90–100	37.9	4.50	61	302	0.91	3.59	9.47	11.87	0.71	4.39
	Infuse 0.45% NaCl at 0.75 ml/kg/min									
100–110	39.0	4.85	71	303	1.14	3.71	9.51	12.43	1.57	4.60
110–120	40.5	5.60	82	295	1.56	4.04	9.97	13.82	2.43	5.52
120–130	40.9	5.20	83	295	1.46	3.74	9.14	12.71	2.20	5.08

All dogs studied during solute diuresis were placed on the same "zero" electrolyte diet to which 50 mEq/day of NaCl was added for 3 days prior to the study. Water was withheld 48 hr and food withheld 24 hr prior to the study. On the day of the experiment, 5 units of vasopressin in oil were injected intramuscularly, 2 hr before the study was started. Thereafter a continuous infusion of 50 munits/kg/hr of aqueous vasopressin was given. Solute diuresis was achieved by infusing 5% saline; the rate of infusion varied from 0.5 to 5.0 ml/min. Collection periods were 10 min in length. Solute diuresis in the normal group of dogs, not given DOCA and ADH, was achieved in same manner.

All studies were performed while the dogs were anesthetized with sodium pentobarbital, 30 mg/kg. Additional anesthetic was given as required to maintain light anesthesia as judged by the maintenance of corneal reflexes. An endotracheal tube fitted with an inflatable cuff was placed in the trachea and connected to a Bird respirator. The pCO_2 was maintained between 35 and 45 mmHg. Arterial blood samples were obtained anaerobically via an arterial catheter in the femoral artery. Urine was collected from an indwelling bladder catheter.

The serum and urine osmolalities were measured with an Advanced Instruments Osmometer. The partial pressure of CO_2 in the blood and glomerular filtration rates were determined by methods previously described (15). Calculations of C_{Osm}, C_{H_2O}, and $T^C_{H_2O}$ were computed in the usual manner.

Results. Free water clearance. Representative experiments during water diuresis in the same dog (IG4) under conditions of mineralocorticoid sufficiency and deficiency are shown in Tables I and II respectively. Only small differences in urine flow (V), C_{Osm}, C_{H_2O}, and GFR for both states are present.

Figure 1 plots C_{H_2O}/100 ml GFR against V/100 ml GFR for each group of animals in both the mineralocorticoid sufficient and aldosterone deficient states. Aldosterone deficiency does not significantly alter the relationship of C_{H_2O}/100 GFR to V/100 GFR. The regression line for the data obtained during the mineralocorticoid sufficient state is $Y = 0.43 + 0.73X$ compared to $Y = 0.54 + 0.71X$ for the aldosterone deficient group. The r^2 value for both groups is 0.94. Paired analysis demonstrated no significant difference in free water excretion between the mineralocorticoid sufficient and deficient groups.

Freewater reabsorption. $T^C_{H_2O}$ was examined in the same dogs utilized for evaluation of solute-free water excretion. Again,

TABLE II. SOLUTE-FREE WATER EXCRETION IN AN ALDOSTERONE DEFICIENT DOG.

Time	GFR	V	U_{Osm}	P_{Osm}	C_{Osm}	C_{H_2O}	$\frac{C_{H_2O}}{GFR} \times 100$	$\frac{V}{GFR} \times 100$	$\frac{C_{Na}}{GFR} \times 100$	$\frac{C_K}{GFR} \times 100$
min	ml/min	ml/min	mOsm/kg	mOsm/kg	ml/min	ml/min	%	%	%	%
Dog IG4, wt 11.6 kg										
0–20	Infuse 0.45% NaCl at 1.0 ml/kg/min									
20–30	Infuse 0.45% NaCl at 0.25 ml/kg/min									
30–40	35.8	2.60	65	281	0.60	2.00	5.58	7.26	0.60	2.34
40–50	38.0	3.30	60	281	0.70	2.60	6.84	8.68	0.71	2.80
50–60	43.8	3.75	62	282	0.82	2.93	6.68	8.56	0.76	2.76
	Infuse 0.45% NaCl at 0.50 ml/kg/min									
60–70	36.6	3.50	56	277	0.71	2.79	7.62	9.56	0.83	3.08
70–80	36.6	3.70	59	277	0.79	2.91	7.95	10.10	1.01	3.36
80–90	35.8	4.05	61	277	0.89	3.16	8.82	11.31	1.13	3.43
	Infuse 0.45% NaCl at 0.75 ml/kg/min									
90–100	32.0	4.10	61	280	0.89	3.21	10.03	12.81	1.57	3.88
100–110	40.3	4.60	67	277	1.11	3.49	8.66	11.41	1.68	4.05
110–120	33.7	4.10	73	287	1.04	3.06	9.08	12.16	1.74	4.06
	Infuse 0.45% NaCl at 1.0 ml/kg/min									
120–130	31.3	4.20	72	274	1.10	3.10	9.90	13.41	2.25	4.44
130–140	33.3	5.00	78	283	1.38	3.62	10.87	15.01	2.71	5.18
140–150	37.0	4.10	77	275	1.43	3.67	9.91	13.78	2.58	4.45

