Renal Handling of Sodium and Water in the Hypothyroid Rat

CLEARANCE AND MICROPUNCTURE STUDIES

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ABSTRACT Hypothyroid rats were examined with conventional renal clearance and micropuncture techniques to elicit the mechanism and site within the nephron responsible for the increased salt and water excretion observed in these animals. When compared with age-matched control rats, a decrease in inulin clearance of 30% ($P < 0.001$) and in Hippuran clearance of 32% ($P < 0.005$) was observed in the hypothyroid rats. Absolute excretion of sodium and water was increased 3-fold ($P < 0.02$) and 2-fold ($P < 0.025$), respectively, while fractional excretion of sodium and water was increased 4.3-fold ($P < 0.02$) and 2.9-fold ($P < 0.05$), respectively, in the hypothyroid animals.

Fractional proximal reabsorption of sodium as assessed from proximal tubular fluid to plasma ratios of inulin ($[TF/P]_{IN}$) was found to be decreased by 28% ($P < 0.001$) in the hypothyroid rats. Superficial single nephron filtration rate was reduced proportionately to the decrease in total filtration rate in the hypothyroid rats.

These data indicate that the proximal tubule is one of the sites of diminished sodium and water reabsorption in the hypothyroid rat. The data also suggest that the observed decrease in glomerular filtration rate in the hypothyroid animals is not caused by a decrease in the number of functioning nephrons and that the observed increase in sodium and water excretion is not caused by a redistribution of filtrate from juxtamedullary to superficial nephrons. Although the exact mechanisms of the observed changes in proximal tubular function remain unknown, the data suggest that they are probably related to the lack of thyroid hormone. Whatever their mechanism, it appears that the enhanced sodium and water excretion observed in the hypothyroid animals must be determined by further reduction in tubular sodium reabsorption in the distal nephron.

INTRODUCTION

Hypothyroidism causes significant changes in renal hemodynamics and the renal handling of salt and water. In the rat, a decrease in glomerular filtration rate (GFR)[1] and in the effective renal plasma flow (ERPF) has been reported (1, 2). Hypothyroid rats drink more water and excrete more urine (3), have a decrease in urinary concentrating ability (2, 4), and waste sodium and die when fed a sodium-free diet (5). In addition, hypothyroid rats exhibit an enhanced natriuresis when salt-loaded (2, 6, 7). Although a decrease in the secretory rate of aldosterone and in the tubular response to aldosterone has been reported in these animals (8, 9), the sodium wasting in hypothyroid adrenalectomized rats was not abolished by addition of mineralocorticoid hormones (2, 10). Furthermore, the abnormalities in GFR and ERPF and in the concentrating ability are

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[1] *Abbreviations used in this paper:* C_{IN}, inulin clearance; C_{PAH}, Hippuran clearance; $C/\pi r^2$, intrinsic reabsorptive capacity; ECFV, extracellular fluid volume; ERPF, effective renal plasma flow; GFR, glomerular filtration rate; PBBO, 2-(4-biphenylyl)-6-phenyl-benzoxazole; PBD, 2-(4'-t-butylphenyl)-5-(4'-biphenyl)-1,3,4-oxdiazole; SSNgfr, superficial single nephron glomerular filtration rate; $(TF/P)_{IN}$, tubular fluid to plasma ratios of inulin.

improved towards normal by the administration of desiccated thyroid or L-thyroxine (1, 4).

The site(s) within the nephron responsible for the observed exaggerated natriuresis were sought by Holmes and DiScala (2). They obtained indirect evidence through clearance studies pointing to the distal and possibly the proximal tubule.

It was the aim of the present study to examine directly the reabsorption of sodium and water in the proximal tubule using conventional micropuncture techniques. Since redistribution of filtrate from juxtamedullary to superficial nephrons is a possible mechanism for an increased natriuresis in the hypothyroid rat, an attempt was made to evaluate this possibility by comparing superficial single nephron filtration rate to total kidney filtration rate.

