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The Renal Clearance of Amino Acids in Cystinuria *

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Summary. The renal clearance of cystine, lysine, ornithine, arginine, and glycine has been compared with the simultaneously determined glomerular filtration rate in nine cystinuric patients. Five were studied before and after stabilization on penicillamine therapy, two were studied only while taking penicillamine, and two were studied only in the absence of penicillamine administration. The renal clearances of cysteine-penicillamine and of penicillamine disulfide were also determined in the patients who were taking the drug.

Amino acids were determined by quantitative ion exchange chromatography, and the reliability of the method has been evaluated in terms of its reproducibility and of the recovery of known amounts of amino acids added to plasma and to urine. The plasma levels of cystine and of the basic amino acids were less than normal in all the patients. Cysteine-penicillamine and penicillamine disulfide were cleared by the kidney at rates similar to that of cystine. Two of the patients had glycine clearances that were considerably above the normal value. The renal clearance of cystine exceeded the glomerular filtration rate in six of the nine patients. The results form a continuum from values approximately equal to the glomerular filtration rate to values about twice this amount. The renal clearances of cystine and of the basic amino acids vary independently of one another in the disease.

The significance of these results is discussed in terms of the nature of the renal tubular transport defect that underlies the disorder.

Introduction

During our investigations of the action of D(−)-penicillamine [1] in the treatment of cystinuria (1, 2) we have measured plasma and urinary amino acids before and during therapy with this drug. Dent, Senior, and Walshe (3) reported that the renal clearance of cystine was close to the glomerular filtration rate (GFR) in the disease; this was confirmed in three out of four patients studied by Arrow and Westall (4) and in three of the four patients studied by Frimpter and associates (5). Dent and Senior (6), Arrow and Westall (4), and Frimpter and co-workers (5) each have also reported single patients in whom the clearance of cystine was significantly greater than the glomerular filtration rate. Glycine excretion has also been reported to be increased in cystinuria (5).

In this paper we present cystine, lysine, ornithine, arginine, and glycine clearance data of nine patients with cystinuria, in six of whom the renal clearance of cystine was greater than GFR. The effect of penicillamine therapy on the blood and urinary concentrations and on the renal clearances of these amino acids and of cysteine-penicillamine and penicillamine disulfide is also reported. Some of these results have been the subject of a preliminary communication (7).

Methods

Determination of the renal clearances of the amino acids and simultaneous measurement of GFR. The amino

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[1] D(−)-penicillamine will be subsequently referred to as penicillamine.

TABLE I

*Reproducibility of the amino acid determination**

Amino acid	Reproducibility (n comparisons)								
	Aqueous solution			Urine†			Plasma‡		
	Mean	SD	n	Mean	SD	n	Mean	SD	n
Cystine	100.0	2.72	12	102.2	6.69	6	101.9	8.11	21
Ornithine	100.4	2.56	12	98.6	3.69	6	104.3	8.58	21
Lysine	99.6	1.79	12	102.0	6.80	6	100.5	6.94	21
Arginine	101.3	1.81	12	101.7	6.09	6	99.9	7.66	21
Glycine	104.1	6.50	11	101.4	4.16	6	102.6	6.66	21

* In the case of aqueous amino acid solutions, a volume (1.0 ml) of the standard amino acid solution, which contained 0.1 μmole of each amino acid, was analyzed and the mean value obtained expressed as a percentage of this. In the case of urine and plasma, each sample was analyzed twice, the value of 100% was arbitrarily assigned to the result of the first determination, the result of the second analysis was expressed as a percentage of this, and the standard deviation of the (n) individual comparisons for each amino acid about the corresponding mean was calculated.

† Cystinuric.

‡ Normal.

acid clearances and GFR were measured simultaneously, the renal clearance of inulin being used to measure GFR in all but one study (Patient 1, study before penicillamine treatment), when the endogenous creatinine clearance was employed. The patients were encouraged to drink water freely during the 9 hours before the test; they were otherwise fasting, and penicillamine was withheld for 12 hours before the investigation. The amounts of inulin infused and the timing of the blood and urine collections were the following: *1*) (Patients 1, 4, and 5). The loading infusion of inulin (4 g dissolved in 500 ml 0.9% NaCl solution) was given over the course of 30 minutes and followed by a maintenance infusion (2 g inulin per 500 ml 0.9% NaCl solution) at a rate of 2 ml per minute for the rest of the study. Timed 30-minute urine collection periods (without catheterization) were begun 30 minutes after the start of the maintenance infusion. Blood samples were obtained immediately before the study and at the midpoint of each clearance period.

