JCI The Journal of Clinical Investigation

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J Clin Invest. 1982;70(4):835-844. https://doi.org/10.1172/JCI110680.

Research Article

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Progenies from SH rats were found to have significantly higher urine Ca/creatinine (micrograms per milligram) (male = 38 vs. 23, $P < 0.05$; female = 79 vs. 60, $P < 0.005$) with 7/20 males and 9/26 females having values 2 SD above the means of normal. After a 12-h fast and during 10% volume expansion with saline, clearance and micropuncture studies were performed on three groups of acutely parathyroidectomized female rats; (*a*) normocalciuric (N) progenies from the normal, (*b*) normocalciuric (NC) progenies from SH, and (*c*) hypercalciuric (HC) progenies from SH rats. Among these groups, there was no significant difference in body weights, glomerular filtration rate, plasma ultrafiltrable Ca (4.5, 4.6 vs. 4.7 mg/100 g), PO_4, and the fractional excretion (FE) of Na or FE_{PO_4}. FE Ca was […]

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ABSTRACT Recently it has been observed that Ca excretion in laboratory rats does not follow a Gaussian distribution, with ~10% of them excreting Ca at a rate of 2 SD above the group mean. This phenomenon has been described as spontaneous hypercalciuria (SH). Our studies were designed to define its mechanism. 48 Wistar rats were subjected to metabolic studies to identify SH, prospectively defined as Ca excretion 2 SD above the group mean during 7 d of dietary Ca deprivation (≤0.03% by analysis), in the absence of hypercalcemia, PO_4 depletion, or exaggerated natriuresis.

Progenies from SH rats were found to have significantly higher urine Ca/creatinine (micrograms per milligram) (male = 38 vs. 23, $P < 0.05$; female = 79 vs. 60, $P < 0.005$) with 7/20 males and 9/26 females having values 2 SD above the means of normal. After a 12-h fast and during 10% volume expansion with saline, clearance and micropuncture studies were performed on three groups of acutely parathyroidectomized female rats; (*a*) normocalciuric (N) progenies from the normal, (*b*) normocalciuric (NC) progenies from SH, and (*c*) hypercalciuric (HC) progenies from SH rats. Among these groups, there was no significant difference in body weights, glomerular filtration rate, plasma ultrafiltrable Ca (4.5, 4.6 vs. 4.7 mg/100 g), PO_4, and the fractional excretion (FE) of Na or FE_{PO_4}. FE Ca was significantly higher in HC rats (13.9%) than N (10.1%) and NC (10.7%). Segmental reabsorption of fluid and Na was comparable among the three groups. Fractional delivery (FD) of Ca was, however, significantly increased in the late proximal tubule of HC rats (62 vs. 49 and 46%, $P < 0.05$). The increased FDCa was no longer apparent in early or late distal tubule (6.9 vs. 6.9 and 7.6%, P = NS). Although FECa exceeded late distal FDCa in all three groups, the increment was significantly greater in HC rats (7.02%) than both N (3.4, $P < 0.05$) and NC rats (3.05, $P < 0.02$).

The effects of chlorothiazide (27.5 mg/kg/d, i.p. × 7 d) were evaluated in the female offsprings of the SH rats. Before chlorothiazide, average urine Ca/creatinine (253 vs. 77.2) and cyclic AMP (26.6 vs. 13.4 μmol/mg creatinine, $P < 0.001$) on days 7 and 8 of the Ca-deprived diet were higher than the normal. On days 6 and 7 of chlorothiazide, average cyclic AMP (cAMP) excretion fell to normal range (11.7 vs. 12.7 μmol/mg creatinine) as Ca excretion was reduced to normal (62 vs. 59.4 μg Ca/mg creatinine).

We conclude: (*a*) SH, as defined in this study, is an inheritable biochemical marker and renal in origin. (*b*) The hypercalciuria is independent of parathyroid hormone, changes in plasma Ca and tubular handling of Na. (*c*) As studied in the PTX and volume expanded conditions of our experiments, decreased Ca reabsorption in superficial proximal convoluted tubule is demonstrable, but the hypercalciuria is probably mediated by diminished Ca transport by the deep nephron. The unlikely possibility of increased secretion by the terminal nephron, however, remains to be excluded. (*d*) In normal rats, there is internephron heterogeneity in regard to Ca transport during saline loading.

INTRODUCTION

The pathogenesis of idiopathic hypercalciuria (IH)[1] in man is incompletely understood (1). Potential mechanisms postulated include primary intestinal hyper-

Portions of this work were presented at the 37th Annual Meeting of the American Federation of Clinical Researchers, May 1980, Washington, DC, and published as an abstract in 1980. *Clin. Res.* 28(2): 454a.

Received for publication 29 October 1981 and in revised form 18 June 1982.