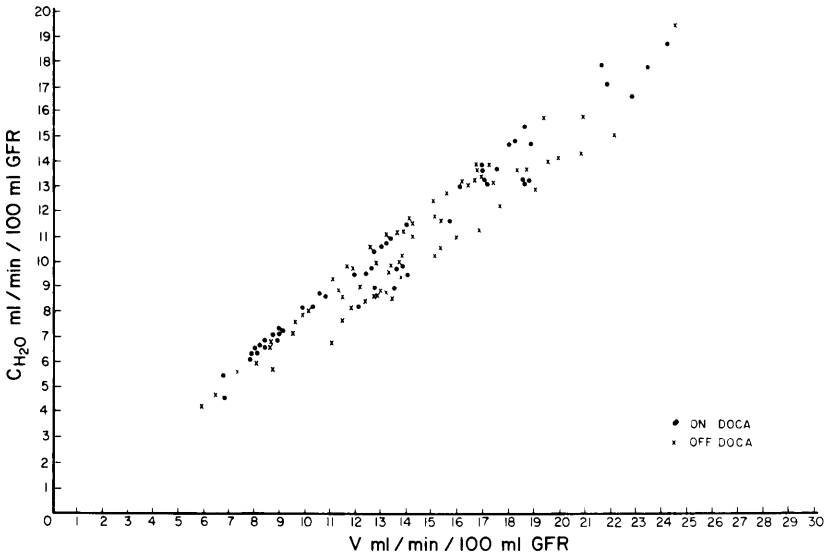

FIG. 1. Comparison of C_{H_2O}/100 ml GFR plotted against V/100 ml GFR for the entire group of animals in both the mineralocorticoid sufficient and aldosterone deficient state.

studies were performed in both the mineralocorticoid sufficient and aldosterone deficient states. Representative experiments during solute diuresis under both conditions of mineralocorticoid sufficiency and deficiency performed on dog IG4 are presented in Table III and IV. C_{Osm}, and $^{T}C_{H_2O}$/100 ml GFR vary only slightly in both states.

Figure 2 plots $^{T}C_{H_2O}$/100 ml GFR against C_{Osm}/100 ml GFR using data obtained from all six dogs. Aldosterone deficiency has no effect on the relationship of $^{T}C_{H_2O}$/100 ml GFR against C_{Osm}/100 ml GFR. The regression line for the data from the

TABLE III. SOLUTE-FREE WATER REABSORPTION IN A MINERALOCORTICOID SUFFICIENT DOG.

