Urol Res (1986) 14:319–322

Urological Research

Ammonium Urate Urolithiasis in the Rat with Portocaval Shunt – Some Aspects of Mineral Metabolism and Urine Composition*

U. Linnemann[1], P. Kuch[2] and P. O. Schwille[1]

Division of Experimental Surgery and Endocrine Research Laboratory[1], Departments of Surgery and Urology, Institute of Mineralogy[2], University of Erlangen (FRG)

Accepted: April 18, 1986

Summary. In the male rat the effects of portocaval shunt (PCS) on mineral metabolism (fractional intestinal absorption, minerals in serum, in bone, in kidney, and in urine) and the effects on urinary constituents were studied. PCS induced a degree of hyperparathyroidism and the formation of ammonium acid urate urinary tract stones. These phenomena were associated with elevated uric acid, ammonium, urinary pH, as well as with elevated supersaturation of urine with struvite, and to a lesser degree with ammonium acid urate. It is suggested that in the rat PCS interferes with both parathyroid gland function and urinary nucleation of ammonium acid urate.

Key words: Portocaval shunt, Mineral metabolism, Hyperparathyroidism, Urinary supersaturation, Ammonium acid urate stones.

Introduction

Portocaval shunt (PCS) in the rat induces a number of metabolic changes. Amino acids accumulate in plasma and in brain [3]. Parathyroidectomy-induced hypocalcemia is reversed [7, 8], and moderate hypocalcemia is induced in the parathyroid intact rat [8]. To our knowledge urine composition after PCS has not been previously reported. Moreover, the study of mineral metabolism was restricted to serum calcium and phosphate, but without taking into account the associated intestinal absorption and bone deposition of minerals. The present work was conceived as a pilot study and suffers from the disadvantage of a small control group (see Animals and Procedures). However, the observation of stone incidence and the accompanying metabolic environment after PCS, is significant.

Animals and Procedures

Male Sprague-Dawley rats were used (Wiga, Coburg; FRG, body weight 240–280 g), and housed for a period of fifteen weeks under conventional conditions (12 h/12 h light/dark cycle; relative humidity 50–60 per cent, room temperature 22 ± 1 °C). After two weeks they were anesthetized (Pentobarbital-Na, 50 mg/kp ip) and were either sham-operated (laparotomy; $n = 3$) or underwent an end-to-side PCS [4]. Three days prior to termination of experiments the rats were separately housed in metabolic cages, and allowed only deionized water. Feces and urine were collected for the last two days in order to allow determination of fractional intestinal absorption [1 – (content in feces/content in food)] of calcium, magnesium, phosphate, and mineral balances. On the last day blood was drawn from the amputated tail stump under general anaesthesia for measurement of blood gases, and a further specimen was taken from the abdominal aorta. Serum was stored at –30 °C until analysed. The left femur was removed, freed of soft tissue, defatted in 70% alcohol, brought to constant dry weight (100 °C), and the bone volume measured using Archimedes' principle. Minerals in feces, urine, serum, bone ash, renal medulla were measured. From serum total protein, urea, creatinine, parathyroid hormone (predominantly N-terminal antibody AS 211/32, Wellcome, Beckenham; UK) and gastrin were estimated. Urinary pH, uric acid, ammonium, citrate, pyrophosphate, and oxalate were measured. Acid-base variables were also estimated. All assays employed routine and established methods, (for details see ref. [9]). Polarisation and scanning electron microscopy, X-ray diffractometry, as well as X-ray dispersion microprobe analysis were used for stone analyses. Because of the limited urine volumes available, which restricted the number of chemical determinations, the relative supersaturation products of the more important crystal- and stone-forming constituents in urine were read from a nomogram [5].

Since the number of animals in the control group precluded conventional statistics we chose to give individual values for fractional absorptions, urinary excretions, and balances of minerals, with pertinent median and range or mean and SEM, as appropriate (Table 1). Results from urine were given as median and range (Table 2), others (section 1, 2, 4 of Results) as mean and SEM. Had the differences shown up in text and tables been tested for significance the majority exceed $p < 0.05$.