METHODS

White male Sprague-Dawley rats (Charles River Breeding Laboratories, Inc., CD strain), with an initial weight of 100–175 g, were used in these experiments. Hypothyroidism was induced by surgical thyroidectomy and additional intraperitoneal injection of 0.2 mCi ^{131}I or by the intraperitoneal injection of 1.0 mCi ^{131}I after feeding the rats an iodine-deficient diet (Remington low iodine-diet, Nutritional Biochemicals Corporation, Cleveland, Ohio) for 10 days. The animals were fed a regular Purina rat chow diet (Ralston Purina Co., St. Louis, Mo.) thereafter, and they received either normal saline or water as drinking fluid. Saline as drinking fluid was chosen initially since the extent of sodium wasting by the hypothyroid rats had not yet been established in our laboratory. Subsequently it was found that the sodium content in the regular Purina rat chow and tap water was sufficient to keep the hypothyroid rats in sodium balance. The approximate daily intake of sodium was 2–4 mEq/24 hr for the rats drinking tap water and it was 7–10 mEq/24 hr for the rats drinking normal saline. Since there was no statistically significant difference in the variables examined including the urinary sodium excretion, all data from saline- and tap water-drinking animals are presented as pooled data. Age-matched rats, fed the same diet and drinking fluids, were used as controls.

The development of hypothyroidism was confirmed from the retardation of growth and the absence of the thyroid gland observed during insertion of the tracheostomy tube and at autopsy of the animals. In addition, one group of randomly selected hypothyroid rats which exhibited the same clinical and autopsy signs observed in the other hypothyroid rats showed a significant decrease in their plasma thyroxine concentration and a significant increase in triiodothyronine (T_3)[2] index when compared to a randomly selected group of age-matched control rats (Table I). Animals were studied 8-16 wk after ^{131}I administration. The weight of the hypothyroid animals at the time of the study was significantly lower (304±22 g) than that of the age-matched controls (389±18 g) (mean ±SEM, $P < 0.01$).

TABLE I

Laboratory Evaluation of Thyroid Function in a Group of Randomly Selected Hypothyroid and Control Rats

	No. of animals	T-4	T-3 index
		μg/100 ml	
Hypothyroid	5	0.3±0.2	0.83±0.03
Range		(0.0–1.1)	(0.76–0.92)
Control	5	6.6±0.9	0.58±0.02
Range		(4.5–9.6)	(0.52–0.63)
P		<0.001	<0.001

Preparation of animals for clearance and micropuncture studies. The animals had free access to their drinking fluid up to the initiation of anesthesia, but food was withdrawn 16 hr before surgery. Anesthesia was induced with 100 mg/kg body weight Inactin (Promonta, Hamburg, W. Germany) intraperitoneally. Thereafter, animals were placed on a temperature-controlled operating table and tracheotomized. One jugular vein was cannulated with polyethylene tubing (Clay-Adams, Inc., Parsippany, N. J., PE 360 pulled out over a small flame to the appropriate diameter) and as soon as the catheter in the jugular vein had been placed, an infusion of Ringer's saline at a rate of 0.6 ml/hr per 100 g body weight was started to replace surgical fluid losses. The right femoral vein was cannulated for the injection of Lissamine green and a catheter, filled with heparinized Ringer's solution, placed in the right femoral artery was connected to a mercury-filled U-manometer for blood pressure measurements. Urine was collected through a PE 50 polyethylene catheter placed in the bladder through a suprapubic incision. In order to keep dead space volume as small as possible, the dome of the bladder was tied off with a ligature. Urine was collected into tared polyethylene containers and volume was estimated by reweighing these containers after the urine collection was completed. The left ureter was interrupted by two ligatures and cutting between them. Another incision was made in the ureter just proximal to the ligature to allow free drainage of the urine until the catheter could be inserted. The left kidney was freed from its fat capsule and was placed into a lucite holder, using utmost care not to injure or compress the hilar vessels and the ureter. Mineral oil warmed by countercurrent flow through a circulating water bath to 37°C was used to bathe in a continuous flow the surface of the kidney. PE 50 polyethylene tubing, slightly pulled out to decrease its outer diameter, was placed through the proximal stump of the ureter directly into the renal pelvis. The kidney was illuminated by a fiber optic system.