2) (Patients 2, 3, 6, 7, 8, and 9). The loading dose of inulin (45 mg per kg body weight dissolved in 200 ml 0.9% NaCl) was infused over the course of approximately 20 minutes and was followed by a maintenance infusion (25 mg inulin per minute per 100 ml approximate GFR, which had been previously determined on the basis of the 24-hour endogenous creatinine clearance). The urine collection periods were timed to the end of micturition and were begun not less than 30 minutes after the maintenance infusion was begun. Each urine collection period lasted approximately 15 minutes. Blood samples were obtained at recorded times immediately before the study, at the beginning of the first clearance period, in the middle of the second and third clearance periods, and immediately after the third clearance period. The plasma inulin concentrations were plotted against time, and the concentration at the midpoint of each clearance period was determined by interpolation. We introduced this modification of the clearance technique to improve the design of the procedure, not because the first method gave unsatisfactory clearance data.

TABLE II

*The recovery of amino acids added to urine and plasma**

	Recovery	
	Urine†	Plasma‡
	%	
Glycine	99.5 (10.5)§	96.3 (15.6)§
Cystine	96.1 (4.1)	100.6 (9.8)
Ornithine	107.0 (4.9)	98.7 (11.0)
Lysine	99.5 (10.1)	111.7 (21.1)
Arginine	99.3 (5.3)	100.3 (11.4)

* A known amount (0.1 μmole) of each amino acid (1.0 ml) of the standard mixture of amino acids in 0.1 N HCl was added to each specimen of urine and plasma. An equal volume of 0.1 N HCl was added to the control samples.

† Cystinuric, 16 determinations.

‡ Normal, 28 determinations.

§ Standard deviation.

Preparation of blood samples for analysis. Blood was drawn from an antecubital vein with a heparinized syringe and immediately centrifuged at approximately 5° C to separate the erythrocytes. Plasma (2.5 ml) was removed, a known amount of norleucine (0.1 μmole, 0.1 ml solution) was added as an internal standard, and the protein was precipitated with salicylsulfonic acid (2.5 ml). The precipitate was removed by centrifugation and washed. The combined supernatants were evaporated to dryness in a rotary evaporator at 40° C.

Analytical methods. Amino acids were determined as described by Crawhall, Thompson, and Bradley (8) with a Technicon amino acid analyzer. The standard amino acid mixtures for calibrating the instrument were also obtained from Technicon Co.[2] Cysteine-penicillamine and penicillamine disulfide were prepared as described previously (2). DL-Ornithine and DL-norleucine were

[2] Technicon Instruments Co., Ltd., Hanworth Lane, Chertsey, Surrey, England.

TABLE III

Plasma and urinary concentrations of cystine, cysteine penicillamine disulfide, penicillamine disulfide, ornithine, lysine, arginine, and glycine, and the rate of urine flow during the renal clearance determinations

