Biochemical and Genetic Studies in Cystinuria: Observations on Double Heterozygotes of Genotype I/II

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ABSTRACT 10 families with cystinuria were investigated by measuring: (*a*) quantitative 24 hr urinary excretion of amino acids by column chromatography; (*b*) endogenous renal clearances of amino acids and creatinine; (*c*) intestinal uptake of ^{14}C-labeled L-cystine, L-lysine, and L-arginine using jejunal mucosal biopsies; (*d*) oral cystine loading tests. All four of these were studied in the probands and the first two in a large number of the family members.

49 members of 8 families were found to have a regular genetic pattern as described previously by Harris, Rosenberg, and their coworkers.

Clinical or biochemical differences between the homozygotes type I (recessive cystinuria) and homozygotes type II (incompletely recessive cystinuria) have not been found. Both types excreted similarly excessive amounts of cystine, lysine, arginine, and ornithine, and had high endogenous renal clearances for these four amino acids. Some homozygotes of both types had a cystine clearance higher than the glomerular filtration rate. Jejunal mucosa from both types of homozygotes exhibited near complete inability to concentrate cystine and lysine in vitro. This was also documented in vivo with oral cystine loads.

The heterozygotes type I were phenotypically normal with respect to the above four measurements. The heterozygotes type II showed moderate but definite abnormalities in their urinary excretion and their renal clearances of dibasic amino acids. Of the four amino acids concerned, cystine was the most reliable marker to differentiate between the heterozygotes type II and the homozygous normals.

In this study, type III cystinuria, as described by Rosenberg, was not encountered.

In two additional families, double heterozygotes of genotype I/II were found. The disease affecting these is clinically and biochemically less severe than that affecting homozygotes of either type I or type II. With respect to the four parameters used in this study, the double heterozygotes type I/II have results which are intermediate between those of the homozygotes type I and II and those of the heterozygotes type II.

INTRODUCTION

The genetic heterogeneity of cystinuria was first recognized in 1955 by Harris, Mittwoch, Robson, and Warren (1, 2) who described a *recessive* and an *incompletely recessive* form on the basis of urinary amino acid excretion patterns in heterozygotes. Their conclusions, reached by measurement of cystine, lysine, and arginine relative to creatinine in random morning urine specimens, have been universally accepted.

Thier, Segal, Fox, Blair, and Rosenberg (3) and Rosenberg and coworkers (4–6) further subdivided the disease into three genetic types mainly on the basis of heterogeneity of the accompanying intestinal transport defect in stone-formers. In their "type I" which corresponds to Harris' recessive type, the stone-formers lack active transport systems in the gut for the four dibasic amino acids, and the heterozygotes excrete normal amounts. In their "type II" in homozygotes, the active intestinal transport of dibasic amino acids was

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also impaired but with respect to cystine the defect was not as complete as in type I. Heterozygotes type II excrete definitely increased amounts of all four dibasic amino acids. In "type III," active intestinal transport of cystine, lysine, and arginine is present in homozygous abnormals and the urinary excretion of the four relevant amino acids is only moderately increased in the carriers. Types II and III are subdivisions of Harris' incompletely recessive variety.

To examine in greater detail the genetic heterogeneity of cystinuria, we have studied renal and intestinal handling of amino acids in cystinuric patients and other family members in 10 kindreds. In 2 of the 10 families, "double heterozygotes" of genotype I/II were found. Detailed investigation of these individuals revealed that the phenotypic expression of the cystinuric trait in them was different from that in homozygous abnormals and from that in heterozygotes type II.

METHODS

Patient material. 72 members of 10 unrelated Caucasian families were studied. The probands were between the ages of 4 and 52 yr. Eight probands had cystine stones, proven by chemical analysis (families CyA, CyB, and CyE–CyH). Two additional families (CyC and CyD) were included in the study when one of their members, while under investigation for another reason (febrile convulsion and coeliac disease), showed an abnormal urinary content of cystine and lysine which was detected by two-dimensional paper chromatography and later confirmed by quantitative measurements. The original proband of family CyC, who had coeliac disease, was replaced as the proband by his eldest healthy brother, who also had abnormal urinary excretion of cystine and lysine, so as to avoid complications in the intestinal uptake studies due to the damaged intestinal mucosa of the child with coeliac disease. The probands of these two families (CyC and CyD) and some of their relatives were found to be heterozygotes type II.

Control material. Normal values for 24 hr urinary excretion of amino acids were derived from tests on 44 healthy individuals, 23 adults (21–50 yr of age) and 21 children (3–11 yr of age). Endogenous renal clearance of amino acids with endogenous creatinine clearance was measured in 10 normal adults between 17 and 45 yr of age, and in 2 children aged 7 and 12 yr.