After the completion of surgery, a loading dose of 125 μCi inulin-^3H and 25 μCi hippurate-^{14}C in 1 ml were given through the cannulated jugular vein followed by a sustaining solution of 125 μCi inulin-^3H and 2 μCi hippurate-^{14}C (New England Nuclear Corp., Boston, Mass.) per ml in Ringer's saline at a rate of 0.6 ml/hr per 100 g body weight replacing the previously started infusion of Ringer's saline. 1 hr was allowed for recovery from surgery and equilibration. Experiments were discontinued when mean blood pressure fell below 80 mm Hg.

Clearance studies. Micropuncture and clearance studies were done simultaneously in most of the rats. Although there was a tendency for the clearance (C_{IN}, C_{PAH}) values

[2] These determinations were done using Res-O-Mat T_3 I^{131} and Res-O-Mat T_4 I^{125} diagnostic kits, produced by Mallinckrodt Chemical Works, St. Louis, Mo. We are indebted to Dr. Frank Gollan and Mrs. Donna Leavelle for advice and generous help with these determinations.

to be slightly lower in the left kidney, the differences between the values from the "manipulated" and "nonmanipulated" kidney were not significant ($P > 0.1$), thus all clearance data are expressed as a sum of both kidneys (Table II). Urine was collected in 20–30-min periods and a blood sample from free-flowing tail blood was taken at the start and end of each clearance period. Portions (5 μl) of urine and plasma samples were transferred into 10 ml of PBD-PBBO in toluene with the addition of Bio-Solv solubilizer (Beckman Instruments, Inc., Fullerton, Calif.) as scintillation fluor. They were counted in a Beckman 3-channel liquid scintillation counter LS-233 to a total of 5000 or more counts.

Urine and plasma sodium and potassium concentrations were measured by flame photometry using lithium as an internal standard. Inulin and hippuran clearance, filtration fraction, filtered load of sodium and potassium, rate of urinary excretion of sodium and potassium, and fractional excretion of sodium and water were calculated from standard equations.

Micropuncture studies. Micropuncture experiments were discontinued when the distribution and disappearance of Lissamine green was not equal in all areas of the kidney surface. Timed samples of tubular fluid for determination of superficial single nephron glomerular filtration rate (SSNgfr) were obtained by injection of an oil droplet about 4–6 tubular diameters in length, allowing the oil to pass the tip of the micropipette. Immediately thereafter a short aspiration with a hand-syringe to overcome tip resistance was initiated and maintained, observing the constant position of the injected oil droplet and the constant diameter of the proximal portion of the punctured tubule. The sample was then quantitatively transferred into scintillation solution and the SSNgfr was calculated from the equation ([TFcpm − Bkg cpm] × 5000 [nl])/([Pcpm − Bkg cpm] × t [min]), where TFcpm = counts per min of tubular fluid, Bkg cpm = background counts, Pcpm = counts of 5000 nl of plasma, and t (min) = time of collection.

Plasma values were interpolated from a curve on graph paper on which the cpm's of the samples, obtained every 20–30 min, were plotted.

Tubular fluid samples for the determination of the tubular fluid to plasma inulin ratios ($[TF/P]_{IN}$) were obtained from the terminal segments of the proximal tubule by injecting a small Halocarbon (Halocarbon Products Corp., Hackensack, N. J.) 4-11 oil (viscosity 8.3 centipoise at 37°C) droplet, 2–3 tubular diameters in length. After the droplet had passed the tip of the micropipette, a short aspiration was initiated to overcome tip resistance. Subsequently, the tubular fluid entered the pipette slowly, while the oil droplet was allowed to drift distally during collection. Collection time was from 2 to 6 min. Strict precaution was observed not to reaspirate after the droplet had flowed distally, to prevent retrograde contamination. Since these collections were not quantitative samples of the filtrate, SSNgfr was not calculated from them. Tubular fluid samples were transferred into constant bore, calibrated quartz capillaries for volume measurement and transferred into scintillation fluid thereafter. All tubular fluid samples were counted to a total of 5000 or more counts.

The terminal segments of the proximal tubule were selected for micropuncture according to three criteria: (*a*) they converged about a vascular "star" (11); (*b*) they were the last proximal convolutions in which intravenously injected Lissamine green appeared before its first disappearance from the surface, and (*c*) a small droplet of colored Halocarbon oil injected into the tubule disappeared from the surface and did not reappear in any other surface convolution. Previous studies by Gertz, Mangos, Braun, and Pagel and Bank, Mutz, and Aynedjian have shown that convolutions selected on this basis are located between 50 and 65% of the total proximal tubular length (11, 12).