Time	GFR	V	U_{Osm}	P_{Osm}	C_{Osm}	$T^C_{H_2O}$	$\frac{C_{Osm}}{GFR} \times 100$	$\frac{T^C_{H_2O}}{GFR} \times 100$	$\frac{C_{Na}}{GFR} \times 100$	$\frac{C_K}{GFR} \times 100$
min	ml/min	ml/min	mOsm/kg	mOsm/kg	ml/min	ml/min	%	%	%	%
Dog IG4, wt 11.8 kg										
0–20	Infuse 5% NaCl at 0.5 ml/min + aqueous vasopressin in saline at 50 munits/kg/hr (0.5 ml/min)									
20–30	36.5	0.35	1020	303	1.10	0.82	3.23	2.24	1.36	17.80
30–40	33.2	0.40	872	303	1.15	0.75	3.46	2.25	1.81	20.30
40–50	29.1	0.45	876	303	1.30	0.85	4.46	2.92	2.40	30.90
	Infuse 5% NaCl at 1.0 ml/min									
50–60	37.8	1.00	669	297	2.25	1.25	5.95	3.30	3.60	39.70
60–70	43.4	1.30	632	303	2.71	1.41	6.24	3.24	4.32	37.90
70–80	40.3	1.65	560	304	3.04	1.39	7.54	3.44	5.62	36.10
	Infuse 5% NaCl at 3.0 ml/min									
80–90	39.5	2.20	522	311	3.69	1.49	9.34	3.77	7.53	43.70
90–100	39.7	2.80	555	319	4.87	2.07	12.20	5.18	10.80	52.80
100–110	45.5	3.80	542	330	6.24	2.44	13.71	5.36	12.70	54.10
	Infuse 5% NaCl at 5.0 ml/min									
110–120	40.7	4.10	538	351	8.28	2.10	15.42	5.35	14.10	52.20
120–130	39.5	5.50	506	368	7.56	2.06	19.44	5.21	17.50	58.40
130–140	39.9	5.90	505	375	7.54	1.94	18.89	4.86	18.70	53.20

TABLE IV. SOLUTE-FREE WATER REABSORPTION IN AN ALDOSTERONE DEFICIENT DOG.

Time	GFR	V	U_{Osm}	P_{Osm}	C_{Osm}	$T^C_{H_2O}$	$\frac{C_{Osm}}{GFR} \times 100$	$\frac{T^C_{H_2O}}{GFR} \times 100$	$\frac{C_{Na}}{GFR} \times 100$	$\frac{C_K}{GFR} \times 100$
min	ml/min	ml/min	mOsm/kg	mOsm/kg	ml/min	ml/min	%	%	%	%
Dog IG4, wt 11.7 kg										
0–20	Infuse 5% NaCl at 0.5 ml/min + aqueous vasopressin in saline at 50 munits/kg/hr (0.5 ml/min)									
20–30	30.0	0.25	1406	302	1.15	0.90	3.83	3.00	1.20	21.20
30–40	33.2	0.35	1171	306	1.37	1.02	4.12	3.07	1.26	30.11
40–50	26.9	0.35	978	306	1.12	0.77	4.16	2.86	1.72	25.42
	Infuse 5% NaCl at 1.0 ml/min									
50–60	41.7	0.40	1208	304	1.59	1.19	3.81	2.85	2.63	39.36
60–70	34.0	0.45	1039	308	1.52	1.07	4.47	3.14	3.20	48.34
70–80	33.5	0.50	1008	307	1.64	1.14	4.89	3.40	3.60	51.18
	Infuse 5% NaCl at 3.0 ml/min									
80–90	41.4	0.95	947	313	2.87	1.92	6.93	4.63	4.10	58.40
90–100	34.8	1.35	723	331	2.99	1.64	8.59	4.71	4.72	67.02
100–110	36.9	2.40	631	334	4.53	2.13	12.61	5.93	8.33	57.04
	Infuse 5% NaCl at 5.0 ml/min									
110–120	41.8	4.10	553	350	6.48	2.38	15.50	5.69	11.45	58.81
120–130	42.0	4.30	525	350	6.45	2.15	15.35	5.11	13.36	34.96
130–140	42.1	5.10	515	341	7.70	2.60	18.28	6.17	15.44	35.46

normal group is $Y = 1.19 + 0.29X$ as compared to $Y = 1.81 + 0.25X$ in the aldosterone deficient group. The r^2 value is 0.93 in the mineralocorticoid sufficient group and 0.92 in the aldosterone deficient group. Paired analysis demonstrated no significant difference between the two groups.

The regression line for the normal dogs not given DOCA or ADH is $Y = 2.13 + 0.26X$. Determination of the difference