Patient	D-penicillamine dose*	Clearance period	Urine flow rate	Plasma amino acids							Urinary amino acids						
				Cystine	Cysteine penicillamine	Penicillamine disulfide	Ornithine	Lysine	Arginine	Glycine	Cystine	Cysteine penicillamine	Penicillamine disulfide	Ornithine	Lysine	Arginine	Glycine
	mg/24 hr		*ml/min*	*μmoles/100 ml*										*μmoles/100 ml*			
1	0	1	8.5	2.37			6.25	11.4	4.22	20.6	31.3			13.3	67.8	17.4	10.7
		2	4.92								44.1			20.4	102	28.2	18.1
	1,090	1	3.9	1.07	0.93	0.25	9.95	19.3	6.02	22.6	24.0	20.4	6.22	29.8	46.8	33.0	12.7
		2	11.9	0.86	1.29	0.44	7.79	14.4	5.98	17.2	7.43	7.54	4.72	24.8	45.3	15.2	19.4
2	0	1	15.4	2.91			5.21	15.1	8.91	16.8	16.7			10.6	45.0	22.3	7.68
		2	14.2	2.72			3.79	13.2	7.07	13.9	17.2			14.1	51.8	22.7	8.33
	727	1	11.4	0.76	1.81	0.70	5.00	10.1	4.41	17.1	5.46	13.3	5.75	17.2	36.3	21.3	10.4
		2	8.77	0.67	1.76	0.67	4.92	10.0	5.49	17.1	8.36	17.3	7.77	25.8	53.1	29.9	14.8
3	484	1	12.8	1.11	0.43	†	2.32	13.2	†	22.4	8.63	2.67	0.48	8.04	46.5	26.5	18.9
		2	7.9	1.17	0.43	0.05	2.51	12.8	4.78	22.4	16.5	3.90	1.14	1.68	†	50.4	29.3
4	0	1	3.17	2.02			10.1	16.8	6.14	24.0	118			140	523	234	76.2
		2	4.33	2.02			7.30	15.6	6.14	23.2	83.5			85.2	384	127	46.4
	0	1	3.07	2.03			5.40	14.4	6.04	20.1	85.7			68.4	215	154	27.0
		2	9.87	2.04			5.21	17.3	7.76	29.2	29.0			17.8	84.0	50.6	9.57
		3	14.6	2.02			6.77	17.3	7.76	22.9	18.9			17.7	57.2	33.6	6.20
	1,091	1	11.7	0.54	0.78	0.15	4.91	15.0	7.23	22.8	11.6	7.90	2.20	12.0	52.6	26.4	8.50
		2	11.6	†	†	†	†	†	†	†	9.26	7.72	1.90	12.2	54.4	32.3	7.10
		3	11.3	0.92	0.89	0.21	4.04	11.3	4.86	18.7	14.1	6.70	1.91	13.1	58.0	38.6	6.25
	1.090	1	13.2	†	†	†	†	†	†	†	13.5	5.30	1.32	16.6	63.0	38.2	10.9
		2	14.7	0.91	0.45	†	3.83	10.7	4.55	19.5	10.1	3.70	0.96	14.6	54.5	35.0	9.60
		3	14.0	1.10	0.45	†	3.42	10.5	4.45	19.1	10.9	2.90	0.68	13.5	51.5	31.0	8.50
5	0	1	8.33	1.83			3.54	10.5	5.66	20.5	41.7			28.6	60.0	79.1	15.4
		2	10.5	1.78			3.25	10.5	5.66	20.3	27.2			20.1	88.5	57.2	8.35
		3	12.5	1.88			4.36	12.3	6.38	22.2	18.8			20.4	94.3	59.0	12.6
	970	1	21.33	0.73	0.78	0.11	2.72	9.23	5.01	23.2	8.17	8.13	1.68	16.7	46.7	29.1	16.2
		2	9.17	0.94	0.47	0.20	2.96	11.2	5.67	27.2	16.7	10.1	0.72	18.1	44.4	61.0	15.8
6	0	1	18.4	2.25			2.69	14.1	6.08	17.5	18.6			8.41	53.0	29.7	16.9
		2	17.2	2.50			2.41	11.1	9.96	16.6	20.4			9.09	48.5	32.0	16.4
		3	19.3	2.37			2.54	11.1	5.96	15.8	17.4			7.73	43.4	25.7	14.7

*Calculated as the free base.
† Either technical fault with the analyzer or a peak too small to be integrated.

TABLE III—(*Continued*)