Before each tubular fluid collection and before puncturing the tubule, 0.05 ml of 10% Lissamine green was injected through the catheter in the femoral vein and the proximal transit time (T) was measured as the time from the diffuse coloration of the kidney to the appearance of the dye front in the tubule convolution selected for puncture.

For the determination of $(TF/P)_{IN}$, plasma inulin concentration was corrected for a plasma water content of 94%.

Intrinsic reabsorptive capacity ($C/\pi r^2$) was calculated according to Gertz from the formula $C/\pi r^2 = (\ln[TF/P]_{IN})/T$ (11).

Calcium concentration in plasma was measured with an Eppendorf spectrophotometer and total plasma protein was determined using an improved biuret method as described by Gornall, Bardawill, and David (13). For statistical analysis data from each animal were averaged and the single average value used in the calculation of overall mean and standard error of the mean (SEM) for the group. The group means were compared using Student's *t* test for unpaired observations as described by Snedecor and Cochran (14). All data are presented as mean ±1 SEM.

RESULTS

Clearance studies (Table II). There was no statistically significant difference in the data obtained from the group of animals which had undergone surgical thyroidectomy and the animals which received ^{131}I only. Similarly, there was no difference in the data in Table II between the animals receiving tap water or saline as drinking fluid.

The inulin (C_{IN}) and hippuran clearance (C_{PAH}) were 30 ($P < 0.001$) and 32% ($P < 0.005$) lower in the hy-

TABLE II

Results of Clearance Studies in Control and Hypothyroid Rats

	Control (15)	*P*	Hypothyroid (25)
C_{IN} (*ml/min per kg body wt*)	6.6 ±0.3	<0.001	4.7 ± 0.3
C_{PAH} (*ml/min per kg body wt*)	23.3 ±2.3	<0.005	16.2 ± 1.1
$\frac{C_{IN}}{C_{PAH}}$	0.32 ±0.03	>0.70	0.31 ± 0.01
V (*μl/min per kg body wt*)	24.3 ±3.4	<0.05	50.9 ±10.6
$U_{Na}V$ (*μEq/min per kg body wt*)	3.4 ±0.9	<0.02	10.6 ± 2.2
$U_{K}V$ (*μEq/min per kg body wt*)	4.9 ±0.6	>0.60	4.7 ± 0.4
FE_{H_2O} (%)	0.42 ±0.06	<0.05	1.18 ± 0.27
FE_{Na} (%)	0.37 ±0.09	<0.02	1.59 ± 0.36

Numbers in parenthesis indicate numbers of animals studied. FE = Fractional Excretion.
The data from tap water- and saline-drinking rats were not different and are combined.

TABLE III

Plasma Determinations, Hematocrit, and Mean Blood Pressure in Control and Hypothyroid Rats

	P_{NA}*	P_K*	P_{Ca}‡	$P_{Protein}$‡	Hematocrit	Blood pressure (mean§)
	mEq/liter	*mEq/liter*	*mg/100 ml*	*g/100 ml*	%	*mm Hg*
Control	150.0±0.9 (20)	5.3±0.1 (20)	10.3±0.1 (8)	5.9±0.1 (8)	48.1±0.4 (20)	113±3 (20)
Hypothyroid	151.7±0.9 (30)	5.1±0.2 (30)	9.1+0.4 (5)	6.3±0.2 (5)	44.8±1.1 (30)	100±3 (30)
P	> 0.1	> 0.2	< 0.01	< 0.05	< 0.025	< 0.005

*Average of the first 2–4 blood sample determinations during clearance study.

‡ Determinations in randomly selected control and hypothyroid rats (treated with ^{131}I irradiation), which did not undergo clearance studies but otherwise were similarly prepared.

§ Readings at the start of the experiment.

Numbers in parenthesis indicate numbers of animals studied.

pothyroid animals, respectively. Consequently, there was no difference in the filtration fraction (C_{IN}/C_{PAH}) between the experimental and control groups.