[1] *Abbreviations used in this paper:* AH, absorptive hypercalciuria; FD, fractional delivery; FE, fractional excretion; HC, hypercalciuric; IH, idiopathic hypercalciuria; JM, juxtamedullary; NC, normocalciuric; PTH, parathyroid hormone; PTX, parathyroidectomy; RH, renal hypercalciuria; SF, superficial; SH, spontaneous hypercalciuria in rats.

absorption (1–3) and diminished renal Ca reabsorption, or "renal leak" (1–4). Because increased Ca absorption is also observed, though, as a secondary or compensatory event in those patients considered to have renal leak, the mere presence of intestinal hyperabsorption is nonspecific. Evidence for these postulates is largely derived from the observed increase in plasma parathyroid hormone (PTH) and/or urinary cyclic AMP (cAMP) in the renal hypercalciurics (RH) (1–6) and decreases in the same parameters in the absorptive hypercalciurics (AH) (1–6). However, documentation for RH as vigorously defined above is scant (3, 5, 7), save for the original description of a few patients (2) and the recent larger series from France (6). The latter unfortunately included an indeterminable number of patients with simultaneously high serum Ca and PTH, making it impossible to exclude the so-called resorptive hypercalciuria (2). Studies by Lau et al. (8) and investigations by Coe and co-workers (1) were unable to segregate patients with IH into absorptive and renal subgroups on the basis of serum PTH. Evaluating the long term effects of the thiazide diuretics on intestinal Ca absorption and cAMP metabolism, Pak et al. offered additional evidence demonstrating the presence of a primary gut defect in the AH (3, 9). As both normal men (10) and rats (11) responded to thiazides with a reduction in urinary excretion of Ca and cAMP and in serum PTH, similar findings in the "RH" by these investigators (3, 9) are too nonspecific to prove the existence of the renal variety.

Nevertheless, there are recent data that indirectly suggest this possibility. Findings have been reported by Lau et al. (8) indicating defects in the reabsorption of Ca and PO_4, and in the proximal fluid reabsorption in patients with IH. Because these tubular dysfunctions are independent of PTH and the status of PO_4 balance, and since they are present in the fasted state, the question arises as to the role of intrinsic renal defects in the pathogenesis of IH. Although studies using pharmacologic manipulations (12) corroborate the evidence for tubular dysfunctions cited above, the lack of a suitable animal model has hampered investigations by more direct techniques.

Recently, it has been observed that Ca excretion in laboratory rats exhibits a bimodal distribution, with ~10% of them excreting Ca at a rate of 2 SD above the mean of the group (13). Because plasma Ca and PO_4 and urine PO_4 excretion were not different from the rest of the group, these investigators coined the term "spontaneous hypercalciuria" (SH) for these animals. The nature of this phenomenon is however, obscure, although the possibility exists that these animals are functionally analogous to the metabolic disorder of IH in man. To characterize the hypercalciuria and to define its mechanism, the present studies were performed on offspring born to rats with SH and their normal contemporaries.

METHODS

Identification of spontaneous hypercalciuric rats. Charles River Wistar rats (Charles River Breeding Laboratories, Wilmington, MA), 24 male (between 220 and 280 g) and 24 female (215–250 g), 4–4.5 mo in age, were housed in individual metabolic cages and fed a Ca-supplemented diet [Ca = 0.6%, prepared by adding $CaCO_3$ to a Ca-deficient diet (≤0.03% by analysis) and supplied by ICN Nutritional Corp., Cleveland, Ohio]. The diet contained 0.36% PO_4, 0.22% Na, 220 IU vitamin D per 100 g. After at least 1 wk of equilibration, over which weight gain was ensured, metabolic studies were performed with daily collections of urine. On day 3, $CaCO_3$ supplements were eliminated from the diet, and urine was collected for an additional 7 d. Throughout the entire study, deionized H_2O and food were allowed ad lib. Blood was obtained from retroorbital sinuses under light ether anesthesia both without fasting (on day 4) and after 14-h fast (on day 7 of the Ca-deficient diet). Urine and plasma were analyzed for Ca, PO_4, creatinine, and Na. On days 5 and 6, urine was collected under oil for determination of pH and NH_3.

Establishment of colonies of normal and hypercalciuric rats. During dietary Ca deprivation, three male rats and two female rats were found to excrete Ca at a rate that exceeded 2 SD over the mean of their respective groups (Figs. 1 and 2). Three hypercalciuric crosses were set up among them and one additional female rat that was borderline hypercalciuric (see rat "G" in Table I). Simultaneously, six rats (three males and three females) from the rest of the group with urine Ca/creatinine ratio within 1 SEM were used to set up three normocalciuric crosses.

Several attempts were made (either between the same pair of breeders or between breeders within the same categories) to yield a total of 15–25 offspring of a given sex within a given group.

Metabolic studies in the progenies. Between 2.5 and 3.5 mo of age, the progenies from the hypercalciuric and normocalciuric crosses were simultaneously subjected to identical metabolic evaluation performed for their parents as outlined above. The mean, SE and SD of the urinary Ca/creatinine ratio were calculated for offsprings from the normocalciuric crosses. At the end of the metabolic evaluation, the rats were replaced on Ca-supplemented diet until 1 wk before the following experiment.

Clearance and micropuncture studies in the female progenies. Three groups of rats were subject to these studies: (*a*) Rats from normocalciuric crosses with urine Ca/creat ratio within 1 SEM of the normocalciuric group (N), (n = 6). (*b*) Rats from hypercalciuric crosses with urine Ca/creat ratio 2 SD above the same mean (HC), (n = 6). (*c*) Rats from hypercalciuric crosses with urine Ca/creat ratio within 1 SE of the same mean (NC), (n = 4).

