Proximal and Distal Tubular Function in Salt-Deprived and in Salt-Loaded Deoxycorticosterone Acetate-Escaped Rats

H. SONNENBERG with the technical assistance of A. MARSDEN-POTTER

From the Department of Physiology, University of Toronto, Toronto 181, Ontario, Canada

ABSTRACT Proximal and distal tubular function was compared with urinary excretion in rats after chronic administration of salt and deoxycorticosterone acetate (DOCA) or during salt deprivation. DOCA rats excreted significantly more sodium than did salt-deprived rats. Measurements of tubular fluid to plasma (TF/P) inulin ratios and concentrations of sodium and potassium in quantitative, timed collections, related to measured tubular length, allowed calculation of absolute reabsorption of fluid and ions in the different nephron segments. Proximal transport was not reduced in DOCA-treated rats compared with salt-deprived animals; in distal tubule the former group reabsorbed more sodium and secreted less potassium than the latter. Calculation of sodium transport in loop of Henle as the difference in flow between the end of the proximal convolution and the beginnings of the distal tubule indicated no inhibition of reabsorption in DOCA animals. Comparison of end-distal tubular flow with simultaneous urinary excretion suggested that sodium load was not the determining factor of enhanced natriuresis in DOCA-treated animals. The data are interpreted as indicating that DOCA-escape in the rat is associated with specific alteration of sodium transport in the collecting duct system.

INTRODUCTION

Chronic administration of mineralocorticoid results in transient sodium retention, followed by increasing natriuresis to control levels (1, 2). The mechanisms of this "escape" from the salt-retaining effects of the hormone remain uncertain. One possibility is development of insensitivity to mineralocorticoids, presumably in distal tubule; another is an increase in circulating levels of an unknown natriuretic substance (3). In dogs, a reduction of proximal tubular reabsorption has been observed during deoxycorticosterone acetate (DOCA) [1]-escape (4), and suggested as a cause for the rise in sodium excretion. Since fractional transport in proximal tubule may be inhibited with little change in natriuresis (5) absolute as well as fractional reabsorptions were studied in proximal and distal tubules of rats showing enhanced urinary sodium excretion despite continued steroid administration. Rats with endogenously elevated levels of aldosterone and continued salt retention were used for comparison.

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METHODS

Male Sprague-Dawley rats (weight range = 217–395 g) were either salt-deprived, receiving the "salt-deficient-diet-modified" of Nutritional Biochemicals Corp. and distilled water for at least 3 wk prior to an experiment (27 NaD rats), or pretreated with deoxycorticosterone acetate (2.5 mg DOCA in oil injected subcutaneously on 5 consecutive days/wk for 3 wk) while receiving Purina rat chow and Ringer's solution to drink (29 DOCA rats). The latter animals were used for experiments during a subsequent 2–3 wk period, while being maintained on Ringer's solution and weekly injections of DOCA. Experiments were usually performed 3–4 days after the last injection of the steroid.

Animals were anaesthetized with Inactin, sodium ethyl-(1-methyl-propyl)-malonyl-thiourea (Promonta, Hamburg, Germany) (10 mg/100 g body weight i.p.) and maintained at a body temperature of 38°C±0.5 on an electrically heated operating table. The left kidney was carefully mobilized (but not decapsulated) through a flank incision and placed in a lucite cup. A flow of prewarmed mineral oil prevented drying or cooling of the exposed kidney surface. Both ureters were cannulated through the flank incision: urine from the right, nonexposed kidney was returned continuously to the circulation via a uretero-venous shunt; that from the left kidney was collected for subsequent analysis. A jugular vein and femoral artery were cannulated for infusion, and arterial pressure measurements and blood sampling, respectively. Animals used for proximal micropunc-

[1] *Abbreviations used in this paper:* C_{In}, glomerular filtration; DOCA, deoxycorticosterone acetate; NaD, salt-deprived; SGFR, single nephron filtration rate; TF/P, tubular fluid to plasma concentration ratio; U_KV, urinary excretion of potassium; $U_{Na}V$, urinary excretion of sodium; V, urinary excretion of fluid.

ture (see below) also had a catheter threaded into the left renal vein (6). On completion of operative procedures a priming dose of [^{3}H]inulin in Ringer's solution (250 μCi in 0.5 ml/100 g body weight) was injected intravenously followed by a maintenance infusion of [^{3}H]-inulin-containing Ringer's at a rate of 0.5 ml/100 g body weight per h. After an equilibration time of ½–1 h, consecutive 15- or 20-min urine collections were taken from the exposed kidney for 1½–2 h; arterial blood samples (0.1 ml) were obtained for each collection period.

Concurrently, quantitative, timed samples of either proximal or distal tubular fluid were collected by micropuncture. A tubule was punctured with a sharpened glass capillary pipette containing colored heavy mineral oil and filled with the oil downstream to the puncture site. Oil displaced proximally was aspired until tubular fluid entered the pipette. This procedure was usually complete within 10-15 s for proximal, and 30–60 s for distal tubule. Fluid was then collected quantitatively for 5–10 or 10–20 min, respectively. Occasional gentle suction was used to ensure continuous fluid flow without tubular distention or movement of the intratubular oil column.