The mean filtered load of sodium ($P_{Na} \times C_{IN}$) was 30% lower in the hypothyroid animals (689±41 μEq/min per kg body weight) than in the control rats (983±47 μEq/min per kg body weight; $P < 0.001$). Since the rate of sodium excretion ($U_{Na}V$) was three times higher in the hypothyroid rats, the fractional excretion of sodium ($FE_{Na} = C_{Na}/C_{IN}$) in these animals was 4.4 times higher than in the control rats. Concomitant with the increase in sodium excretion was an increase in the absolute (V) and fractional (FE_{H_2O}) excretion of water. The rate of potassium excretion was not different in the hypothyroid and control animals in spite of a lower filtered load of potassium ($P_K \times C_{IN}$) in the hypothyroid animals (23.0 + 1.8 μEq/min per kg body weight) than in the control rats (33.6 + 1.8 μEq/min per kg body weight; $P < 0.001$).

There were no differences in plasma sodium and potassium concentrations between the groups of hypothyroid and control animals (Table III). Plasma calcium concentration was lower and plasma total protein concentration was higher in a group of randomly selected hypothyroid rats compared with a randomly selected group of age-matched controls (Table III). Arterial hematocrit and mean arterial blood pressure were 6 and 11% lower, respectively in the hypothyroid rats (Table III).

Micropuncture studies. The superficial single nephron filtration rate (SSNgfr) was significantly lower in the hypothyroid animals ($P < 0.001$) while the ratio of SSNgfr to total GFR was not different ($P > 0.60$). (Table IV).

Fractional fluid reabsorption in the proximal tubule $[1 - (P/TF)_{IN}]$ was 28% lower in the hypothyroid animals ($P < 0.001$). Proximal transit times of Lissamine green were not different between the control and hypothyroid groups ($P > 0.7$), presumably due to the fact that the absolute fluid volume remaining in the end proximal tubule was similar in the control and hypothyroid group (estimated to be 13.1 nl/min vs. 12.0 nl/min, respectively). Intrinsic reabsorptive capacity ($C/\pi r^2$) was 41% lower in the hypothyroid rats ($P < 0.001$) (Table V).

DISCUSSION

The finding of a decrease in inulin clearance (C_{IN}) in the hypothyroid rats is in good agreement with the data of previous observers, reporting a decrease of 35% (1) and 36.6% (2), respectively. The clearance of Hippuran (C_{PAH}) in this study was found decreased to about the same degree as C_{IN} in the hypothyroid animals, a finding at variance with that of Osorio and Zadunaiski (1) who found a decrease in Diodrast clearance (C_D) of 67% and in C_{IN} of 35% resulting in the calculation of a significantly increased filtration fraction C_{IN}/C_D, while there was no difference in C_{IN}/C_{PAH} between control and hypothyroid rats in the present study. This discrepancy is best explained by a difference in methodology,[3] which tended to underestimate their calculated C_D, thus resulting in an elevated C_{IN}/C_D (57%) (1). While the decrease in glomerular filtration rate may be a consequence of the decrease in renal blood flow, morphological changes in the hypothyroid kidney should be considered. In man,

[3] Osorio and Zadunaiski (1) used the method of Braun-Menendez and Chiodi (15) for the determination of C_D. This is a subcutaneous single dose technique with only one urine collection period and with blood obtained at the end of this single urine collection at a time when plasma levels of iodine are still increasing. Most important, however, there is no indication of how much Diodrast was injected into their animals (1) and it is possible according to the original clearance method (15) that plasma levels of Diodrast iodine might have reached the range of self-depression of C_D. In contrast, due to the high specific activity of the Hippuran-^{14}C used in the present study, Hippuran levels in the plasma never exceeded 0.2 mg/100 ml so that maximal extraction of the PAH can be assumed.

thickening of the basement membrane of the glomeruli and tubuli, prominent capillary walls with small capillary lumina, and increased mesangium have been observed (16, 17). Porte, Fonck-Cussac, Stoebner, Reville, and Stephan (18) have examined the ultrastructural changes in the kidney of rats treated with propylthiouracil or radioiodine and did not observe the thickening of the glomerular basement membrane. It appears that these changes are of no functional significance in relation to the observed decrease in GFR in the hypothyroid animals.