The following experiment was performed taking rats from these groups in an alternate manner. 1 wk before the experiment, the rat was fed the Ca-deficient diet again. After a 12–14-h fast, the rat was anesthetized and then surgically prepared as previously described (14). After insertion of the venous cannula, NaCl 150 mM/liter containing 5 mg/100 ml Ca was infused at a rate of 10 ml/100 g per h for the duration of the experiment. Methoxy [^{3}H]inulin was added to the infusate to monitor glomerular filtration rate. Para-

thyroidectomy (PTX) was performed by electric cautery as previously described (14). After an equilibration period of 120 min, clearance and micropuncture were performed over the ensuing 90–120 min, over which collections of 3 to 4 urine and 10 to 15 tubular fluid samples were obtained. At least three samples from each of the late proximal, early distal, and late distal segments were taken per rat. Tubular sites of micropuncture were identified and localized as previously described (14). Plasma was obtained every 30 to 45 min for determination of hematocrit, [^{3}H]inulin, Na, Ca and PO_4. Urine and tubular fluid were analyzed for similar parameters. At the end of the experiment, aortic blood was drawn under oil and plasma ultrafiltrate obtained by centrifuging with an Amicon cone (Amicon Corp., Scientific Sys. Div., Lexington, MA) as was previously described (14).

Effects of chlorothiazide administration on the excretion of cAMP and Ca. Two pairs each of SH and N rats from the progeny procured above were crossed. Their offspring were similarly evaluated as described. Urine was analyzed for Ca, creatinine, and cAMP. As the low Ca diet was continued, chlorothiazide (Merck, Sharp and Dohme, West Point, PA) was administered at 27.5 mg/kg per d, i.p. for the subsequent 7 d to the 10 SH females and 7 N females. Urine was similarly analyzed.

Specimen analyses. [^{3}H]inulin in plasma, urine, and tubular fluid was analyzed by liquid scintillation counter (Hewlett Packard Co., Palo Alto, CA). Ca, PO_4, Na, and creatinine in plasma and urine were determined by methods previously described (14). Urine pH was determined by the Beckman pH meter (Beckman Instruments, Inc., Fullerton, CA) and NH_3 analyzed by indophenol color reaction modified after methods described (15). Cyclic AMP was determined by a modification of methods of Steiner et al. (16) using a radioimmunoassay kit by New England Nuclear, Boston, MA.

Tubular fluid electrolytes were analyzed by an ARL (Applied Research Laboratory Division of Bausch & Lomb, Sunland, CA) electron microprobe. Except for the substitution for the Cameca model, details of sample processing and sample analysis were identical to published reports (14, 17). Unknowns were accepted only if the standard curves yielded correlation coefficient ≥0.999 between micro (by the probe) and macroanalysis (by conventional methods described above).

Calculations. The clearance (C) of substance X is determined by U_xV/P_x where U_x and P_x represent the concentration of x in urine and plasma (in the case of Na and inulin) or plasma ultrafiltrable (in the case of Ca and PO_4) and V denotes the urine flow rate. Glomerular filtration rate is estimated by C_{In}. Fractional excretion (FE) of x is calculated by the ratio of C_x/C_{in}. Fractional delivery (FD) of Ca is calculated by the ratio of tubular fluid:plasma ultrafiltrable Ca over tubular fluid:plasma inulin. FD Na is similarly derived. Titratable acid (TA) is approximated by the urinary concentration of $H_2PO_4^-$ derived from the formula, total urine PO_4/ [antilog (pH-pK_2) + I] where pK_2, the dissociation constant of $H_2PO_4^-$, is assigned the value of 6.8. Within the prevailing urine pH of these specimens, the error introduced by assigning the fixed value of 6.8 in this estimate of TA was ≤10%. All findings are expressed as the mean±SE unless otherwise stated using the average results from each rat as one data point. Data were subject to statistical analysis using the Student's *t* test, paired or nonpaired, whichever appropriate. Difference is considered significant if *P* value ≤0.05. Where more than two groups were compared, one-way analysis of variance was first applied to determine whether there was evidence for statistical difference.

RESULTS

Identification of SH rats. The metabolic profile of the parent generation and the selected breeders are summarized in Table I, Fig. 1 (female) and Fig. 2 (male). On 0.6% diet, none of the 24 female rats excreted Ca at rates outside 2 SD of the group mean [336 = 150 + 2 × (93), Table I)]. On a 0.03% Ca diet, however, two of the 24 female rats (M and W) had a urine Ca/creat ratio 2 SD above the group mean (179 = 89 + 2 × 45). Rat (G) (urine Ca/creat 148) was used for the third hypercalciuric cross even though it was only border-line HC. In terms of plasma Ca (nonfasted), plasma PO_4 (fasted), urinary Na/creat, and urinary PO_4/creat, these three HC rats were within 2 SD of the group mean (Fig. 1). Their body weights, urine pH, and urinary titratable acid and NH_3 were also within 2 SD of the group mean except for rat M, which had a high rate of NH_3 excretion (Table I). Thus, the hypercalciuria in these three rats was spontaneous and could not be attributed to hypercalcemia, PO_4 depletion, exaggerated rates of natriuresis, or accelerated rates of acid metabolism characteristic of increased acid consumption. Three other rats with urine Ca/creat ratio closest to the group mean were selected for the purpose of breeding a normocalciuric colony. There was no difference between the normocalciuric and hypercalciuric female breeders in any of the parameters depicted on Fig. 1 and Table I. Although the individual values for the three SH rats were within 2 SD of the group mean when placed on 0.6% Ca diet (Table I), as a subgroup, their urine Ca/creat ratio was greater than the remaining 21 [223±28 (SE) vs. 132±17, $P < 0.05$]. Additionally, as dietary Ca was reduced from 0.6 to 0.03%, the percent fall in this ratio was attenuated in these three SH female rats (−30.1±4.3 vs. −51.4±3.7%, $P < 0.05$). Taken together, these findings suggest that the hypercalciuria is relatively independent of diet Ca.