* Parts of this work were presented at the XIIth Stone Symposium, series Bonn-Vienna, March 20–22, 1986 (Urol Res (1986) 14:86

Table 1. Effect of PCS or sham-operation on fractional absorption, urinary excretion, and balance of minerals. For further explanation see section on Animals and Procedures

No.	Fractional intestinal absorption; %			Urinary excretion; mg/mg creatinine			Mineral balance per 24 h; mg		
	Calcium	Magnesium	Phosphate	Calcium	Magnesium	Phosphate	Calcium	Magnesium	Phosphate
I. PCS rats; $n = 6$									
1	38	16	34	0.15	0.48	1.02	4.41	–0.07	34.25
2	41	23	15	0.21	0.76	0.56	6.57	0.36	18.6
3	35	17	16	0.12	0.69	0.25	4.49	0.12	16.7
4	33	–6	–1.6	0.84	0.94	0.41	5.75	–0.87	–3.8
5	19	4	6	0.21	0.45	1.04	1.8	–0.24	0.5
6	41	19	24	0.23	0.66	1.16	6.77	–0.05	27.7
$\bar{x}_{1-6}$	34.5	12.2	15.6	$\tilde{x}_{1-6}$ 0.21	0.68	0.79	$\bar{x}_{1-6}$ 4.96	–0.14	19.8
SEM	3.4	4.5	5.2	Range 0.12–0.48	0.45–0.94	0.25–1.16	SEM 0.75	0.17	5.8
II. Sham-operated rats; $n = 3$									
7	50	26	35	0.08	0.40	1.42	10.81	0.83	52
8	45	14	32	0.09	0.32	0.76	9.96	0.39	54
9	47	16	17	0.15	0.42	0.40	10.04	0.34	34
$\bar{x}_{7-9}$	47.3	18.7	28.0	$\tilde{x}_{7-9}$ 0.09	0.40	0.76	$\bar{x}_{7-9}$ 10.27	0.52	44.3
SEM	1.4	2.5	5.6	Range 0.08–0.15	0.32–0.42	0.40–1.42	SEM 0.27	0.16	8.7

Table 2. Effect of PCS or sham-operation on data in urine, which are of relevance in stone-forming processes. For further explanation see sections on Animals and Procedures

	PCS; $n = 6$	Sham; $n = 3$
I. Urine volume, pH and substances[a]		
Volume; ml[b]	29 (11–40)	10 (6–10)
pH	7.54 (6.76–8.16)	7.36 (7.09–7.49)
Uric acid; mg/mg	1.45 (0.78–2.38)	0.23 (0.20–0.27)
Ammonium; mM/mg	67 (50–93)	6.1 (4.2–6.2)
Citrate; µg/mg	25.5 (20.5–29.0)	5.1 (4.4–5.2)
Pyrophosphate; nM/mg	66 (66–115)	105 (31–125)
Oxalate; µg/mg	48.19 (28.68–102.11)	72.55 (61.06–100.90)
II. Relative supersaturation products		
Calcium oxalate	0.73 (0.59–0.99)	1.20 (1.25–1.5)
Brushite	0.11 (–0.23–0.19)	0.53 (0.50–0.62)
Octacalcium-Phosphate	0.22 (–0.61–0.57)	0.76 (0.55–0.93)
Ammonium acid urate	0.53 (0.46–0.55)	–0.28 (–0.11––0.32)
Struvite	1.2 (–1.2––2.4)	–0.05 (–0.25–0.2)
Sodium acid urate	0.05 (–0.4–0.4)	0.17 (–0.4–0.35)

[a] in mg or mM per mg creatinine
[b] per 24 h; mean of the last two experimental days

Results

1. General Data

During the entire observation period PCS animals ($n = 6$) consumed much less food than controls ($n = 3$); the values during the last three days were 16.4 ± 1.4 g and 23.8 ± 0.6 g per day, respectively. In contrast, PCS rats consumed more water (76.4 ± 15.1 and 43.8 ± 2.5 ml per day, respectively). The net result of this eating behaviour was an absence of weight gain in the PCS rats (252 ± 7.6 and 479 ± 36 g, respectively).