One healthy adult and four children between 2 and 5 yr of age, were used as controls in studying the uptake of L-cystine-^{14}C and L-lysine-^{14}C by jejunal mucosal biopsies. The adult was the father of one of our heterozygote type II probands and had a normal 24 hr urinary excretion and clearance of amino acids. The four children were under investigation for chronic diarrhoea and were accepted as controls when their jejunal biopsies were judged to be normal under the dissecting microscope and subsequently by light microscopy.

Three normal young adults served as controls for the L-cystine loading tests.

Clinical methods. The probands and members of their kindreds under the age of 4 yr were studied in the Clinical Investigation Unit of the hospital. At the time of the study, only one proband (CyF, II-2) had clinical or roentgenological evidence of calculi and none had urinary tract infections, as determined by examination of the urinary sediment and culture. D-penicillamine and sodium bicarbonate treatment was discontinued for a week before and during the investigation, but high fluid intake was maintained. For the first 2 days, the patients were stabilized on the normal ward diet while routine laboratory tests were performed. Consecutive quantitative 24-hr urinary collections were then performed for 2 or 3 days. Endogenous renal clearances of amino acids and creatinine were determined simultaneously on the next day, and an intestinal biopsy and a cystine loading test were performed on the subsequent 2 days. 8 of the 10 probands were investigated on two or more occasions, at least 3 months apart.

The 24-hr urine samples of the older family members and controls were collected at home according to specific written instructions while these individuals were on their usual diet. All renal clearances were done in the Clinical Investigation Unit.

Clearance procedures. Simultaneous endogenous clearances of creatinine and amino acids were measured after a 12 hr overnight fast. Adequate urine flow was assured by increasing the water intake for 2–3 hr before and during the test. An exactly timed urine collection was made over a period of 2½–4 hr, the specimens being voided spontaneously. Blood samples were drawn for amino acid and creatinine determinations both at the beginning and at the end of the timed urine collection. The mean plasma levels of the two amino acids determinations and the mean of the two creatinine determinations were used for the calculation of the clearances.

Preparation of samples. For amino acid determinations, venous blood was drawn into a syringe containing a minute quantity of crystalline EDTA to prevent clotting. The plasma was separated by immediate centrifugation and the proteins precipitated within 15 min by adding 1 volume of plasma, drop by drop, with constant mixing, to 5 volumes of saturated picric acid solution. The supernatant obtained by centrifugation was stored in a refrigerator. The technique for the prevention of oxidation of cysteine to cystine in plasma was not used (7). During the collection periods, urines were kept refrigerated and thereafter frozen. For creatinine and uric acid determinations, serum was used which was stored frozen.

Analytical procedures. Amino acids in plasma and urine were measured by the Piez-Morris procedure of ion exchange column chomatography (8) using a 140 cm micro column in an automatic amino acid analyzer (Technicon Corp., Tarrytown, N. Y.). The method was standardized by the use of analytical grade L-forms of amino acids.

In the case of plasma, the picric acid was removed by a Rexyn AG_1 column (Fisher Scientific Co., Pittsburgh, Pa.). The eluate was then evaporated to dryness in a vacuum evaporator, redissolved in an acid-sucrose solution (0.02 N HCl and 12.5% sucrose) and a portion corresponding between 0.5 and 1 ml of plasma was loaded on the column. Urine was acidified with an equal volume of 0.02 N HCl, evaporated to dryness, and redissolved in acid-sucrose solution as described above. A volume corresponding to ½ or 1 min excretion was loaded on the column.

Creatinine in the serum was measured by a modification (9) of the method of Owen, Iggo, Scandrett, and Stewart (10). With this method, we found that the ratio of endogenous creatinine clearance to inulin clearance was 1:1 in one study consisting of three short clearance periods. Other investigators have shown that the true endogenous creatinine: inulin clearance ratio is only slightly greater than

1.0 (11, 12). Creatinine in the urine was determined by an AutoAnalyzer (13).

Urinary calcium and magnesium were measured by emission and atomic absorption flame photometry (14, 15), urinary inorganic phosphate by an automated adaptation of the Gomori method (16). Uric acid in serum and urine was measured by the carbonate-phosphotungstate method (17). Urinary oxalate was determined by Archer's method (18), by a commercial laboratory.[1]

Calculations. The glomerular filtration rate was calculated from the true endogenous creatinine clearance. The filtered load of each amino acid (P_{AA})[2] was obtained from the product of the plasma concentration of each amino acid and the creatinine clearance. The net reabsorption of each amino acid per minute was obtained by subtracting its rate of excretion in micromoles per minute (UV_{AA}) from its filtered load. The percentage of reabsorption was calculated by dividing the amount reabsorbed by the filtered load, times 100. The clearance of each amino acid (UV_{AA}/P_{AA}) is reported in milliliters per minute per 1.73 m^2 BSA (body surface areas).