Similarly, when fed the 0.6% Ca diet, it was not possible to identify any HC male rats (Table I). However, when fed the 0.03% Ca diet, three of them (2, 6, and 10) had urine Ca/creat ratio 2 SD above the group mean (112 = 58 + 2 × 27) (Fig. 2 and Table I). Since all the measured plasma and urinary indices known to affect Ca excretion were within 2 SD of the group (Fig. 2 and Table I), the hypercalciuria in these three male rats was considered spontaneous. They were used as breeders. Three male rats with urine Ca/creat ratio closest to the mean were chosen to breed a NC colony. Except for their Ca excretion, there was no difference between the NC and SH breeders in any of the parameters measured (Table I and Fig. 2). Like the female, as a subgroup, the three SH male breeders excreted more Ca than the remaining 21 rats when fed

TABLE I

Weight and Urinary Excretion of Ca and H^+ Ion in the Parent Generation and the Selected Breeders

			Urine Ca/creatinine (μg/mg)								
			0.6%	0.03% diet Ca				Urine pH	Urine TA	Urine NH_3	Urine TA + NH_3
		Body wt (g)	Last 2 d	Days 1 & 2	3 & 4	5 & 6	1 → 6		(mM/mg creatinine)		
Male											
(n = 24)	Mean	257	78	64	58	53	58	5.8	0.26	0.094	0.36
	SD	±18	±42	±31	±32	±28	±27	±0.1	±0.04	±0.015	±0.05
	2° (★)‡	257	112	101	129§	113§	113§	5.8	0.21	0.080	0.29
	6 (■)	261	158	122	123§	110§	118§	5.8	0.27	0.120	0.39
	10 (●)	238	124	121	130§	120§	120§	5.9	0.21	0.074	0.29
	3 (☆)	269	88	59	55	51	56	5.7	0.26	0.082	0.33
	22 (□)	273	73	66	61	65	64	5.6	0.24	0.098	0.37
	23 (○)	266	97	69	65	70	68	5.7	0.25	0.092	0.34
Female											
(n = 24)	Mean	229	150	102	86	77	89	5.8	0.20	0.067	0.27
	SD	±13	±93	±57	±54	±35	±45	±0.3	±0.04	±0.014	±0.04
	G° (★)‡	215	171	217§	102	128	148	5.8	0.25	0.089	0.34
	M (■)	245	247	209	239§	175°	207§	6.1	0.17	0.104§	0.27
	W (●)	241	251	244§	197§	168°	203§	5.8	0.19	0.075	0.27
	C (☆)	234	126	73	68	63	68	5.6	0.27	0.094	0.36§
	N (□)	250	200	97	77	75	83	5.9	0.21	0.057	0.27
	R (○)	223	152	116	99	79	98	5.7	0.24	0.066	0.31

° Numbers refer to male rats, letters refer to female rats.

‡ Solid symbols refer to hypercalciuric and open symbols refer to normocalciuric breeders.

§ Outside 2 SD of mean of the group.

the 0.6% Ca diet [139±21 (SE) vs. 70±8, $P < 0.005$] (Table I), even though their individual values were not outside 2 SD of the group mean. Furthermore, as dietary Ca was reduced, the percent fall in the urine Ca/creat ratio was attenuated in these three HC rats (−10.9±9.8 vs. −34.8±3.9%, $P < 0.05$). These results are therefore suggestive of a hypercalciuria relatively unaffected by dietary Ca.

Establishment of hypercalciuric and normocalciuric progenies. Four breeding attempts were made between the three pairs of hypercalciuric breeders and four between the normocalciuric breeders. These yielded a total of 48 female progenies (Fig. 3) and 34 male progenies (Fig. 4). At 2.5–3.5 mo of age, they were similarly evaluated for spontaneous hypercalciuria as their parents. Figs. 3 and 4 represent a plot, respectively, for the female and male progenies of their average urine Ca/creat ratio during the last 3 d of 1 wk of 0.03% Ca diet. Each square or circle represents one rat. As a group, the progenies from HC parents had a significantly higher ratio than that from NC parents [79±5 (SE) vs. 60±3 for the female and 38±5 vs. 23±2 for the male]. 9 of the 26 female and 7 of the 20 males from SH parents had values 2 SD above the mean of the offspring from NC parents. These 16 (9 + 7) SH rats did not differ from their 30 (17 + 13) NC siblings or from the 36 (22 + 14) N rats born to NC parents in any of the following parameters measured: plasma Ca, plasma PO_4, urinary Na or PO_4, or urinary pH. These findings indicate that they have inherited SH from their parents.

Clearance and micropuncture studies. Having defined the biochemical parameters of these progenies, we chose the female offspring for clearance and micropuncture studies simply because there appeared to be more of them from every birth and because they did not grow as fast as the male. Three groups were selected (*a*) N rats born to NC parents (left hand panel, Fig. 3) that had urine Ca/creat ratio within 1 SE of the mean of the entire group (60±3, Fig. 3), (*b*) NC offspring from SH parents (lower half of the right hand panel, Fig. 3) that had urine Ca/creat ratio within 60±3, 1 SE from the same mean, (*c*) SH offspring from SH parents (upper half of the right hand panel, Fig. 3) that had urine Ca/creat ratio 2 SD above the same mean [84.7 = 60 + 2 × 12.4].