Proximal tubules were studied in 21 DOCA and 22 NaD rats; distal micropuncture was performed in 13 DOCA and 11 NaD rats. These groups include five and six animals, respectively, in which both proximal and distal collections were taken. Simultaneous clearance measurements were not obtained in five DOCA and four NaD rats of the proximal series. On completion of the experimental procedures the majority of animals were subsequently used for studies on blood volume regulation (6, 7). At the end of the experiment punctured tubules were filled with liquid latex, the puncture site marked with Sudan black, and lengths from glomerulus to collection site and last surface convolution (proximal tubule) or from macula densa to puncture site and first confluence of two distal tubules (distal tubule) were measured on the microdissected casts. Occasionally, microdissection revealed a confluence of two distal tubules proximal to the collection site. Quantitative data from such collections were halved and an average length of the two tubules was determined.

Concentration of sodium and potassium in urine and plasma was determined by flame photometry, that of [^{3}H]-inulin by liquid scintillation counting. Urinary excretion of fluid (V) and ions ($U_{Na}V$, U_KV) as well as glomerular filtration (C_{In}) were calculated. The volume of tubular fluid collections was measured with a calibrated micropipette of constant-bore capillary glass and expelled either directly into the scintillation mixture (proximal fluid) or under oil (distal fluid). In the latter case 10–20-nl portions were used for determination of [^{3}H]inulin, as well as for Na and K by helium glow photometry. Tubular fluid osmolality was measured with a Clifton nanoliter osmometer (Clifton Technical Physics, New York). Using the tubular fluid to plasma (TF/P) concentration ratio of inulin and the measured rate of quantitative collection of tubular fluid, single nephron filtration rate (SGFR) and fluid reabsorption to the proximal or distal puncture site were calculated. Filtered load and tubular reabsorption of sodium were then determined for each distal tubule, using in addition, the plasma and tubular fluid concentrations of the ion. Similarly, filtered and distal tubular load of potassium were calculated. Standard statistical methods were used for data reduction and tests of significance (8, 9).

RESULTS

Excretory data for DOCA and NaD rats used for proximal and distal micropuncture are given in Table I. Both proximal and distal collections were made in some animals of the "distal" group. Excretion in both proximal groups was somewhat depressed compared with that of the corresponding distal groups, probably reflecting the more extensive operative procedure in the former (6). In either case, however, DOCA animals excreted significantly more sodium ($P < 0.05$) than NaD rats. Despite the difference in dietary intake, plasma sodium concentrations were not statistically different, while plasma potassium values of DOCA rats were reduced compared to NaD animals. The difference in kidney weights reflects the relative weight gains on the two diets.

TABLE I

Renal Excretion of DOCA and NaD Rats Used for Proximal and Distal Micropuncture

	V	$U_{Na}V$	U_KV	C_{In}	P_{Na}	P_K	KW*
	µl/min/gKW	*neq/min/gKW*	*neq/min/gKW*	*ml/min/gKW*	*meq/liter*	*meq/liter*	*g*
DOCA							
Proximal (n = 16)‡	4.10±1.18§	239±49	413±73	0.88±0.08	146±1	4.1±0.3	1.75±0.08
Distal (n = 13)	7.69±2.43	670±277	592±102	0.88±0.03	142±2	4.2±0.2	1.93±0.09
NaD							
Proximal (n = 18)	3.50±0.60	44.0±10.8	585±116	0.75±0.07	144±1	5.9±0.1	1.39±0.06
Distal (n = 11)	4.18±0.60	53.1±15.8	673±77	1.00±0.04	140±1	5.6±0.1	1.52±0.10

* Experimental kidney weight.

‡ Number of animals.

§ Mean±SE, expressed per gram kidney wieght.

TABLE II

Intratubular Flow, Tubular Fluid to Plasma Insulin Ratio, and Length from Glomerulus to Proximal Collection Site in Individual Rats