Patient	D-penicillamine dose*	Clearance period	Urine flow rate	Plasma amino acids							Urinary amino acids						
				Cystine	Cysteine penicillamine	Penicillamine disulfide	Ornithine	Lysine	Arginine	Glycine	Cystine	Cysteine penicillamine	Penicillamine disulfide	Ornithine	Lysine	Arginine	Glycine
	mg/24 hr		*ml/min*				*μmoles/100 ml*							*μmoles/100 ml*			
	1,090	1	17.8	0.95	0.91	0.28	2.41	11.6	5.34	17.8	6.29	6.19	2.87	9.30	42.8	26.3	21.1
		2	19.3	0.77	0.78	0.25	2.25	11.6	4.63	14.8	6.57	4.13	1.88	8.43	43.2	24.3	21.3
7	1.450	1	17.2	0.64	1.43	0.38	3.94	15.8	5.28	20.5	7.05	11.5	2.51	11.3	58.0	23.9	14.7
		2	15.7	0.64	1.42	0.33	2.65	16.1	6.07	20.3	8.10	11.5	2.88	12.0	63.8	25.5	13.7
		3	16.6	0.42	1.12	0.34	3.49	12.4	4.36	29.1	8.33	12.4	3.90	21.1	64.3	26.3	13.6
8	0	1	18.1	1.73			2.75	11.2	5.27	20.2	14.4			11.0	49.8	30.5	28.6
		2	14.9	1.89			2.94	11.8	5.14	23.4	14.2			10.6	49.3	29.2	31.1
		3	10.1	1.83			2.87	11.7	5.49	22.0	15.9			11.2	53.4	32.8	33.6
		4	14.7	1.83			2.93	11.6	5.56	21.1	15.6			10.7	51.8	30.8	32.2
9	0	1	8.65	2.15			4.50	14.9	8.30	23.3	19.5			3.57	27.4	1.81	5.17
		2	4.84	2.14			4.08	13.7	7.86	20.5	26.8			5.13	40.0	4.28	6.89
		3	7.77	2.17			4.26	13.0	8.20	20.1	25.1			4.78	35.0	3.93	6.62

obtained commercially.[3] The reproducibility of this method was tested for aqueous amino acid solutions, urine, and plasma (Table I). The recoveries of known amounts of the amino acids for which we report analytical data are shown in Table II.

Creatinine and inulin were estimated by the methods of Edwards and Whyte (9) and Schreiner (10), respectively.

Patients. The clinical states of the patients are summarized in the Appendix. They were hospitalized for these investigations. Patients 2, 3, 6, 7, and 9 ate a repetitive diet for at least 6 days before the studies; Patients 1, 4, and 8 ate an unrestricted diet.

Results

The concentrations of glycine, cystine, lysine, ornithine, and arginine in plasma and urine of the nine patients during the clearance periods are presented in Table III. Clearance measurements were not made on Patients 3 and 7 before penicillamine therapy was begun or on Patients 8 and 9 while they were on penicillamine treatment. Patient 4 was studied twice before and twice while taking penicillamine. The fasting plasma cystine concentrations agree with the values obtained in this disease by previous investigators (4, 5, 11), and penicillamine treatment reduced it by more than half in all cases. The concentrations of cysteine-penicillamine sometimes exceeded the concentrations of cystine, and the concentrations of penicillamine disulfide were considerably lower than those of the other disulfides. The precision of the penicillamine disulfide analysis is, therefore, less than that of the other amino acids. The concentrations of lysine and arginine in the plasma agree with the data of Frimpter (5, 12), being rather lower than earlier reported values in normal subjects (11, 12). The concentration of ornithine is more variable among different patients and within the normal range (2.95 to 10.6 μmole per 100 ml) (13) in some of the studies. Penicillamine administration did not alter the plasma concentrations of the basic amino acids.

The amino acid clearances and the simultaneously determined GFR are shown in Table IV. The ratio of cystine clearance ($C_{cystine}$) to GFR was unaltered by penicillamine administration (Patients 1, 2, 4, 5, and 6), and varies between about one and two. In six of the nine patients the cys-

[3] Hopkin and Williams, Ltd., Chadwell Heath, Essex, England.

TABLE IV

Glomerular filtration rate (GFR) and renal clearance (C) of cystine, cysteine-penicillamine, penicillamine disulfide ornithine, lysine, arginine, and glycine