Except for a slightly lower body weight in the NC group (262 vs. 276 g) (Table II), there were no differ-

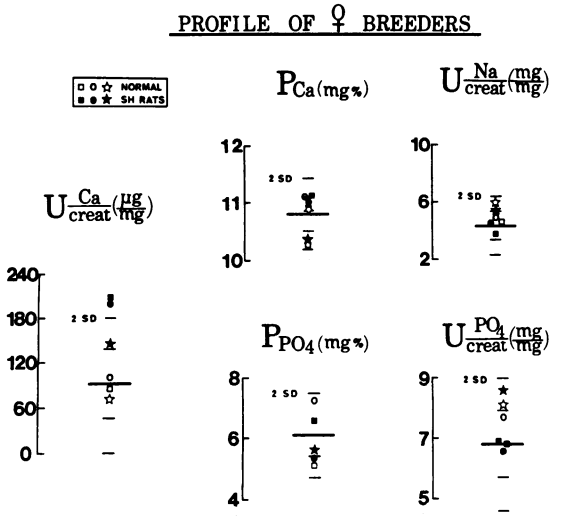

FIGURE 1 Biochemical profile of the parental generation (n = 24) from which three female hypercalciuric (solid symbols) and three female normocalciuric rats (open symbols) were identified and chosen as breeders. Heavy horizontal bars represent the mean of the group of 24 rats and lighter bars SD of the mean. U, urine; P, plasma. Only plasma PO_4 was obtained after 12-h fast.

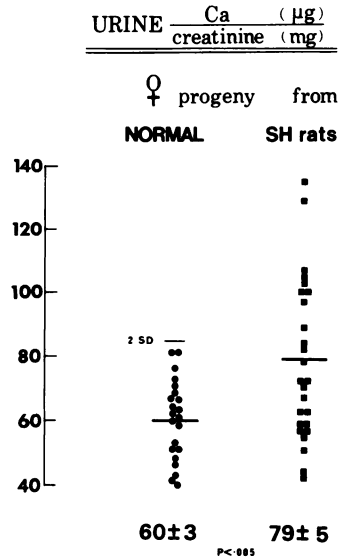

FIGURE 3 Urinary Ca/creatinine ratio for the female progenies from normal rats and rats with spontaneous hypercalciuria (SH). Each circle represents the average value for one rat born to the normal parents obtained during the last 3 d of 1 wk of 0.03% Ca diet. Each square represents the same but from a rat born to SH parents.

ences among the normal, SH, and NC rats in glomerular filtration rate, plasma ultrafiltrable Ca, plasma PO_4, or in their FENa and $FEPO_4$ (Table II). The moderately depressed plasma PO_4, uniformly for all three groups, probably reflects the low Ca diet on which these rats were chronically stabilized before the acute experiment. The markedly reduced $FEPO_4$ in all three groups is indicative of the acute PTX imposed at the start of the experiment. FECa was significantly higher in the SH rats (13.9%) compared with either the normal (10.1%, $P < 0.001$) or their normocalciuric sibling (10.7, $P < 0.05$). Thus, hypercalciuria in these rats persisted in the fasted state and did not depend on the presence of the parathyroid gland. Confirming the results of the metabolic studies, this phenomenon was not mediated by hypercalcemia or by abnormalities in the rates of Na or PO_4 excretion.

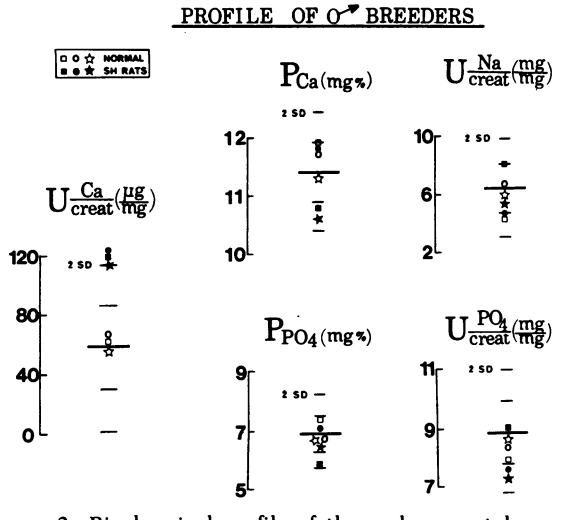

FIGURE 2 Biochemical profile of the male parental generation (n = 24) from which three hypercalciuric (solid symbols) and three normocalciuric rats (open symbols) were identified and chosen as breeders. Abbreviations and symbols are as defined in Fig. 1.

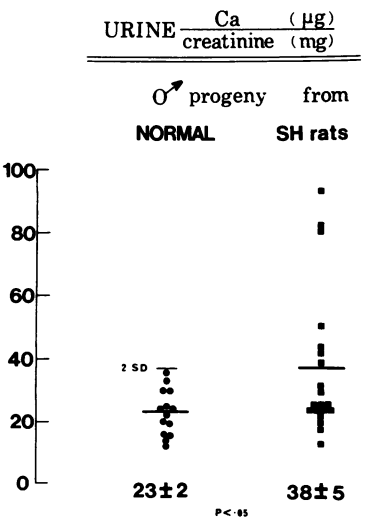

FIGURE 4 Same as Fig. 3 except that the data refer to the male progenies.