Osorio et al. (1) and we have observed a proportionally greater reduction in kidney weight (KW) than in body weight (BW) in the hypothyroid animals: ($KW/BW = 2.9 \pm 0.06 \times 10^{-3}$ in the hypothyroid vs. $3.3 \pm 0.07 \times 10^{-3}$ in the control rats; $P < 0.001$). That this finding cannot account entirely for the observed decrease in GFR in hypothyroidism is illustrated by the fact that GFR per kidney weight was also lower in the hypothyroid animals: 0.72±0.03 ml/min per g vs. 0.91±0.04 in controls; $P < 0.001$.

TABLE IV

Superficial Single Nephron Filtration Rate (SSNgfr) and Ratio of Superficial Single Nephron Filtration Rate over Kidney Filtration Rate (SSNgfr/GFR) in Control and Hypothyroid Rats

	Rat No.	SSNgfr	SSNgfr/GFR ($\times 10^{-6}$)
		nl/min	
Control	1*	43.5	35.1
	3*	43.7	47.0
	4*	41.1	42.8
	8*	38.8	35.9
	9*	29.9	27.7
	12	24.6	43.0
	27	39.4	44.2
	32	46.1	30.7
	41	40.7	26.3
Mean±SEM		38.6±2.3	37.0±2.6
Hypothyroid	2*	15.1	20.7
	5*	13.4	21.6
	6*	31.3	35.5
	7*	30.3	32.5
	11*	15.7	39.5
	20	21.2	23.3
	35	19.0	67.0
	39	26.7	40.8
	40	30.3	35.0
Mean±SEM		22.6±2.4	35.1±4.7
	P	<0.001	>0.60

* Rats received 0.9% saline as drinking fluid for at least 8 wk before experiment; the remaining animals received tap water as drinking fluid. There was no statistical significant difference in SSNgfr and SSNgfr/GFR between the animals who received saline (*) or tap water as drinking fluid. Data reported represent the average of 2–6 tubular punctures.

TABLE V

Proximal Water Movement in Control and Hypothyroid Rats

	Rat No.	$(TF/P)_{IN}$	Transit time	$C/\pi r^2$
			sec	*sec*$^{-1}$
Control	13	2.7	11.7	0.089
	14*	3.4	12.2	0.101
	16*	4.1	12.4	0.117
	17*	2.8	10.9	0.088
	18*	2.6	10.4	0.089
	19*	2.9	13.0	0.084
	21	2.5	11.1	0.078
	29	2.3	7.5	0.109
	31	2.9	7.2	0.135
Mean±SEM		2.9±0.2	10.7±0.7	0.099±0.006
Hypothyroid	15*	2.3	12.9	0.063
	22	2.2	13.6	0.059
	23*	2.4	14.5	0.061
	24	1.7	9.0	0.058
	25	1.4	10.0	0.042
	26*	1.2	10.2	0.016
	27*	1.8	9.0	0.068
	28*	2.1	13.0	0.058
	30*	1.8	8.5	0.062
	33	2.1	8.3	0.091
	34	1.4	8.6	0.038
	35	1.7	8.0	0.054
	36	1.9	8.3	0.078
	37	2.5	10.0	0.085
	38	1.9	13.5	0.047
Mean±SEM		1.9±0.1	10.5±0.6	0.059±0.005
	P	<0.001	>0.7	<0.001

* Rats received 0.9% saline as drinking fluid for at least 8 wk before experiment; the remaining animals received tap water as drinking fluid. There was no statistical significant difference in $(TF/P)_{IN}$, transit time, or $C/\pi r^2$ between the animals who received saline (*) or tap water as drinking fluid. Data reported represent the average of 2–6 tubular punctures.

The possibility then exists that the decrease in GFR in the hypothyroid rats may be caused by a decrease in the number of functioning nephrons. Studies on the remnant kidney have clearly demonstrated that superficial single nephron filtration rate (SSNgfr) is increased, while total kidney filtration rate (GFR) is decreased in this model of reduction of the number of functioning nephrons (19, 20). The proportional decrease of both SSNgfr and GFR observed in our study excludes a decrease in the number of functioning superficial nephrons as a cause for the decrease in GFR observed in the hypothyroid rats.