Patient	D-penicillamine dose	GFR	Cystine		Cysteine-penicillamine		Penicillamine disulfide		Ornithine		Lysine		Arginine		Glycine	
			C_{Cys}	C_{Cys}/GFR	$C_{Cys\text{-}pen}$	$C_{Cys\text{-}pen}$/GFR	$C_{Pen\text{-}pen}$	$C_{Pen\text{-}pen}$/GFR	C_{Orn}	C_{Orn}/GFR	C_{Lys}	C_{Lys}/GFR	C_{Arg}	C_{Arg}/GFR	C_{Gly}	C_{Gly}/GFR
	mg/24 hr	*ml/min*	*ml/min*		*ml/min*		*ml/min*		*ml/min*		*ml/min*		*ml/min*		*ml/min*	
1	0	121	110	0.91					19.4	0.16	53.5	0.44	39.4	0.33	4.93	0.04
	1,090	105	109	1.04	88.7	0.84	122	1.16	38.3	0.36	47.7	0.45	35.8	0.34	14.8	0.14
2	0	94	104	1.11					49.2	0.52	57.7	0.61	47.4	0.50	9.12	0.10
	730	101	110	1.09	101	1.00	117	1.16	50.3	0.50	49.9	0.49	52.8	0.52	8.52	0.08
3	484	113	119	1.05	85.4	0.76	†	†	54.5	0.48	59.4	0.53	85.6	0.77	13.1	0.11
4	0	74.5	166	2.23					43.0	0.58	93.3	1.25	105	1.41	8.54	0.11
	0	73.2	122	1.67					41.6	0.57	47.8	0.65	72.1	0.98	4.04	0.06
	1.090	69.7	133	1.91	92.5	1.33	99.1	1.42	32.2	0.46	51.1	0.73	70.0	1.00	4.10	0.06
	1,090	86.2	151	1.75	114	1.32	†	†	51.1	0.59	67.1	0.78	97.6	1.13	6.32	0.07
5	0	97.2	141	1.45					57.4	0.59	67.2	0.69	104	1.07	5.28	0.08
	970	111	180	1.62	188	1.69	†	†	43.3	0.39	64.3	0.58	99.5	0.90	13.3	0.12
6	0	114	142	1.25					60.4	0.53	73.4	0.64	86.1	0.75	17.6	0.15
	1,090	120	158	1.32	122	1.02	198	1.65	70.5	0.53	68.9	0.64	102	0.75	24.5	0.15
7	1,450	103	188	1.83	127	1.23	116	1.13	60.8	0.59	58.2	0.57	66.8	0.65	10.5	0.10
8	0	95.6	129	1.34					68.4	0.71	69.4	0.72	90.2	0.94	22.8	0.23
9	0	72.3	105	1.45					10.4	0.14	21.4	0.30	3.77	0.05	2.79	0.04

* Glomerular filtration rate was measured by the clearance of inulin except where indicated by the second footnote symbol, when the endogenous creatinine clearance was used. These results are average values derived from the data given in Table III and adjusted to a standard body surface area of 1.73 m^2.

† Determination unsatisfactory for technical reasons.

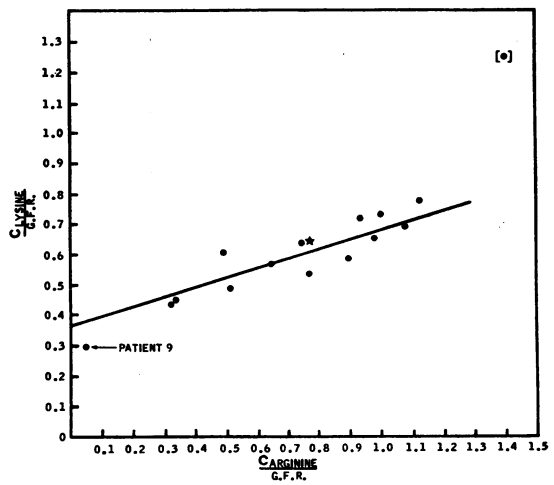

FIG. 1. RELATIONSHIP BETWEEN THE RATIOS C_{lysine}/GFR AND $C_{arginine}$/GFR. The regression line was calculated by the method of averages (the value enclosed in brackets was omitted from the calculation). The star indicates two identical values. C = clearance; GFR = glomerular filtration rate.

tine clearance is greater than the glomerular filtration rate. In general, the clearance of cysteine-penicillamine tends to parallel the cystine clearance, but the clearances of the basic amino acids do not necessarily parallel the clearances of cystine. The present values for the ratio $C_{arginine}$/GFR range from 0.33 to 1.13 except for Patient 9, in whom this value was 0.051. The ranges of the ratios C_{lysine}/GFR and $C_{ornithine}$/GFR are 0.44 to 0.78 and 0.36 to 0.59, respectively. Patient 9 is of special interest because her high cystine clearance ($C_{cystine}$/GFR = 1.47) is associated with smaller increases in the basic amino acid clearances than in the other patients, this being particularly marked for arginine ($C_{arginine}$/GFR = 0.05, C_{lysine}/GFR = 0.30, $C_{ornithine}$/GFR = 0.14).