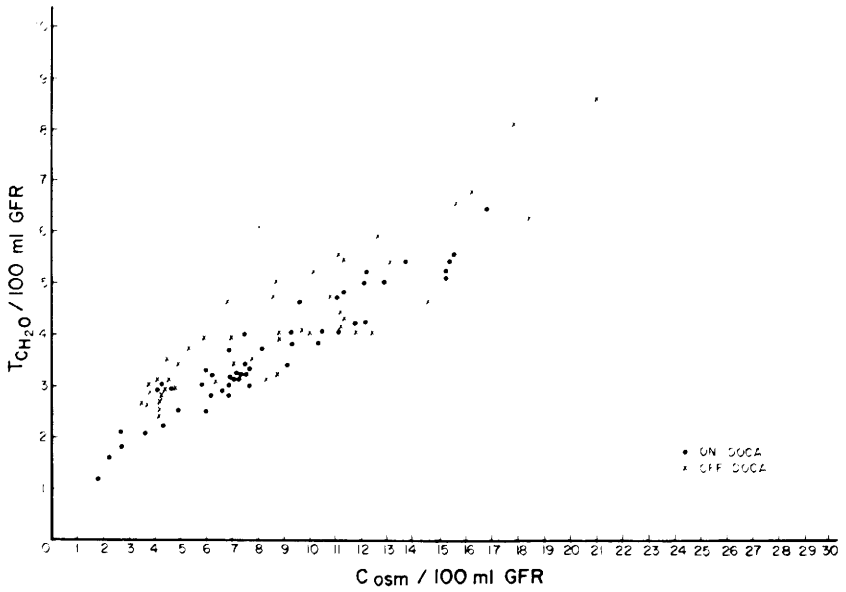

FIG. 2. Comparison of $^{TC}H_2O$/100 ml GFR plotted against ^{C}Osm/100 ml GFR for the entire group of animals in both the mineralocorticoid sufficient and aldosterone deficient state.

between the two linear regressions from the mineralocorticoid sufficient dogs, given DOCA and ADH and the normal group of dogs, not given DOCA and ADH, using the Student's *t* test failed to demonstrate any statistical difference between the two groups. Thus, the physiologic doses of DOCA administered to the normal dogs studied in the mineralocorticoid sufficient state resulted in no impairment of sodium reabsorption and $^{TC}H_2O$ formation as measured with these clearance techniques. The administration of ADH during solute diuresis also apparently had no effect on $^{TC}H_2O$ formation since regression analysis showed no difference between the mineralocorticoid sufficient dogs given 50 munits/kg/hr ADH during the study and the normal dogs in which the ADH infusion was not administered.

Maximal urinary concentration in the mineralocorticoid sufficient group was 1356 mOsm/kg ± 254 (SD) and 1386 mOsm/kg ± 331 (SD) in the aldosterone deficient group. This difference is obviously not significant. DOCA treatment did not result in weight gain in any of the dogs studied.

Discussion. Although the effects of aldosterone on electrolyte excretion are well known (16–20), there has been much controversy with respect to the specific site of action of aldosterone in the nephron. Several investigators have suggested a defect in proximal tubular sodium reabsorption following adrenalectomy (21–23). Recent studies by Kurtzman *et al.* (1) have provided inferential evidence that aldosterone deficiency results in no significant net depression of proximal sodium reabsorption. Wright *et al.* (3), using direct measurements failed to demonstrate any effect of selective aldosterone deficiency on sodium reabsorption in the proximal tubule; however, Gill and coworkers (4) have recently shown that sodium reabsorption in the proximal tubule is increased with the administration of DOCA.

Since there is suggestive evidence that aldosterone may stimulate sodium reabsorption in the ascending limb of Henle's loop (8, 9, 11), further studies were indicated to determine if this segment of the nephron is responsive to the influence of aldosterone.

This study was designed to measure the effect of aldosterone deficiency on the ascending limb of the loop of Henle, in the nephron of the dog, by measurement of solute-free water clearance and reabsorption. If aldosterone has a stimulatory effect on

sodium reabsorption in the ascending limb of Henle's loop, the clearance of solute-free water, during water diuresis, and the reabsorption of solute-free water, at any level of urinary flow or osmotic clearance respectively, would be depressed in its absence. Since our data failed to demonstrate any defect of aldosterone deficiency on free water clearance and reabsorption, or on maximal urinary concentration, we conclude that aldosterone exerts no gross effect of sodium reabsorption in the ascending limb of Henle's loop. It is possible, however, that the clearance techniques used in this study might fail to detect a small, but physiologically significant, effect of aldosterone on ascending limb transport.

A depressive effect of aldosterone deficiency on ascending limb sodium reabsorption could possibly have been missed by DOCA administration and/or saline administration with expansion of extracellular volume. This volume expansion might result in depressed sodium reabsorption by the ascending limb of the loop of Henle so that the results obtained in our control dogs were really depressed results, secondary to volume expansion. When these results were compared with those obtained from the aldosterone deficient animals no difference would be discernible.