The increased excretion of sodium and water seems to be a characteristic of renal function in the hypo-

thyroid animals receiving a saline load (2, 5–7). This characteristic has also been observed in the present study examining nondiuretic hypothyroid rats. The excretion of sodium (absolute and fractional) was greater in these animals in spite of a reduction in the filtered sodium load, indicating a decreased tubular sodium reabsorption in the hypothyroid rats. The micropuncture data demonstrate that the proximal tubule is one of the sites of decreased absolute and fractional reabsorption of fluid in these animals. These data give strong support to the suggestion of Holmes and DiScala (2) of proximal tubular involvement in the exaggerated natriuresis of the hypothyroid rat. These authors observed that three out of nine hypothyroid animals excreted more than 45% of the filtered Na^+ load at the peak of the diuresis that followed the infusion of 5% saline.

In another recent paper by the same authors (21), oxygen consumption in cortical slices of hypothyroid rat kidneys was compared with that of control rats and found to be significantly decreased in hydropenia as well as during volume expansion with 5% saline. Considering the close correlation between tubular sodium transport and oxygen consumption in the kidney (22) and the fact that proximal tubules constitute the bulk of cortical tissue (23), these data constitute further evidence that proximal tubular sodium reabsorption is decreased in hypothyroid rats. Estimation of the absolute amounts of sodium delivered out of the end proximal tubule in the present study indicate that these were similar in the control (2.0 nEq/min) and the hypothyroid animals (1.8 nEq/min). Since the absolute amount of sodium excreted into the urine ($U_{Na}V$) was 300% higher in the hypothyroid rats, it is obvious that there also must exist a defect of tubular reabsorption of sodium further distal in the nephron to account for the enhanced sodium excretion.

Several factors, known to influence proximal tubular reabsorption of sodium should be considered. It is conceivable that the reduction in proximal sodium reabsorption in hypothyroid animals may represent an adaptive phenomenon to the chronic reduction in single nephron filtration rate. Acute reductions in GFR, however, are accompanied by an unaltered or increased, not a decreased proximal fractional reabsorption of filtrate (24–27). No definite comment can be made on the relative importance of the reduction of GFR on the decrease in proximal tubular reabsorption of fluid in the hypothyroid animals from our experimental data. That factors other than GFR are operative in the decreased reabsorption of sodium and water may be apparent from the exaggerated natriuresis that ensued without changes in GFR during the administration of a hypertonic saline load to hypothyroid rats (2).

Although a decrease in the secretory rate of aldosterone and in the tubular response to aldosterone has been reported in hypothyroid rats (8, 9), the sodium wasting in hypothyroid adrenalectomized rats was not abolished by the administration of mineralocorticoid hormones (10). In addition, exogenous D-aldosterone did not abolish the exaggerated natriuresis in response to a saline load in hypothyroid rats (2) even though it might have delayed its onset. Lastly, aldosterone has not been unequivocally shown to have an effect on proximal tubular sodium reabsorption (28).

Chronic expansion of the extracellular fluid volume (ECFV) in the hypothyroid animals cannot be ruled out. However, others (2) have found a significantly decreased ECFV in the hypothyroid rat. The measurements were not done in our animals. In addition, the infusion rates were controlled and chosen according to the body weight of the animals to minimize changes in ECFV during the experimental procedure.

Peritubular oncotic pressure and peritubular hydrostatic pressure are factors known to influence proximal sodium reabsorption. Peritubular oncotic pressure, if lower in the hypothyroid animals could account for our findings. However, the observation of an increased plasma protein concentration in the presence of a filtration fraction not different from that in the controls suggests an increased, not a decreased peritubular protein concentration in the hypothyroid rats. This presumably will facilitate an increase, not a decrease, in proximal reabsorption of filtrate.

Koch, Aynedjian, and Bank (29) have shown that an increase in mean arterial blood pressure decreases proximal reabsorption. Mean blood pressure, however, was lower in the hypothyroid rats, a fact which would have had the opposite effect on proximal reabsorption from that observed.