	DOCA				NaD		
n*	$\dot{V}_t$	TF/P inulin	Length	n	$\dot{V}_t$	TF/P inulin	Length
	nl/min		*mm*		*nl/min*		*mm*
3	9.5±0.3‡	2.99±1.00	3.15±0.90	3	19.5±1.0	1.97±0.29	1.06±0.32
6	8.4±2.4	3.64±0.66	3.15±0.50	4	16.7±0.8	2.47±0.07	3.78±0.39
1	25.6	1.85	2.43	5	14.3±2.0	2.12±0.26	2.74±0.30
2	8.7±1.9	1.90±0.12	1.72±0.20	1	23.9	2.09	2.08
1	14.4	1.86	2.63	1	9.4	2.32	2.98
2	18.2±4.2	2.05±0.06	3.18±1.62	3	11.7±1.4	2.14±0.21	2.67±0.78
3	15.1±3.2	1.98±0.11	3.36±0.49	3	13.8±3.0	2.38±0.29	3.02±0.51
3	19.1±3.2	1.67±0.14	3.58±0.28	2	12.6±4.0	2.49±0.32	3.60±0.44
4	21.4±3.3	1.71±0.08	2.67±0.65	1	19.6	1.66	1.70
3	5.2±0.9	3.94±0.32	3.69±0.44	3	9.1±1.5	1.72±0.18	1.97±0.47
5	23.6±1.8	1.64±0.14	2.20±0.21	5	13.5±2.2	1.97±0.18	2.17±0.56
4	18.8±3.8	2.03±0.47	3.46±0.64	1	6.7	3.33	3.50
4	34.5±9.4	2.00±0.27	4.12±1.36	4	6.4±3.3	3.47±0.61	3.23±0.35
8	32.7±2.7	2.22±0.12	5.12±0.60	4	13.5±0.8	2.80±0.22	3.57±0.59
3	35.1±12.7	2.20±0.44	3.17±1.17	5	10.8±1.7	2.81±0.41	2.85±0.61
3	38.7±4.9	2.11±0.22	4.83±1.88	3	22.4±7.6	3.37±0.90	3.16±1.21
2	43.2±1.3	2.08±0.33	6.62±0.62	2	27.0±4.6	1.83±0.12	3.75±0.25
2	42.6±0.8	2.06±0.06	6.50±0.50	1	22.1	2.10	4.40
1	31.9	1.45	3.62	2	20.0±0.8	2.32±0.02	3.62±0.12
2	30.0±0.1	1.79±0.01	4.69±0.31	2	23.1±0.3	1.60±0.24	4.04±0.91
1	28.5	2.41	3.50	1	15.6	1.39	2.12
				1	10.4	1.88	3.50
$\bar{x}$§	23.5±1.7	2.27±0.12	3.73±0.22		14.8±0.9	2.37±0.11	2.93±0.15

* Number of samples per animal.

‡ Average values ±SE in each animal.

§ Mean ±SE of all samples.

Averaged micropuncture data for proximal tubules in each animal (Table II) show that intratubular flow was higher ($P < 0.01$) in the DOCA group. Since fractional reabsorption was not different from the NaD series, a higher absolute rate of proximal transport is suggested for the high-salt animals. Comparable data for distal tubules (Table III) indicate that the higher fluid reabsorption persisted to this nephron segment. However, the differences in intratubular flows are related in large part to differences in kidney weights between the two groups (see Table V). In addition, absolute length of proximal and distal convolution is the same for DOCA and NaD rats when factored by respective kidney weight. Surprisingly, distal intratubular concentration of sodium was lower ($P < 0.001$) in DOCA rats, as were the concentrations of potassium and total solute ($P < 0.05$). Sodium concentrations in individual proximal tubules of these animals averaged 143±4 SE ($n = 17$) and 133±3 ($n = 14$) meq/liter for DOCA and NaD groups, respectively. Potassium values were 4.89±0.19 (DOCA, $n = 18$) and 5.76±0.22 meq/liter (NaD, $n = 12$); corresponding proximal tubular osmolalities were 299±3 ($n = 21$) and 303±2 ($n = 15$) mosmol/liter.

From the calculated single nephron filtration rates in individual punctured tubules an average value of SGFR was obtained for each rat[2] and related to the corresponding value of inulin clearance (Fig. 1). For quantitative comparison single nephron filtration was multiplied by 30,000, the estimated number of glomeruli in a rat kidney (10). Despite the large variability, significant correlation ($P < 0.02$) of SGFR and C_{In} is seen in DOCA and NaD rats used for proximal micropuncture (DOCA: $r = 0.67$; NaD: $r = 0.56$). No such correlation was evident in animals used for distal micropuncture, due mainly to the smaller range of filtration

[2] If there is a reciprocal relationship between intratubular flow and TF/P inulin (Tables II, III) an erroneously high SGFR is obtained by multiplying average flow by average inulin ratio in a given animal.

TABLE III

Intratubular Flow and Concentration with Length from Macula Densa to Distal Collection Site in Individual Rats