TABLE II
Plasma and Clearance Data in the Female Progenies (Mean±SE)

	Wt	GRF	Plasma UF calcium	Plasma PO_4	FENa	$FEPO_4$	FECa
	gm	*ml/min*	*mg%*	*mg%*	%	%	%
Normal	267	3.0	4.5	5.0	11.4	1.5	10.1
(n = 6)	±11	±0.2	±0.2	±0.2	±0.6	±0.8	±0.3
P*	NS	NS	NS	NS	NS	NS	<0.001
Spontaneous hypercalciuria	276	3.1	4.7	5.0	11.2	0.97	13.9
(n = 6)	±4	±0.2	±0.3	±0.2	±0.3	±0.57	±0.6
P*	<0.002	NS	NS	NS	NS	NS	<0.05
Normocalciuria	262	2.7	4.6	4.7	10.9	1.3	10.7
(n = 4)	±4	±0.1	±0.2	±0.4	±0.5	±0.9	±1.4

* P refers to statistical comparison between groups above and below.
NS = not significant.

Fluid and Na reabsorption were comparable among the three groups in the proximal convoluted tubule, within the loop of Henle and the distal convolution of the superficial nephrons subject to micropuncture studies (Table III). FDCa was significantly increased in the late proximal tubule of SH rats (62 vs. 49% in normal and 46% in NC rats, Table III), indicating reduced Ca transport within the proximal convoluted tubule of the superficial nephron. This reduction in Ca transport was not due to diminished fluid or Na reabsorption since FDCa/FDNa was still significantly greater in SH rats than in the normal (1.32±0.10 vs. 1.06±0.04, $P < 0.05$). However, Ca reabsorption by the pars recta and/or the loop of Henle of the SH rats was able to reclaim the increased load delivered, resulting in comparable FDCa to early distal tubule (15.4 vs. 14.4 and 14.6% P, NS, Table III). Transport within the distal tubule was also not different, as FDCa exiting the late distal puncture sites was similar (6.92 vs. 6.85 vs. 7.6%, P, NS, Table III). Compared with the fractional Ca delivery to the final urine, there was an apparent addition of Ca to the tubular fluid exiting the superficial distal tubule in all three groups ($\Delta = 7.02 \pm 1.03$, $P < 0.005$ in SH; $\Delta = 3.4 + 1.2$, $P < 0.04$ in N; $\Delta = 3.05 \pm 0.67$, $P < 0.05$ in NC rats). However, the magnitude of this apparent Ca addition was significantly greater in SH rats than in the normal or NC rats [7.02 vs. 3.4 ($P < 0.05$) and 3.05 ($P < 0.02$)].

Effects of chlorothiazide (Table IV). Before the thiazide, urine Ca, and cAMP excretion was significantly higher in the SH rats than the normal. During the thiazide administration, cAMP excretion fell to

TABLE III
Tubular Fluid Data (Mean±SE)

	Late proximal			Early distal			Late distal (LD)			ΔFDCa	Urine
	$\frac{TF}{P}$ In	FDNa	FDCa	$\frac{TF}{P}$ In	FDNa	FDCa	$\frac{TF}{P}$ In	FDNa	FDCa	LD-Urine	FECa
		%	%		%	%		%	%	%	%
Normal	2.2	45.1	49	3.3	14.9	14.4	6.1	9.5	6.85	−3.4	10.1
(n = 6)	±0.1	±2.7	±4	±0.3	±1.5	±1.7	±0.8	±1.7	±0.84	±1.2	±0.3
P*	NS	NS	<0.05	NS	NS	NS	NS	NS	NS	<0.05	<0.001
SH	2.1	47.7	62	3.7	14.8	15.4	6.4	10.2	6.92	−7.02	13.9
(n = 6)	±0.1	±3.0	±4	±0.2	±2.5	±1.9	±0.6	±2.1	±0.56	±1.03	±0.6
P*	NS	NS	<0.03	NS	NS	NS	NS	NS	NS	<0.02	<0.05
NC	2.2	44.9	46	3.7	16.4	14.6	6.7	11.0	7.6	−3.05	10.7
(n = 4)	±0.2	±4.3	±2	±0.1	±0.9	±2.4	±0.9	±0.6	±0.7	±0.67	±1.4

* P denotes statistical comparison between values above and below.
NS = not significant.

TABLE IV

Effects of Chlorothiazide on the Average Daily Excretion of Ca and Cyclic AMP (cAMP) in Female Progeny Equilibrated on a Low (0.03%) Ca Diet (Mean±SE)

Low Ca diet	Days 7 and 8			Days 17 and 18		
Chlorothiazide	None			Days 6 and 7		
	Urine Ca/Creat	Urine cAMP	Urine cCAMP/Creat	Urine Ca/Creat	Urine cAMP	Urine cAMP/Creat
	μg/ml	*μmol/d*	*μmol/mg*	*μg/ml*	*μmol/d*	*μmol/mg*
Normal rats (n = 7)	77.2 ±6.7	135 ±28	13.4 ±2.5	59.4* ±6.2	129 ±23	12.7 ±1.2
SH rats (n = 10)	253 ±19	258 ±19	26.6 ±1.2	62.0‡ ±9.0	113‡ ±20	11.7‡ ±1.3
P§	<0.001	<0.005	<0.001	NS	NS	NS

* $P < 0.03$.

‡ $P < 0.001$ vs. days 7 and 8 of low Ca diet within the same group.

§ P values refer to differences between groups.