If volume had not been expanded, there would have been a clear cut difference between the control and the aldosterone deficient groups. We think that this series of events played no role in our study for the following reasons. First, none of our animals gained weight while receiving DOCA. These animals received only 1 mg/day of DOCA. Our experience indicates that for adrenalectomized animals weighing 7.5–15 kg a maintenance dose of DOCA is 0.5–1.0 mg/day. The dose of DOCA required for volume expansion is 1 mg/kg or more. Thus, it is unlikely that volume expansion occurred in any of our animals.

Second, if the lines we obtained from plotting $^{C}H_2O$/GFR versus V/GFR are compared to those of other investigators who did not give DOCA to their animals and administered 0.45% saline, 0.125% saline, or 2.5% glucose (24, 25, 27), one finds that in each instance $^{C}H_2O$/100 ml GFR at any level of V/100 ml GFR is equal or greater in our animals than in the others, indicating that ascending limb reabsorption in our animals was not depressed. Similar results are obtained if one compares our $^{TC}H_2O$ data with that of others (24, 25).

Third, Barton and coworkers (28) have recently demonstrated that in the dog extracellular volume expansion (with DOCA and saline) does not depress sodium reabsorption in the diluting segment of the ascending limb, indicating that even if volume had been expanded, it would not have influenced our results.

To further assess the effect of DOCA on sodium reabsorption in the ascending limb, free water reabsorption was studied in a group of normal dogs to which no DOCA was administered. As previously stated, there was no statistical difference in the regression lines for $^{TC}H_2O$/100 ml GFR between the two groups. These data are in agreement with that of Barton and colleagues (28), suggesting that DOCA induced volume expansion does not inhibit sodium reabsorption in the ascending limb of the loop of Henle.

The information presented in this study, when added to that which demonstrates no effect of aldosterone on proximal sodium reabsorption, suggests that aldosterone exerts its sole effect on renal sodium reabsorption in a localized segment of the distal tubule (presumably the sodium for potassium exchange site). Thus, under the influence of aldosterone, sodium is reabsorbed in exchange for either potassium or hydrogen ion. Sodium may also be reabsorbed as sodium chloride in this site of the nephron. Without aldosterone there is persistent loss of small amounts of sodium, chloride, and bicarbonate resulting in the progressive contraction of extracellular volume, mild metabolic acidosis, and hyperkalemia characteristic of adrenal insufficiency (1).

This study also provides further evidence that the inability to excrete a water load in adrenal insufficiency is unrelated to mineralocorticoid deficiency. The ability of glucocorticoid hormones to restore the ability to excrete a water load to subjects with adrenal insufficiency may be the con-

sequence of either inhibition of vasopressin release from the neurohypophysis (29) or a direct effect of glucocorticoids on renal tubular permeability. The recent studies of Ufferman and Schrier (30) provide further evidence for this belief. They showed that urinary dilution was unimpaired in mineralocorticoid deficient animals in which volume contraction was prevented by salt administration.

Summary. To examine the effect of mineralocorticoid deficiency on sodium transport by the ascending limb of the loop of Henle, free water clearance and reabsorption were measured in the same six dogs under conditions of aldosterone deficiency and mineralocorticoid sufficiency. Aldosterone deficiency was induced by bilateral adrenalectomy with dexamethasone replacement. $^{C}H_2O$/100 ml GFR ranged from 4.0 to 19.5 in the aldosterone deficient dogs and 4.5–18.6 in the mineralocorticoid sufficient dogs. $^{C}H_2O$/100 ml GFR plotted against V/100 ml GFR showed no significant difference between the two groups. $^{Tc}H_2O$/100 ml GFR ranged from 1.2 to 6.5 in the mineralocorticoid sufficient group and 2.4–8.5 in the aldosterone deficient group. $^{Tc}H_2O$/100 GFR plotted against ^{C}Osm/100 ml GFR revealed no significant difference between the two groups. Maximal urine concentration in the mineralocorticoid sufficient group was 1356 mOsm/kg ± 254 (SD) and 1386 mOsm/kg ± 331 (SD) for the aldosterone deficient group; the difference is not significant. This study failed to demonstrate any effect of aldosterone deficiency on renal concentrating and diluting capacity and thus provides inferential evidence against an effect of aldosterone on ascending limb sodium reabsorption.

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