n*	$\dot{V}_t$	TF/P inulin	$[Na]_t$	$[K]_t$	$[osmol]_t$	Length
	nl/min		*meq/liter*	*meq/liter*	*mosmol/liter*	*mm*
DOCA						
5	5.5±1.2‡	9.85±2.28	34±14 (*n* = 3)*	—	126±7 (*n* = 4)	1.68±0.11
4	12.1±2.2	5.47±0.84	25±6	2.28±0.75	112±5	1.20±0.42
5	4.9±1.0	10.65±2.67	14±4	6.53±1.59	176±27	1.50±0.39
3	3.4±0.6	18.65±2.23	38±7	6.67±1.40	201±16	1.46±0.47
5	8.1±1.9	11.16±1.95	10±2	4.71±1.82	134±22	1.96±0.12
6	8.9±1.7	10.20±3.11	16±3	2.51±0.24	140±7 (*n* = 5)	1.69±0.27
6	9.2±2.2	7.56±1.59	20±2	3.68±1.54	164±19	1.21±0.29
6	11.7±2.4	5.00±0.70	38±6	2.26±0.65	126±6	1.09±0.29
5	7.2±1.4	9.04±0.16	20±4 (*n* = 4)	2.54±0.38 (*n* = 4)	98±14	2.53±0.12
3	11.4±3.7	6.27±1.14	24±4	1.92±0.34	104±5	1.60±0.51
7	12.6±2.7	6.72±1.57	29±3	4.19±1.08	161±23 (*n* = 6)	1.30±0.32
6	6.7±1.2	9.74±1.80	18±4	3.50±1.05	160±24	1.74±0.39
6	7.4±2.0	11.30±3.31	31±8	3.08±0.97	196±28	1.94±0.26
$\bar{x}$§	8.6±0.7	9.10±0.96	24±2	3.62±0.45	148±10	1.59±0.09
NaD						
4	7.7±1.5	5.82±0.77	56±10	—	112±10	0.84±0.35
5	3.0±0.6	13.74±2.74	34±14 (*n* = 2)	—	191±14	0.83±0.20
5	2.8±0.7	11.40±2.41	97±15 (*n* = 4)	11.72±0.56 (*n* = 4)	233±21 (*n* = 4)	1.48±0.07
4	2.7±0.7	10.58±1.42	66±13		172±8	0.98±0.37
5	5.7±1.2	9.57±2.65	50±5 (*n* = 4)	3.65±0.74 (*n* = 3)	158±2	0.63±0.15
5	6.0±1.3	7.79±1.20	34±6	9.24±0.63	175±18	1.23±0.25
5	3.2±0.8	12.98±1.93	52±12	5.87±2.05	217±26 (*n* = 4)	0.80±0.29
5	4.7±1.3	9.50±2.42	68±12	5.35±0.81	203±17	0.90±0.39
5	7.3±1.0	5.31±0.49	28±2 (*n* = 4)	1.85±0.86	138±8	0.39±0.03
5	3.6±0.7	7.52±1.10	73±10	5.58±0.60	216±27	0.80±0.26
5	2.7±0.5	11.72±1.47	44±2	7.90±1.67	196±6	.51±0.25
$\bar{x}$	4.5±0.05	9.68±0.83	56±5	5.78±0.98	183±10	0.94±0.08

* Number of samples per animal.

‡ Average values ±SE in each animal.

§ Mean ±SE of all samples.

in these groups. Paired *t* test analysis showed no significant difference between SGFR and C_{In} in DOCA animals (proximal collection: SGFR = 1.62±0.16 SE, C_{In} = 1.51±0.14 ml/min, distal collection: SGFR = 1.65 ±0.07, C_{In} = 1.69±0.09 ml/min), or in NaD rats used for proximal puncture (SGFR = 0.97±0.08, C_{In} = 1.07 ±0.11 ml/min). In contrast, the difference in distally punctured NaD rats (SGFR = 0.99±0.08, C_{In} = 1.51 ±0.10 ml/min) was statistically significant ($P < 0.001$). To determine if the site of collection could account for this discrepancy, single nephron filtration rates obtained from proximal and distal micropuncture data in the same animal were compared (Table IV). No significant differences in SGFR were evident, leading to the con-

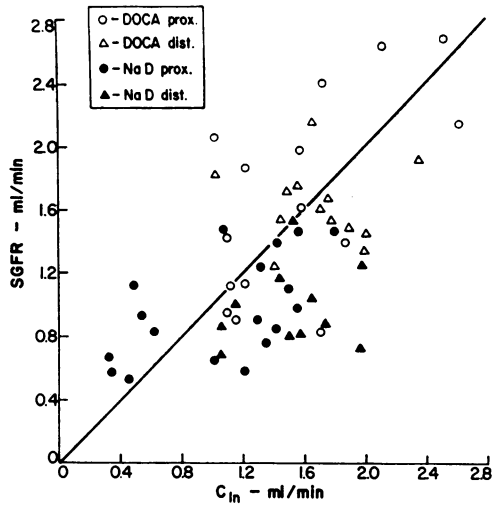

FIGURE 1 Average single nephron filtration rate (SGFR) and inulin clearance (C_{In}) in individual rats. SGFR values obtained from proximal and distal micropuncture data are multiplied by the estimated number of nephrons/kidney for quantitative comparison. Theoretical line of identity is indicated.

clusion that filtration rate in some but not all salt-deficient rats is distributed preferentially to deep nephrons not accessible to micropuncture.

Inulin TF/P ratios (Fig. 2) revealed no difference between DOCA and NaD rats in fractional fluid reabsorption with tubular length in individual proximal tubules. Average inulin ratios in the first 50% of the proximal convolution were 1.87 and 1.83 for DOCA and NaD groups, respectively. Comparable averages for lengths between 50% and 100% were 2.48 (DOCA) and 2.69 (NaD). In distal tubule, an initially lower

TABLE IV

*Comparison of Average Single Nephron Filtration Rates Obtained from Proximal and Distal Collection in Individual Rats**

DOCA SGFR‡		NaD SGFR	
Proximal	Distal	Proximal	Distal
nl/min		*nl/min*	
45.0	44.7	34.9	28.8
44.6	41.4	27.6	26.7
53.5	57.7	32.7	24.3
74.3	72.1	42.6	41.7
84.1	63.1	45.9	39.1
82.6	49.8	43.8	34.4
43.7	48.4	43.8	32.5
50.6	55.6	34.8	34.8
57.1	60.6	22.3	22.8
		18.4	26.9
$\bar{x}\pm$SE 59.5±5.5	54.9±3.3	34.7±3.0	31.2±2.0

* Complete measurements of proximal tubular length were unavailable in four animals of each group; proximal data from these animals is therefore not included in other tables or figures.