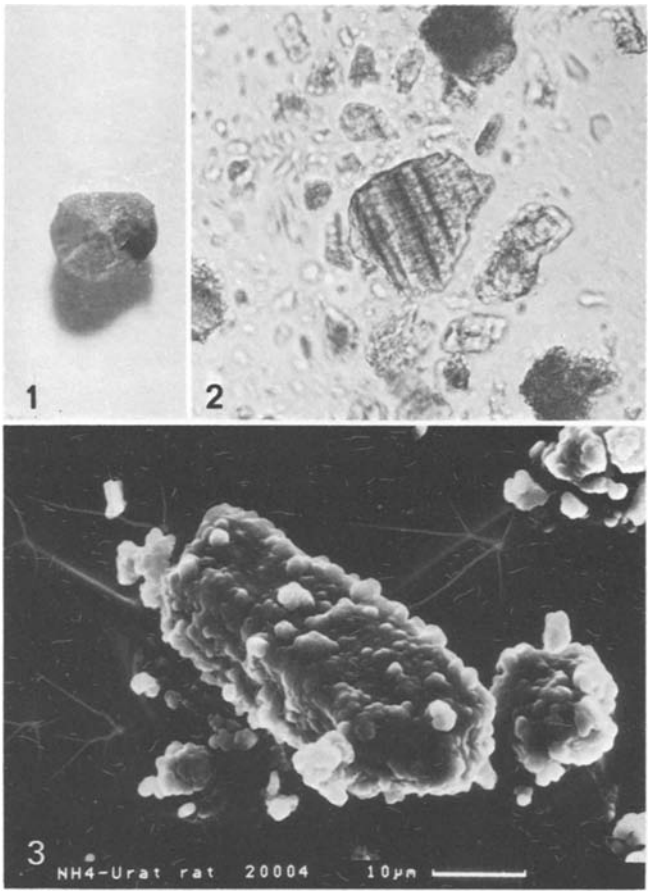

Fig. 1. Ammonium acid urate stone found in the urinary tract of rats fifteen weeks after PCS. Diameter: 1.5 mm. Note that the main morphological property is concentric layering (onion-like structure); a radial fibrous structure is visible

Fig. 2. Ammonium acid urate as in Fig. 1. Micrograph taken from the polarisation microscope (x 380). Note that the centrally located powder corn reflects the inner structure as shown by the native stone (Fig. 1)

Fig. 3. Ammonium acid urate as in Fig. 1. Micrograph of stalky aggregate with bulbous surface taken from the scanning electron microscope (x 1140)

There were no signs of urinary tract infection. When examining the urinary tract locally all PCS, but none of the control rats, had concretions in either the bladder or the pyelon. Analysis revealed ammonium acid urate, with minor admixtures of phosphorus, magnesium, potassium, calcium, the latter proven by micro-analysis. In Figs. 1–3 are shown the onion-like inner structure of one of these stones (Fig. 1), the powder when evaluated by the polarisation microscope (Fig. 2), and the crystals as documented by the scanning electron microscope (Fig. 3).

2. Data in Blood and Serum

The acid-base status, as reflected by the analysis of blood gases, was not changed by PCS. Total protein, an indicator of protein synthesis, and urea and creatinine, two indicators of protein catabolism, were all decreased after PCS. The respective values were 40.8 ± 1.6 and 52.3 ± 3.8 g/l, 281 ± 21 and 357 ± 24 mg/l, 2.60 ± 0.2 and 4.0 ± 0.2 mg/l (PCS versus controls).

The previously observed tendency toward PCS mediated low total calcium [8] was confirmed (2.15 ± 0.05 and 2.29 ± 0.09 mM/l, respectively), and also a clearly higher parathyroid hormone (728 ± 63 and 360 ± 77 pg-equiv/ml, respectively). Gastrin, a hormone so far not implicated in urinary stone disease, was considerably lowered by PCS (43 ± 5 and 116 ± 18 pg-equiv/ml respectively).