NS = not significant.

normal (113±20 vs. 129±23 μmol/d) as urine Ca excretion was normalized (62 vs. 59.4 μg/mg creatinine). Thus, the hypercalciuria of SH rats was associated with increased excretion of cAMP, presumably a reflection of PTH activity, which could be normalized as the hypercalciuria was abolished. These findings are highly suggestive of a "renal leak" mechanism.

DISCUSSION

The documentation of SH in rats by Favus and Coe (13) has offered unique opportunities for shedding light on the human disorder of IH. The initial study suggests that intestinal hyperabsorption of Ca is present in rats with SH (13), but whether the hyperabsorption is primary (as was implied) or secondary to renal wastage of Ca (not vigorously excluded) remains unclear. Thus, it is uncertain to what extent "renal leak" also plays a role.

We sought to evaluate the existence of RH in the rat model by modifying the original definition of SH to include only those rats excreting excessive Ca when placed on low Ca diet. The rationales for such a prospective definition are two. First, we would like to focus our studies on a subgroup of SH rats least likely to be influenced by dietary Ca. The restrictive definition ought to improve the homogeneity of the population. It should be immediately emphasized that this approach did not entirely exclude rats with AH or resorptive hypercalciuria. Calculations indicate that even on a 0.03% Ca diet, the amount of Ca ingested per rat (average of 15–20 g of food) ranged between 4 and 6 mg/d, whereas the amount of Ca excreted varied from 0.6 to 2.0 mg/d. Accordingly, on this diet urine Ca accounts for only 10–50% of the diet Ca, values that are more in the same order of magnitude as for the adult man. Similarly, rats with resorptive hypercalciuria (2) would not be excluded by our definition since their hypercalciuria stems from abnormal rates of resorption of bone Ca(2). The second reason for our restrictive definition is that the low Ca diet ought to minimize the magnitude of diet and fecal contamination.

Our metabolic data indicate that it is possible to identify a small percentage of laboratory rats in both sexes that excrete Ca at rates outside 2 SD of the group mean. Results from breeding the HC rats demonstrate the feasibility of establishing such a colony with very similar phenotypic biochemistries as the parents. Approximately one-third of these progenies were hypercalciuric (Figs. 3 and 4). Similar to the male rats with SH previously described (13), the hypercalciuria observed by us could not be attributed to increases in plasma Ca, decreases in plasma or urine PO_4, or abnormal rates of Na excretion (Figs. 1 and 2).

Pathophysiologically, hypercalciuria could be resorptive, absorptive or renal (2). Lack of evidence for hypercalcemia (Table II), PO_4 depletion (Figs. 1 and 2), or abnormal acid-base metabolism (Table I) in these rats argues against a resorptive etiology. We could not absolutely exclude intestinal hyperabsorption. However, in response to marked reduction in diet Ca from 0.6 to 0.03%, Ca excretion fell far less in the hypercalciuric male (−11 vs. −35%) and female (−30 vs. −51%), which argues against AH (see Results). Furthermore, the associated increases in urinary excretion

of cAMP in the SH rats are inconsistent with an absorptive etiology according to current concepts of hypercalciuria (1–7, 9).

On the other hand, our studies provided strong evidence for a renal etiology. First, clearance studies indicate that after a 12-h fast and at comparable plasma ultrafiltrable Ca, FECa in SH rat was significantly higher (Table II). The clearance data further indicate that the hypercalciuria was not dependent on differences in plasma level of or renal sensitivity to PTH, as both the SH and normal rats had been PTX before studies. Persistence of increased rates of Ca excretion after fasting has previously been noted in SH rats (13) and support our interpretation of a renal origin. In this regard, the findings in SH rats are strikingly similar to IH man studied after Ca infusion to suppress endogenous PTH (8). Secondly and independently, the increased cAMP excretion in the SH rats and its normalization with chlorothiazide treatment in association with elimination of the hypercalciuria are strongly indicative of a primary renal leak.

From the renal standpoint, no known factors were present, to which the hypercalciuria could be attributed. These include the comparable rates of Na excretion (FENa = 11.2 vs. 11.4%, Table II), the lack of any evidence for PO_4 depletion or excessive acid ingestion. We did not definitively exclude renal tubular acidosis but considered this possibility remote, because of the known effect of increased tubular load of HCO_3^- on Ca reabsorption (18) and because of the comparable urine pH between normal and SH rats (Table I). It should be emphasized that intestinal hyperabsorption may be present and play a minor role in the pathogenesis of SH, which our studies could not absolutely exclude.

We next evaluated the tubular mechanism by the micropuncture techniques. It could be argued that our SH rats and the normal represented two ends of one continuous spectrum. To circumvent this criticism, we took two precautionary steps. First, the so-called normals had urine Ca/creat ratio within 1 SE of the group mean, so that they were not hypocalciuric. Secondly, another set of control was studied drawing from the NC siblings of the hypercalciuric progeny, which again had urine Ca/creat ratio within 1 SE from the mean of normal.

Results from these experiments indicate that there was diminished Ca reabsorption by the superficial proximal convoluted tubule (Table III). However, as this increased delivery of Ca was no longer apparent in early distal tubule, nephron segments between these two points (pars recta and/or ascending limb of Henle's loop) must have reclaimed the increment in Ca rejected by the pars convoluta.