‡ Single nephron filtration rate.

TF/P value in DOCA rats (TF/P 0–50%: DOCA = 5.32, NaD = 7.97; $P < 0.05$) approached that of NaD rats in the second half of the tubule (TF/P 50–100%: DOCA = 10.21, NaD = 12.19; NS) suggesting greater fractional reabsorption of fluid in the DOCA group. Combined with the differences in single nephron filtration rates the inulin ratios indicate that absolute fluid reabsorption in both proximal and distal tubule was greater in DOCA-treated rats.

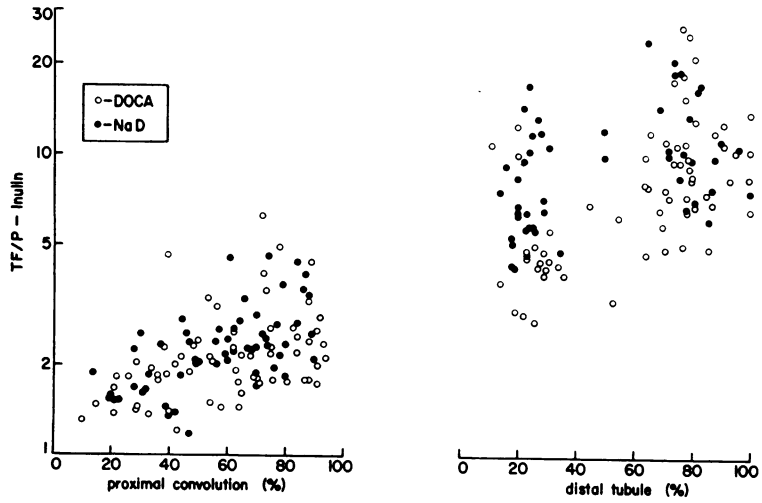

FIGURE 2 Tubular fluid to plasma (TF/P) ratios of inulin concentration in proximal and distal tubule of DOCA and NaD rats.

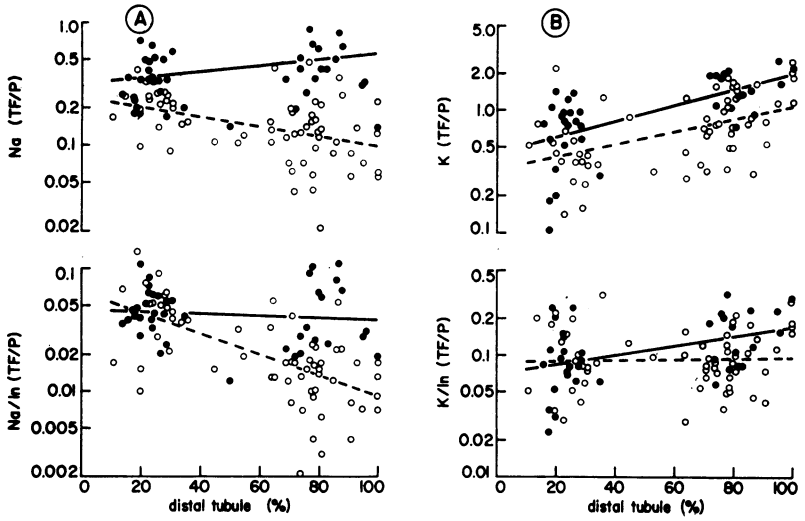

FIGURE 3 Tubular fluid to plasma concentration ratios (upper panels) and fraction of filtered load remaining in tubule (lower panels) with distal tubular length in DOCA and NaD rats. (*A*) sodium, (*B*) potassium. Solid and broken lines indicate statistical regression lines for NaD (●) and DOCA (○) series, respectively.

Both the intratubular sodium concentration and the fraction of filtered sodium remaining in the tubule (Fig. 3*A*) showed significant decrease with distal tubular length only in DOCA rats; corresponding data for potassium (Fig. 3*B*) indicated that distal secretion of potassium was somewhat greater in salt-deprived animals. Since filtered load of ions was greater in the DOCA group, absolute reabsorption of sodium in the distal tubule of salt-excreting rats was enhanced relative to that of salt-conserving animals.

No statistically significant relationship between distal length and intratubular osmolality was seen in either DOCA or NaD rats. However, length is only one of several factors (Na transport, water permeability, intratubular flow) determining distal osmolality, any one of which may predominate under different conditions. Correlations of osmolality and distal tubular flow rate were significant ($P < 0.01$) for both groups (Fig. 4), indicating that flow rate had a major influence on the equilibration of water across the tubular wall in these experiments.