Lowering the hematocrit has also been observed to decrease proximal reabsorption (30–33). It appears that this effect is mediated through changes in blood viscosity (33), which in turn influence those physical factors that modify proximal tubular sodium reabsorption. In the studies in which they were simultaneously determined (30, 33) the decrease in proximal tubular reabsorption resulting from a decrease in hematocrit was consistently accompanied by a fall in filtration fraction. However, filtration fraction in the control and hypothyroid animals in our study was similar, and therefore it seems unlikely that the slightly lower hematocrit in the hypothyroid animals was the causative factor for the decreased sodium reabsorption in the latter.

Redistribution of filtrate from juxtamedullary ("salt conserving") to superficial ("salt losing") nephrons may result in an increased excretion of sodium. Indirect evidence from our study, however renders this possibility less likely. If this were the case, we would

have expected an increase in the ratio of superficial single nephron filtration rate to total filtration rate. This ratio, however, was the same in control and hypothyroid animals, making redistribution of filtrate, as judged by this indirect criteria, an unlikely major factor in the decreased sodium reabsorption.

Finally, the action of a humoral natriuretic factor cannot be excluded. Besides any as yet unidentified natriuretic humoral substance per se, the effects of calcitonin and parathyroid hormone merit some consideration because of their relationship with the thyroid gland. Calcitonin has been shown to have natriuretic properties in man (34) and rabbit (35). In the rat the calcitonin-producing parafollicular cells are located in the thyroid gland (36) and are removed or destroyed by surgery or radioiodide irradiation (39). For that reason it could not have played any role in the natriuresis observed in the hypothyroid rats. Decreased reabsorption of sodium in the proximal tubule has been observed in the rat (37) and in the dog (38) after the administration of parathyroid hormone. Even though the parathyroid glands were not completely removed by surgery in the animals used in our study, parathyroid tissue was probably reduced by this procedure. Irradiation with radioiodide in a dose of 875 μCi induces not only destruction of thyroid tissue in the rat but also affects the parathyroid glands with distortion of epithelial cells and infiltration with fibrin and neutrophil leukocytes (39). The dose of ^{131}I used to induce hypothyroidism was even higher in our study (1.0 mCi) making parathyroid damage a possibility. The observed 11% decrease in plasma calcium concentration in the hypothyroid rats may be consistent with this. Nevertheless, according to the above data (37, 38) a low activity of parathormone would not be expected to decrease proximal sodium reabsorption or increase sodium excretion.

Thus, we conclude that none of the discussed mechanisms can explain the decrease in fractional proximal reabsorption in the hypothyroid animals and that it is the chronic deficiency of thyroid hormone itself which causes the observed changes. The possible mechanisms whereby thyroid hormone deficiency alters proximal tubular fluid reabsorption are not known. Several mitochondrial enzyme systems have been reported to be decreased in content and/or in activity in the tissues of hypothyroid rats. Cytochrome a b, and c content as well as the activity of β-hydroxybutyrate dehydrogenase and glycerol-1-phosphate dehydrogenase in the kidney of hypothyroid rats are diminished (40). These enzymes, known to influence energy metabolism and glycolytic activity, could well be responsible for some of the observed changes in sodium transport.

In addition, Ismail-Beigi and Edelman (41) have recently reported on the effects of triiodothyronine (T^3) on $Na^+ - K^+$-activated adenosine triphosphatase (ATPase) in kidney homogenates of thyroidectomized rats. These authors found a 69% increase in $Na^+ - K^+$ ATPase activity after three subcutaneous injections of 50 μg T^3/100 g body weight (41). Furthermore, transmembrane Na^+ and K^+ concentration differences in liver and diaphragm of thyroidectomized rats were also significantly increased by the administration of T^3 (42). While thyroid hormone could have a simultaneous effect on active and passive transport of Na^+ and K^+ a dominant effect on the Na^+ pump is indicated by their studies (42).

Thus, although the exact mechanism of the observed decreased proximal sodium reabsorption in the hypothyroid rat remains unknown, the data best suggest that it is probably directly related to the lack of thyroid hormone. Whatever its mechanism, it appears that the natriuresis observed in the hypothyroid animal must be determined by a further decrease in tubular reabsorption in the distal nephron.

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