When the clearance ratios $C_{arginine}$/GFR and C_{lsyine}/GFR are compared graphically (Figure 1), the results for Patient 9 lie on the line describing the results for the other patients.

Discussion

Accuracy of the method of amino acid analysis. Ion exchange chromatography with automated methods of determining the amino acids in the column effluent has been widely applied to the analysis of blood and urine. Its reliability for the determination of amino acids in aqueous solutions and in protein hydrolyzates is well documented, but there have been few detailed investigations of its validity when applied to biological fluids, which often present a more complicated analytical problem. Preliminary experiments confirmed that the reproducibility of the present method (Table I) when applied to aqueous solutions was satisfactory, the standard deviations of the individual values about their mean (expressed as 100%) being 2.72% or less for cystine and the basic amino acids. Similar values are reported by Spackman, Stein, and Moore (14) and Dickinson, Rosenblum, and Hamilton (13). We have no explanation for the larger standard deviation that we observed for glycine. The results obtained with urine and plasma showed a wider spread of values about the corresponding means, lysine and ornithine having the largest standard deviations in urine and plasma, respectively. This could have been due to small amounts of interfering substances that behaved inconsistently on the ion exchange column. Average recoveries within 5% of the theoretical results were obtained for glycine, cystine, lysine, and arginine in urine and for glycine, cystine, ornithine, and arginine in plasma. The recovery of ornithine from urine and of lysine from plasma was usually high, suggesting in this case also that there was interference by another Ninhydrin-reacting compound, which was not being separated from the amino acid concerned; this was tryptophan in the case of plasma lysine. Urine contains no tryptophan, so that this effect is not observed when it is analyzed. The range of values for the recovery data is wider for plasma than for urine, possibly due to the formation of small and variable amounts of low molecular weight substances during the deproteinization procedure, loss of free amino acids by absorption onto the denatured protein precipitate, or both. Cystine is known to be absorbed onto plasma proteins before precipitation (13), but loss due to this can be minimized by precipitating the plasma proteins within 15 minutes of drawing the blood, as was done in the present studies. Salicylsulfonic acid is a convenient protein precipitant because it does not have to be removed before the protein-free supernatant is applied to the column of ion exchange resin. Dickinson and associates (13) concluded that it was at least as satisfactory as any of the other methods that they studied; however,

Gerritsen, Rehberg, and Waisman (15) think that it leads to significant loss of some amino acids as compared with ultrafiltration. The cystine determinations on both urine and plasma were among the most satisfactory from the point of view of recovery and reproducibility (Tables I and II); the possible discrepancies that we have enumerated would not explain the relatively large differences between, for example, cystine clearance and glomerular filtration rate that we report.

Significance of $C_{cystine}/C_{inulin}$ greater than 1. While investigating the renal amino acid clearance of nine patients with cystinuria, we have encountered six patients in whom the renal clearance of cystine was greater than the GFR. Only one of these was of a magnitude comparable to the patient reported by Frimpter and associates (5), and the others appear to represent a continuous range from clearances equal to the GFR to clearances twice the GFR. No attempt was made in the present work to fractionate cysteine from cystine. However, the former amino acid cannot be detected in urine, so that even if it is assumed that all the plasma cysteine was oxidized to cystine during the preparation and analysis of the plasma samples, the resulting error would reduce and not increase the ratio $C_{cystine}$/GFR.