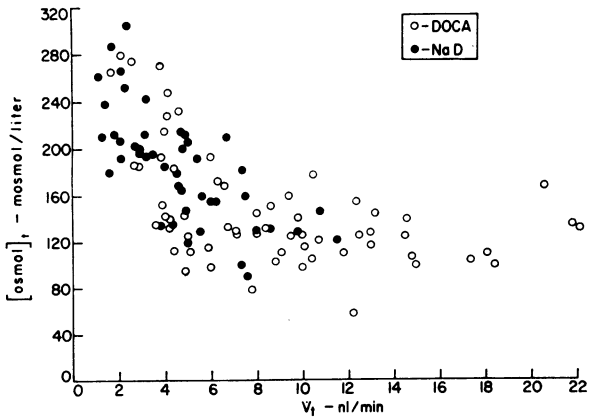

FIGURE 4 Relationship between intratubular osmolality and flow in distal tubules of DOCA and NaD rats.

Absolute transport rate of fluid and ions was calculated for each micropuncture collection and related to the two known determinants, filtered load and tubular length as outlined in the appendix. Average intratubular loads of ions and water at beginning and end of distal tubule were calculated for each distally punctured animal by substituting SGFR and absolute distal length in the derived equations. In addition, end-proximal loads were estimated by substituting the distally determined SGFR of each animal and an average proximal tubular length in the regression equations derived from proximal micropuncture data. Values were factored by respective kidney weights, allowing direct comparison of function between DOCA and NaD groups. Results are shown in Table V. Associated with significantly enhanced filtered load of fluid and sodium ($P < 0.01$), reabsorption in proximal tubule was greater in DOCA rats. The difference in load persisted to the beginning of the distal tubule, indicating proportional reabsorption in both groups. At the end of this nephron segment, however, intratubular sodium load in DOCA rats was lower ($P < 0.001$) than the comparable value for salt-deprived animals, demonstrating the greatly enhanced Na reabsorption in the former.

A comparison between end-distal delivery and urinary excretion of sodium was made for each punctured dis-

TABLE V

Average Load per Nephron of Fluid and Ions in Different Tubule Segments of Distally Punctured Rats

	Filtered	End-proximal	Beginning distal	End-distal
DOCA				
V (nl/min/gKW*)	29.24±1.91‡	10.62±1.01	7.64±0.47	2.59±0.33
Na (neq/min/gKW)	4.124±0.292	1.502±0.151	0.250±0.015	0.021±0.009
K (neq/min/gKW)	0.124±0.007	0.045±0.004	0.014±0.001	0.015±0.001
NaD				
V (nl/min/gKW)	21.65±1.78	8.33±0.74	3.71±0.31	1.96±0.21
Na (neq/min/gKW)	2.933±0.253	1.118±0.112	0.158±0.012	0.126±0.010
K (neq/min/gKW)	0.117±0.006	0.045±0.004	0.013±0.001	0.022±0.001

* Kidney weight.

‡ Mean±SE of average single nephron values in each animal.

tal tubule. Expected reabsorption from puncture site to first confluence of two tubules was calculated using the appropriate partial regression coefficient β_2 (see Table VII). This reabsorption was then subtracted from the value of intratubular flow. End-distal delivery was then related to urinary excretion calculated for the time of collection and is given for each nephron in Table VI. It is evident that the magnitude of end-distal delivery of sodium was not related to simultaneous urinary excretion of the ion in either DOCA or NaD series. In addition, the greater natriuresis in the former group occurred despite an almost uniformly lower end-distal load. Negative values for Na delivery in the DOCA series occurred when calculated reabsorption for the remainder of the distal tubule exceeded intratubular flow of the ion at the puncture site. Since these values were associated with extremely low sodium concentrations and/or fluid flow rates Na supply might be considered as having been rate-limiting under these circumstances.

DISCUSSION

The salt- and DOCA-treated rats excrete more, and the salt-deprived rats less sodium than anaesthetized normal rats (11). Since both groups presumably have high levels of circulating mineralocorticoids, the first due to injection of DOCA and the second due to endogenous aldosterone release, the difference in natriuresis demonstrates the modification of hormone-induced salt retention by high salt intake (DOCA escape).

Superficial nephron filtration rates of DOCA rats are higher than those of salt-deprived animals. Although absolute fluid reabsorption in proximal tubule is increased proportionately, indicating the operation of glomerulo-tubular balance, end-proximal delivery in the high-salt group remains elevated. This latter finding is in agreement with results obtained in DOCA-escaped dogs (4, 12). The difference in tubular load persists to the beginning of the distal tubule, despite increased sodium transport in loop of Henle of DOCA-treated rats, calculated as the difference in load between end of proximal and beginning of distal tubule. However, calculated rates of distal Na reabsorption are higher by almost an order of magnitude in the DOCA group, so that the end-distal sodium delivery is less than that of the low-salt group. The salt-retaining action of the administered mineralocorticoid thus appears to be greater than that seen with physiologically raised levels of aldosterone.

In view of the usually positive relationship between distal Na reabsorption and K secretion (13) the lack of net secretion of potassium in DOCA rats appears disconcerting. However, if one assumes that simultaneous potassium reabsorption (14) may also be enhanced, a combination of the two opposing effects could account for the absence of net transfer of the ion in this series.