3. Intestinal Absorption, Urinary Excretion, and Balance of Calcium, Magnesium, Phosphate; Minerals in Bone and Kidney

From the data in Table 1 there was clear evidence that PCS reduced intestinal absorption of all three minerals, but increased urinary excretion per unit creatinine, albeit with phosphate there may have been no real increase. The balance of minerals was reduced by PCS; the greatest decrease was observed with magnesium. Analysis of the three minerals in bone ash failed to detect differences between PCS and controls, when the measured mineral was related to bone volume (mM/ml; data not shown). In contrast, the calcium content of renal medullary tissue, an area known to accumulate stone forming substances, was twice as high after PCS than in controls whereas phosphate remained stable (data not shown).

4. Urine Volume, pH, Other Urine Constituents; Relative Supersaturation of Urine (Table 2)

Among the measured variables there was an increase after PCS with volume (factor 3), uric acid (factor 6), ammonium (factor 11), citrate (factor 5), whereas pyrophosphate and oxalate were decreased to 63 and 61 per cent, respectively, of the values from control rats. The pH remained stabile.

The calculated RSPs are unexpected in as much as the only stone forming phase exceeding the upper limit of the range of metastability was struvite, contrasting with ammonium acid urate as the major constituent of the stones formed by PCS rats (see above). The RSPs of other stone forming phases, like calcium phosphates and sodium acid urate, which are all known to precipitate along increasing urinary pH, were reduced by PCS, as is the case with calcium oxalate.

Discussion

In the rat PCS regularly induces urinary tract stones composed of ammonium acid urate, a stone phase found seldom in humans (approx. 0.5 per cent; [2]). The spectrum of associated anomalies in PCS, as opposed to control rats, relates to body weight, minerals, hormones, urinary uric acid, ammonium and citrate. They clearly indicate that this type of stone formation is metabolic in nature, and not merely due to the dietary behavior of these animals.

There is less clarity, however, on the mechanisms underlying nucleation and growth of ammonium urate, although PCS induces a striking increase in urinary ammonium and uric acid. Thus, in PCS rats a certain degree of hyperparathyroidism may prevail, as indicated by elevated parathyroid hormone. In humans uric acid lithiasis has been found associated with primary hyperparathyroidism [12], and in the rat hyperparathyroidism arising from dietary magnesium deficiency induces nephrocalcinosis [10], most likely due to enhanced deposition of calcium phosphate. Unfortunately, the relative supersaturation product of hydroxyapatite, a phase known to form in more alkaline urine [6], present in hyperparathyroidism, could not be calculated because of the small amounts of urine available for analyses. Also, the ability of hydroxyapatite to induce heterogeneous nucleation of other stone forming phases, e.g. calcium oxalate, has been documented [6], but the influence upon ammonium urate remains unknown. Thus, at present the possible roles of parathyroid hormone and urinary supersaturation with hydroxyapatite as causes or mediators of ammonium urate stones formed in the presence of intestinal malabsorption of minerals after PCS await further investigation. One argument for such a role may be the higher content of calcium in the renal medulla after PCS.

The reduction of blood gastrin concentration following PCS in the present study suggests that PCS alters, in addition to parathyroid hormone, other peptide hormones, mainly from the gastrointestinal tract [13]. Interestingly, an association was found of low gastrin and high urinary pH in renal calcium stone patients [11], and in the present work there is a similar tendency toward higher pH in stone-forming PCS rats.

The interpretation of ammonium urate lithiasis is further hampered by the fact that its relative supersaturation product in urine does not exceed the upper limit of metastability, above which spontaneous nucleation can be expected [5]. In contrast, the relative supersaturation product of struvite, another phase known to precipitate in alkaline urine, like hydroxyapatite, exceeds this limit. Together with the minute amounts of magnesium and phosphorus found within a number of stones, it may be suggested that struvite may play some role in the nucleation of ammonium acid urate. However, more direct studies on possible interactions of the two phases are not available. During the course of our experiments it was reported that the increased uricosuria following PCS (see Table 2) may have provoked ammonium urate nucleation, but these authors did not give data on the physico-chemical environment in urine [14]. According to the present data factors other than simple supersaturation of urine with urate, ammonium or both may have contributed. Citrate, an inhibitor of nucleation of calcium containing stone phases (owing to its complexation of calcium ions) and of crystal growth and aggregation [1], substantially increases after PCS. For further studies one could speculate that citrate also interferes with struvite formation thereby stimulating ammonium acid urate. Some answers to unsolved questions as delineated here are expected from current studies in this laboratory aimed at the prevention of ammonium acid urate stones.