Because Ca transport in both the proximal straight tubule (19) and the cortical thick ascending limb (20) is load-dependent, it is not unexpected that compensation for the defective proximal convoluted tubule within one or both of these nephron segments could normalize the amount of Ca delivered to the early distal tubule. Similar compensation by these segments has been observed for the increased rejection by the proximal tubule of PO_4 depleted rats (14). We could not differentiate which of these nephron segments was responsible, nor could we exclude the possibility of similar defects in the pars recta as in the pars convoluta. Since transport within the distal tubule was strikingly similar between the normal and SH rats (Table III), increased Ca excretion in the latter (Table II) must be mediated by changes beyond the late distal puncture site or outside the superficial nephron population.

At least two potential mechanisms must be considered. First, diminished reabsorption of Ca in the SH rats by the cortical collecting tubule, the medullary and/or the papillary collecting duct (collectively designated as the terminal nephron in subsequent discussions) would in theory adequately explain the hypercalciuria. Ca transport in the granular portion of the cortical collecting tubule (21) and by the inner medullary collecting duct (22) have recently been substantiated. However, there was an apparent addition of Ca between the late distal tubule and the urine (6.92–13.9% of the filtered load, Table III). Such an increment in FDCa could not be attributed solely to reduced reabsorption by the terminal nephron. Additional factors or a second and entirely different mechanism must be invoked. For the latter, two possibilities exist: secretion, or increased Ca delivery by the juxtamedullary nephron (JM) to the terminal nephron.

Similar but smaller increment in FDCa was found for the normal (3.4±1.2%) and the NC rats (3.05±0.67%). Thus, the hypercalciuria can be ascribed to either increased Ca secretion by the terminal nephron or increased Ca rejection by the JM nephron of SH rats. Presently, we cannot differentiate between these two possibilities, although there are several reasons to favor the interpretation of increased JM nephron rejection. First, the evidence for significant secretion of Ca under physiological conditions is scant, despite demonstrations for such a possibility in response to pharmacologic agents (23, 24).

Second, it is unusual for a metabolic disorder to be the consequence of an increased, rather than defective, transport. Third, functional heterogeneity between superficial and deep nephrons has been amply documented in regard to a growing list of solutes transported by the kidney: Na (25), Cl (26), K (27), and PO_4 (28). Similar phenomenon for Ca would not be unreasonable.

Indeed, the few data that are available concerning

Ca transport are compatible with this formulation. First, studies in young rats (29) and the Psammomys (30) indicate more intense reabsorption of Ca than Na by tubular segments proximal to the hairpin turn of the JM nephron; quite different from the close linkage between Ca and Na in the SF proximal convoluted tubule (31). Secondly, low dose furosemide reduced the transport of Ca, but not of Na, in the same tubular segments of only the JM nephron (29). Thirdly, recent in vitro microperfusion of the rabbit thick ascending limb suggests that the medullary portion transports Ca by a passive mechanism that can be inhibited by furosemide and increased by calcitonin, whereas the cortical portion transports Ca by an active mechanism that is insensitive to furosemide or calcitonin, but increased by PTH (32, 33). Because the loop of Henle of SF nephron traverses only a short distance into the medulla, whereas that of the JM nephron resides deep and primarily in the medulla, these observations indicate that transport of Ca by the JM nephron is less active in mechanism, more limited in capacity, and possibly more vulnerable to inhibitors like furosemide, than SF nephron.

Considered in this light, the increment in FDCa between SF late distal tubule and final urine in the normal rats is most likely the result of greater Ca delivery from JM nephron. Accordingly, the greater increment in FDCa in SH rats would represent greater rates of Ca rejection by the JM nephron relative to the normal JM nephron. In other words, Ca transport by the JM nephron as a whole is diminished in SH rats. Only studies in the Munich-Wistar rats could further evaluate this postulated hypothesis of functional heterogeneity. Furthermore, because the tubular fluid results were obtained from PTX rats, additional studies in the presence of PTH must be performed before extrapolation to the intact animal can be made.

The location of the defect in Ca reabsorption in the JM nephron cannot be answered by our studies. It is tempting to speculate that dysfunctions found in SF nephrons are also present in the proximal convoluted tubule of JM nephrons. If IH in man and SH in rats could be meaningfully compared, an unproven assumption, the proximal tubule dysfunctions in IH (8, 12) would corroborate our present observations in the SH rat.

Finally, the familial nature of SH in rats has been demonstrated, although the precise genetics of this metabolic disorder await specific studies. Despite the superficial resemblance between IH in man and SH in rats [familial (34), the presence of defects in proximal tubule transport (8)], more data are needed to establish a functional analogy.

In summary, we have documented the presence of a subgroup of SH rats that is renal in origin and familial in nature. The hypercalciuria is associated with increased urinary cAMP excretion that is correctable by thiazide diuretics. Furthermore, it is independent of plasma Ca, PO_4 depletion, PTH and the renal handling or excretion of Na. In the volume expanded and PTX condition of our experiments, excessive Ca excretion appears to be mediated by diminished transport in the JM nephron although there was an associated decrease in Ca transport in the SF proximal tubule. We have also demonstrated the existence of functional heterogeneity in the normal rats in regard to Ca transport under the saline loading conditions of our experiments.

ACKNOWLEDGMENTS

The authors would like to thank Edward Ku, Anna Ku, and Lisa Haberstrolh for their technical assistance, and Ms. G. Agas and Ms. C. Corson for their secretarial support.

These studies were supported by U. S. Public Health Service National Institutes of Health National Institutes of Arthritis, Metabolism and Digestive Diseases grant 1 ROI AM28035, a grant from the Michigan Kidney Foundation, and the American Heart Association grant 80-849. Dr. Lau is a recipient of National Institutes of Health Career Development Award 1 KO4 AM00843.

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