Calculations of distal tubular transport depend on the validity of distally measured single nephron filtration rates. Comparison with proximally determined SGFR in the same animals (Table IV) revealed no significant differences, indicating that in these experiments the site of puncture did not introduce systematic error into the calculation of individual nephron filtration rate. Since on the average distal intratubular flow in NaD rats was lower than in DOCA rats (Table III), a small but consistent contamination of collected sample with fluid moving retrograde past the distal oil block could still conceivably account for the difference in intratubular sodium concentration. Distal collections at flow rates between 3 and 8 nl/min for both groups were therefore compared (see Fig. 3). 26 samples from DOCA rats had an average flow rate of 5.4±0.3 SE nl/min, while 22 collections from NaD rats averaged 5.2±0.3 nl/min. Intratubular Na concentration, in contrast, was significantly different (DOCA = 20.8±2.6, NaD = 51.2±4.8

TABLE VI

End-Distal Delivery of Sodium, Compared with Simultaneous Natriuresis

	DOCA		NaD	
	Na_{e-d}*	$U_{Na}V$‡	Na_{e-d}	$U_{Na}V$
	neq/min		*neq/min*	
	—	11,347	—	49
	0.084	9,504		
	0.155	8,109	—	28
	−0.002	6,639	—	27
	—	7,637	0.219	25
			—	32
$\bar{x}$§	0.079	8,647	—	32
SE	0.045	817	0.031	25
			$\bar{x}$ 0.125	27
			SE	1
	−0.062	3,977	0.096	81
	0.041	2,502	0.280	71
	0.078	1,290	—	81
	0.320	1,290	0.530	63
			0.220	93
	0.094	2,265		
	0.081	638	0.282	78
			0.091	5
	−0.131	32	0.065	52
	−0.039	46	0.141	56
	−0.025	45	0.233	47
	−0.063	48	0.118	54
	‖ 0.050	57		
			0.139	52
	−0.046	48	0.035	2
	0.029	6		
	−0.031	244	—	46
	−0.207	211	0.244	48
	0.041	161	0.414	51
			0.223	50
	−0.065	205	0.236	50
	0.073	24		
			0.279	49
			0.045	1
	−0.024	705	0.271	50
	‖ 0.120	580	0.061	50
	0.011	580	0.218	46
	−0.104	530	‖0.100	45
	−0.041	437	0.263	37
	−0.008	566	0.183	46
	0.037	43	0.043	2
	−0.010	98	0.156	31
	−0.047	196	0.053	31
	−0.033	257	0.129	27
	0.063	292	0.116	31
	0.035	292	0.122	27
	0.006	312		
			0.115	29
	0.002	241	0.017	1
	0.017	33		
	−0.100	232	0.428	63
	−0.001	250	0.068	49
	−0.060	250	0.214	41
	−0.359	269	0.466	31
	0.026	330	0.149	26
	0.091	334		
			0.265	42
	−0.067	278	0.078	7
	0.064	18		
	0.215	1,172	0.223	282
	0.095	1,155	0.098	292
	0.040	1,155	0.197	292
	0.384	1,587	0.053	300
	0.946	1,587	0.197	264
	−0.035	1,670		
			0.154	286
	0.274	1,388	0.033	6
	0.147	102		
	−0.059	464	0.243	38
	−0.002	633	0.232	149
	—	759	0.264	188
	0.035	846	0.314	32
	0.122	961	0.070	33
	0.024	733	0.225	88
	0.038	86	0.041	33
	0.208	533	0.106	82
	0.063	766	0.088	101
	0.020	467	0.135	101
			0.130	95
	0.097	589	0.000	95
	0.057	91		
			0.092	95
			0.024	3
	‖ 0.120	2,063		
	0.163	2,360		
	0.008	2,537		
	0.405	2,705		
	‖ 0.110	2,674		
	0.398	2,674		
	0.113	2,634		
	0.118	2,521		
	0.058	88		
	−0.295	272		
	−0.088	284		
	0.053	299		
	−0.052	323		
	0.155	305		
	−0.100	292		
	−0.054	296		
	0.062	7		
	0.030	312		
	−0.025	328		
	0.105	428		
	0.085	428		
	−0.029	333		
	−0.020	333		
	0.024	360		
	0.024	22		
Mean¶	0.046	1,409	0.187	79
SE	0.022	279	0.018	11
Mean/gKW**	0.024	730	0.123	52
SE	0.013	151	0.012	7

* Calculated end-distal flow of sodium per nephron.
‡ Urinary Na excretion during each micropuncture collection.
§ Average values per animal.
‖ Averaged values from two distal tubules.
¶ Overall sample mean.
** Sample mean per gram kidney weight.

meq Na/liter; $P < 0.001$), demonstrating a real difference in sodium transport.