It has been suggested (4, 6) that the occurrence of cystine clearances that are greater than the glomerular filtration rate in cystinuria could be due to cystine being dissolved from calculi, and that this would be particularly likely to occur during a diuresis. Further studies of cystinuric stone-forming patients have shown that a consistent increase in the cystine excretion occurred only in one out of six patients during a diuresis of comparable magnitude to that achieved in the present studies (16). The rates of urine flow and the urinary cystine concentrations vary during some of the clearance studies (e.g., study 2 on Patient 4, Table III) without a corresponding change in the cystine clearance or in the glomerular filtration rate (Table IV), and this supports the view that the occurrence of $C_{cystine}$/GFR ratios greater than unity cannot be explained by particularly high rates of urine flow during part of the investigation. In addition, we do not think that stone dissolution explains the cystine clearance values that exceeded the glomerular filtration rate in the present work because some of the patients (1, 2, 6, 7, and 8) did not have radiologically demonstrable calculi.

Frimpter and co-workers (5) have discussed the significance of bidirectional flow of amino acids across the tubule cell as a possible mechanism to account for cystine clearances greater than GFR in cystinuria. Transport from the lumen of the renal tubule involves an influx pathway (I_{RT}) from the renal tubule into the cell and an efflux pathway (E_{PC}) from the cell into the peritubular capillaries. Some reverse flow can also be envisaged along the pathway (E_{RT}) from the cell into the lumen of the tubule and along an influx pathway (I_{PC}) from the peritubular capillaries into the cell. In this system, the experimentally determined ratio $C_{cystine}$/GFR depends upon the activities of I_{RT} and E_{RT}, and the occurrence of ratios greater than unity could be accounted for by a partial or complete defect that slows the rate of transport along I_{RT} without affecting E_{RT}. This concept of the disease is in accord with the *in vitro* demonstration that kidney cortex slices from cystinuric patients seem capable of actively transporting cystine (17), but it is not known if any of the patients whose renal tissue was examined in this way (17) had cystine clearance that was greater than GFR.

It has been shown that cystine does not exist as such in the human renal cortex but is wholly present in the reduced form (i.e., as cysteine) (18). Thus, it may be cysteine rather than cystine that is transported along the pathways E_{PC} and E_{RT}. The appearance of more cystine in the urine than can be accounted for by cystine filtered at the glomerulus could be due to increased *de novo* synthesis of cystine in the renal tubular epithelium. Some support for this idea is provided by the observations of Frimpter (12) and of Rosenberg, Durant, and Holland (19), who found that the cystine concentrations in renal artery and renal venous blood were the same. Frimpter (12) also reported that the concentration of cysteine in renal venous blood was less than that in the blood that reached the kidney, producing a high extraction of this amino acid. This observation was not, however, confirmed by Rosenberg and associates (19).

The renal clearances of cysteine-penicillamine and penicillamine disulfide are of the same order

of magnitude as the cystine clearance and do not depend on the dose of penicillamine under the conditions of these studies. This suggests that the cystinuric kidney may handle cystine and these "foreign" disulfides by the same, or similar, mechanisms and that some of these disulfides appear in the urine not only by glomerular filtration, but also by I_{PC} and E_{RT}. It is also possible, by analogy with cysteine (18), that penicillamine disulfide and cysteine-penicillamine may be reduced to thiols in the renal tubular cells.

Penicillamine administration lowered the plasma and urinary cystine concentrations, but the corresponding values for the basic amino acids were unaltered. The renal clearance values for the basic amino acids were, like the cystine clearance, unaltered by penicillamine administration. Thus, there is no increased loss of basic amino acids, and the over-all excretion of cystine plus the cysteine moeity of cysteine-penicillamine is decreased by penicillamine therapy (7).

Scriver, Efron, and Schafer (20) reported that the normal renal clearance of glycine was 4.7 (SD 1.3) ml per minute per 1.73 m^2. Some of the present group of patients had glycine clearances that were above this range, but only Patients 6 and 8 had glycine clearances that approached the value (23 ml per minute per 1.73 m^2) reported by Frimpter and co-workers (5) for their patient with a cystine clearance greater than the glomerular filtration rate.