Acknowledgement. We are grateful to G. Wölfel, B. Schreiber, I. Goldberg, K. Schwille, for excellent technical and secretarial assistance. The study was supported by research funds of the University Hospital, Erlangen.

References

1. Bisaz S, Felix R, Neuman WF, Fleisch H (1978) Quantitative determination of inhibitors of calcium phosphate precipitation in whole urine. Miner Electrolyte Metab 1:74–83
2. Hesse A, Bach D (1982) Form und Zusammensetzung von Harnsteinen. In: Hesse A, Bach D (Hrsg) Harnsteine, Pathobiochemie und klinisch-chemische Diagnostik. Thieme, Stuttgart New York, S 4
3. Jeppson B, James IH, Edwards LL, Fischer JE (1985) Relationship of brain glutamine and brain neutral amino acid concentrations after proto-caval anastomosis in rats. Eur J Clin Invest 15:179–187
4. Lee S (1985) Portocaval shunt. In: Lee S (ed) Manual of microsurgery. CRC Press, Boca Raton, p 69
5. Marshall RW, Robertson WG (1976) Nomogramm for the estimations of the saturation of urine with calcium oxalate, calcium phosphate, magnesium ammonium phosphate, uric acid, sodium acid urate, ammonium acid urate, and cystine. Clin Chim Acta 72:253–260
6. Nancollas GH (1983) Crystallization theory relating to urinary stone formation. World J Urol 1:131–137
7. Pfeffermann R, Sakai A, Kashiwabara H, Fisch H, Taha M, Goldson H, Kountz SL (1977) The prevention and correction of hypocalcemia in the parathyroidectomized rat by portocaval shunt. Surgery 82:266–270
8. Schwille PO, Linnemann U, Issa S, Klein P (1985) Portocaval shunt decreases serum calcium and corrects partially parathyroidectomy induced hypocalcemia. In: Norman A, Schäfer K, Grigoleit HG, v Herrath D (eds) Vitamin D. A chemical, biochemical and clinical update. Walter de Gruyter, Berlin New York, pp 1085–1086
9. Schwille PO, Weippert JH, Bausch W, Rümenapf G (1985) Acute oral alkali citrate load in healthy humans. Response of blood and urinary citrate, mineral metabolism, and factors related to stone formation. Urol Res 13:161–168
10. Schwille PO, Brandt G, Brunner P, Ulbrich D, Kömpf W (1975) Pankreasinseln, Plasma-Glucagon und renale Verkalkungen unter verschiedener Grunddiät bei der Ratte. Urologe A 14:306–314
11. Schwille PO, Rümenapf G, Köhler R, Weippert JH (1987) Fasting gastrinemia and elevated supersaturation with hydroxyapatite of fasting urine – observations in renal calcium stone patients and controls Urol Res 15 (in press)
12. Scott JT, Dixon ASJ, Bywaters EGL (1964) Association of hyperuricemia and gout with hyperparathyroidism. Br Med J:1070–1073
13. Shurberg JL, Resnick RH, Koff RS, Ros E, Baum RA, Palotta JA (1977) Serum lipids, insulin and glucagon after porto-caval shunt in cirrhosis. Gastroenterology 72:301–304
14. Thomas J, Castaing D, Miramand JL, Gaultier J, Thomas E (1985) Augmentation de l'uricurie du rat après anastomose porto-cave. Action du clofibrate. Ann Urol 19:129–131

Prof. Dr. Dr. P. O. Schwille
Universitäts-Krankenhaus
Chirurgische Klinik
Maximiliansplatz
D-8520 Erlangen