The lack of correlation between end-distal sodium delivery by superficial nephrons and urinary Na excretion (Table VI) demonstrates that the final level of natriuresis is dependent on mechanisms located farther downstream in the tubular system. One such possible mechanism is a differential delivery of sodium to the collecting duct system by superficial and deep nephrons. In DOCA rats, in which the identity of inulin clearance and SGFR multiplied by total nephron number indicates homogeneity of filtration rate, increased Na delivery by deep nephrons requires the assumption of inhibition of transport in these structures. Conversely, in NaD rats deep nephrons would be expected to reabsorb most or all of their sodium load prior to the collecting duct. Although such "salt-conserving" function of juxtamedullary nephrons has been predicted (10, 15), there appears to be no a priori reason that chronic DOCA administration should reverse this effect while simultaneously enhancing transport in superficial nephrons. It is concluded, therefore, that alterations in net sodium reabsorption in the collecting duct system itself have a determining influence on urinary Na excretion. Since it has been shown that passive influx of sodium into collecting duct fluid may occur (16), an increase in permeability, together with higher medullary sodium concentration as a result of enhanced ascending limb transport (17) could account for the escape phenomenon in the DOCA rats. However an effect of aldosterone on collecting duct epithelium is to reduce passive permeability to sodium (16), suggesting a specific inhibition of sodium transport in the terminal nephron segment as a mechanism of DOCA escape.

APPENDIX

In proximal tubule, absolute fluid reabsorption is dependent on both filtration rate and on the length of the tubule available for reabsorption. Although a linear relationship between reabsorption and filtration is often assumed, this relation may be either linear or nonlinear. Similarly the dependence of reabsorption on tubular length may be linear or nonlinear. An equation which takes into consideration these possibilities may be written as follows:

$$y = a + bx_1^{\beta_1}x_2^{\beta_2}, \quad (1)$$

where y represents proximal reabsorption in nanoliters per minute, x_1 and x_2 are filtration rate in nanoliters per minute and tubular length in millimeters, respectively, and a and b are constants. The exponents of β_1 and β_2 describe the nature of the relationship of x_1 and x_2 to y: if $\beta = 0$ there is no relationship, if $\beta = 1$ the relationship is linear. Since the constant a is theoretically equal to zero the log transformation of the equation is given as:

$$\ln y = \ln b + \beta_1 \ln x_1 + \beta_2 \ln x_2. \quad (2)$$

Using multiple linear regression analysis (8) the constants β_1 and β_2 may be evaluated and substituted in equation 1. Simple orthogonal regression (9) is then used to correlate reabsorption with the new independent variable $x_1^{\beta_1}x_2^{\beta_2}$. Results of such calculations for both groups of animals are given below:

$$\text{DOCA: } y = 1.1 + 0.67x_1^{0.76}x_2^{0.46}; \ (r = 0.918) \quad (3)$$

$$\text{NaD: } y = 0.8 + 0.38x_1^{0.96}x_2^{0.35}; \ (r = 0.838). \quad (4)$$

Correlation coefficients, r, are indicated. By substituting values for SGFR and proximal tubular length, reabsorption may be calculated.

In the distal convolution measured intratubular flow was related to filtered load and absolute length of tubule with the equation:

$$y = \beta_0 + \beta_1 x_1 + \beta_2 x_2, \quad (5)$$

where y represents tubular flow of fluid in nanoliters per minute or of Na or K in nanoequivalents per minute, x_1 is filtered load of fluid or ions in nanoliters per minute or nanoequivalents per minute, x_2 equals distal tubular length in millimeters, and β_0, β_1, and β_2 are constants. Using linear multiple regression analysis (8), the partial regression coefficients β_1 and β_2 (which indicate the degree of change of the dependent variable, y for a unit change in one inde-

TABLE VII

Multiple Regression Analysis of Distal Transport of Fluid, Sodium, and Potassium in DOCA and NaD Rats

	β_0	β_1	β_2	$r_{1y.2}$*	$r_{2y.1}$*	r‡	n§
DOCA—distal							
V (nl/min)	3.88	0.191	−3.740	0.550	−0.578	0.686	67
Na (neq/min)	0.206	0.036	−0.173	0.353	−0.577	0.591	64
K (neq/min)	−0.006	0.139	0.0002	0.453	0.012	0.465	61
NaD—distal							
V (nl/min)	1.30	0.124	−1.268	0.517	−0.380	0.613	50
Na (neq/min)	0.070	0.038	−0.026	0.403	−0.146	0.430	44
K (neq/min)	−0.002	0.124	0.0037	0.418	0.340	0.504	37

* Partial correlation coefficients for y with x_1 and x_2, respectively.

‡ Multiple correlation coefficient for y with x_1 and x_2 combined.

§ Number of samples.

pendent variable x_1 or x_2 when the other independent variable remains constant) may be calculated. No appreciable increase in correlation was obtained by transformation of data to test for nonlinearity of the relationship between tubular flow and filtered load or length, justifying the use of equation (5). Data obtained from the multiple regression analysis are given in Table VII. As indicated by the partial correlation coefficients $r_{1y.2}$, at constant distal tubular length there is significant correlation ($P < 0.01$) between filtered load and intratubular flow of fluid, Na, and K in DOCA as well as NaD rats. Similarly, at constant filtered load, significant correlation ($P < 0.05$) with distal length is found in every case, except for potassium in DOCA rats and sodium in NaD rats. Data were substituted in the appropriate equations (Table VII) to obtain flow of ions and water at beginning and end of distal tubule.

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