Dissociation of the amino acid transport defects in cystinuria. There are striking differences in the ratios $C_{cystine}/GFR$, C_{lysine}/GFR, $C_{ornithine}/GFR$, and $C_{arginine}/GFR$ from patient to patient and in the relative magnitudes of these ratios within a given patient. These differences were not related to the administration of D-penicillamine. Thus, it appears that within a group of cystinuric patients there exist variations in the degree of expressivity of the abnormal genetic mechanism, which does not affect the transport of the four amino acids equally. Such variations of expressivity with respect to a single structural gene directing the synthesis of a carrier for cystine and the basic amino acids in the kidney would be expected to give rise to variations in the degree of the amino acid transport defect in which all four amino acids would be affected to the same extent. The present observation that the magnitude of the transport defect varied among the different amino acids as well as among different patients raises the possibility that the control of the formation of these mechanisms may depend upon the activity of more than one gene. This situation could prevail either because the individual amino acids have separate carrier proteins or because there is a modifying effect from other genes that influences the activity of a single carrier protein in such a way as to alter its affinity for the individual amino acids differentially.

Patient 9 is of particular interest because she shows an extreme degree of dissociation of the amino acid transport defects; thus, although the ratio $C_{cystine}/GFR$ was 1.49, the clearances of the basic amino acids were relatively lower than those observed for any of the other patients. In particular, the excretion of arginine was extremely low relative to that of cystine. Another patient with similar clearance data has recently been encountered in this laboratory (21), and patients with high cystine excretions but low arginine excretions have been reported by Crawhall, Saunders, and Thompson (22) and by Harris, Mittwoch, Robson, and Warren (23). When the clearance ratios $C_{arginine}/GFR$ and C_{lysine}/GFR are compared graphically (Figure 1), the results for Patient 9 lie on an extension of the line that describes the results for the other patients. This suggests that the manifestations of the disease in this patient represent an extreme degree of the same genetic abnormality as exists in the other patients rather than a separate genetic variant. Extrapolation of the regression line for $C_{arginine}/GFR$ on C_{lysine}/GFR cuts the latter axis at a finite value. These results indicate that at low rates of basic amino acid clearance the defect in lysine transport is more marked than the defect in arginine transport, although at high rates of basic amino acid clearance the arginine clearance can be greater than the lysine clearance (Figure 1). This is compatible with Harris and Robson's (24) generalization that when the reabsorptive capacity for the four amino acids is partially limited, arginine is reabsorbed preferentially to cystine and lysine.

A few preliminary studies suggest that the patients with $C_{cystine}/GFR$ greater than unity may

or may not have parents with detectable abnormalities of cystine and basic amino acid excretion. Similarly, the occurrence of high basic amino acid clearances does not appear to be correlated with the types of genetic pattern previously described by Harris and his colleagues (21, 23).

Appendix

Clinical data on the patients studied

Patient 1 is a twenty-three-year-old female with bilateral renal calculi for 4 years. Her urinary tract was radiologically normal.

Patient 2 is a twenty-five-year-old female who had right nephrectomy for recurrent calculi 15 years ago. Her left kidney and lower renal tract were radiologically normal.

Patient 3 is an eleven-year-old female who had right nephrolithotomy and left renal colic 6 and 3 years ago, respectively. Her right kidney contained a small calculus and had just detectable function on excretion pyelography.

Patient 4 is a twenty-one-year-old male with an 18-year history of bilateral renal colic who needed surgical treatment on several occasions. Calculi were present in his left kidney.

Patient 5 is a thirty-year-old male with a 16-year history of recurrent bilateral renal calculi who required surgical treatment on several occasions. Bilateral calculi were present. Pretreatment renal clearance studies were performed while he had thyrotoxicosis, which was treated by subtotal thyroidectomy before D-penicillamine was given.

Patient 6 is a nineteen-year-old male who had been passing stones and "gravel" for 5 years. His renal tract was radiologically normal.

Patient 7 is a twenty-nine-year-old male. He had renal colic and an impacted urethral calculus 5 years ago; his left kidney was not functioning then. He had repeated right renal colic with an impacted calculus and uremia on one occasion. No calculi were present at the time of the present studies.

Patient 8 is a twenty-two-year-old female (sister of Patient 6). She had symptoms of renal calculi for 4 years and bilateral nephrolithotomies. No calculi are now present.

Patient 9 is a ten-year-old female who had bilateral renal calculi and an impacted left ureteric calculus. The latter was removed surgically 5 months before the present studies.

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