The 24 hr urinary excretion of amino acids is expressed as micromoles per kilogram of body weight. After having expressed the values obtained from 44 normals (23 adults and 21 children) in micromoles: (*a*) per kilogram of body weight per 24 hr; (*b*) per 1.73 m^2 BSA/24 hr; (*c*) per gram of creatinine; (*d*) per liter of urine, we found that the results in adults and in children showed good correlation only when they were expressed in μmoles/24 hr per kg of body weight, or per gram of creatinine. However, the correlation based on weight was better than that based on creatinine. Expressing the 24 hr amino acid excretion on the basis of kilogram body weight, the *P* values are: cystine, 0.66; lysine, 0.62; ornithine, 0.5; arginine, 0.04. We have no explanation for the lack of correlation in the case of arginine. Because we had no controls of our own under 3 yr of age, values from family members less than 3 yr of age were not included.

Finally, when arginine or ornithine peaks, or both, were too small to be integrated on the chromatogram, values representing the minimum amount calculable (7.5 μmoles for the 24 hr urinary excretion, and 0.01 μmoles per min for clearance) were assigned.

Oral loading studies. After an overnight fast, the subject quickly drank L-cystine (0.5 mmole per kg of body weight) mixed with 100 ml of tap water. The glass container was then rinsed four times with 25 ml of tap water which the subject drank to ensure complete ingestion of the dose. Blood samples for quantitative measurement of amino acids were obtained from the antecubital vein, before and at ½, 1, 1½, 2, 3, and 4 hr after the cystine load.

Intestinal uptake of amino acids. After an overnight fast, jejunal mucosal biopsies were performed by the oral route using a Crosby capsule which was placed at the ligament of Treitz under fluoroscopic control.

The technique for handling these specimens and their incubation with ^{14}C-labeled amino acids and inulin-^{14}C was as described by Thier et al. (3) and Fox, Thier, Rosenberg, Kiser, and Segal (19), including estimation of inulin space and calculation of distribution ratios for labeled amino acids. The total tissue water was considered to be 80% of the initial wet tissue weight (20). The extracellular water as measured by labeled inulin was 28 ±9.3% of the wet tissue weight.

The radioactive compounds used were L-cystine-^{14}C,[3] L-arginine-^{14}C,[4] L-lysine-^{14}C,[5] and inulin-carboxyl-^{14}C of molecular weight 5000–5500.[6] The purity of these was verified by paper chromatography and radioautography. Incubations were all aerobic (95% O_2 and 5% CO_2) and for 45 min.

At the end of the incubation the biopsy material was withdrawn from the flasks as quickly as possible. It was dipped twice in isotonic saline to rinse off surface radioactivity, blotted, weighed on a torsion balance, and then placed in a counting vial containing 1.0 ml of NCS[7] and left overnight in the dark at room temperature; 0.1 ml of the medium was treated the same way. The following day, 9 ml of a solution containing 0.457% PPO (2,5-diphenyloxazole), 0.010% POPOP (*p*-bis[2-(5-phenyloxazolyl)]benzene) in toluene was added to the NCS solution. The vials were then sealed, shaken, and counted in a liquid scintillation spectrometer with an efficiency of 70–75% for this system.

Statistical treatment. Standard statistical procedures (Student's *t* test and the modified *t* test) were used (21).

RESULTS

Genetic material

In this study 8 of the 10 families (CyA–CyH) conformed to the genetic classifications of cystinuria of Harris et al. (1, 2) and Rosenberg et al. (4, 6). The two atypical families (CyI and CyJ) will be discussed separately.

Typical families. Genotypes were assigned to 49 members of the first 8 families based on their 24 hr excretion and renal clearances of amino acids and on their pedigrees (Fig. 1).

A subject with the constellation of cystine stone-formation, marked hyperexcretion of cystine, lysine, arginine, and ornithine, and grossly abnormal endogenous clearances of these four amino acids, is classified as a homozygote. A clinically unaffected parent or a clinically unaffected offspring of a known homozygote is classified as a heterozygote. A heterozygote is considered as type I (genotype +/I) if both the 24 hr urinary excretion of cystine and the cystine clearance are normal; as type II (genotype +/II) if the urinary excretion of cystine is above 3.4 μmoles per kg of body weight/24 hr (the upper 99% confidence limit in controls) and/or if the percentage of tubular reabsorption calculated from the clearances is below 98.8% (the lower 99% confidence limit in controls). Homozygous abnormals were considered to be type I (genotype I/I) when both parents were +/I, and homozygotes type II (genotype

[1] Bio-Science Laboratories, Van Auys, Calif.

[2] *Abbreviations used in this paper:* BSA, body surface area; P_{AA}, filtered load of each amino acid; UV_{AA}, rate of excretion in micromoles per minute; UV_{AA}/P_{AA}, clearance of each amino acid.

[3] New England Nuclear Corp., Boston, Mass.

[4] New England Nuclear Corp.

[5] Radiochemical Centre, Amersham, England.

[6] New England Nuclear Corp.

[7] Nuclear-Chicago solubilizer, Nuclear-Chicago Corp., Des Plaines, Ill.

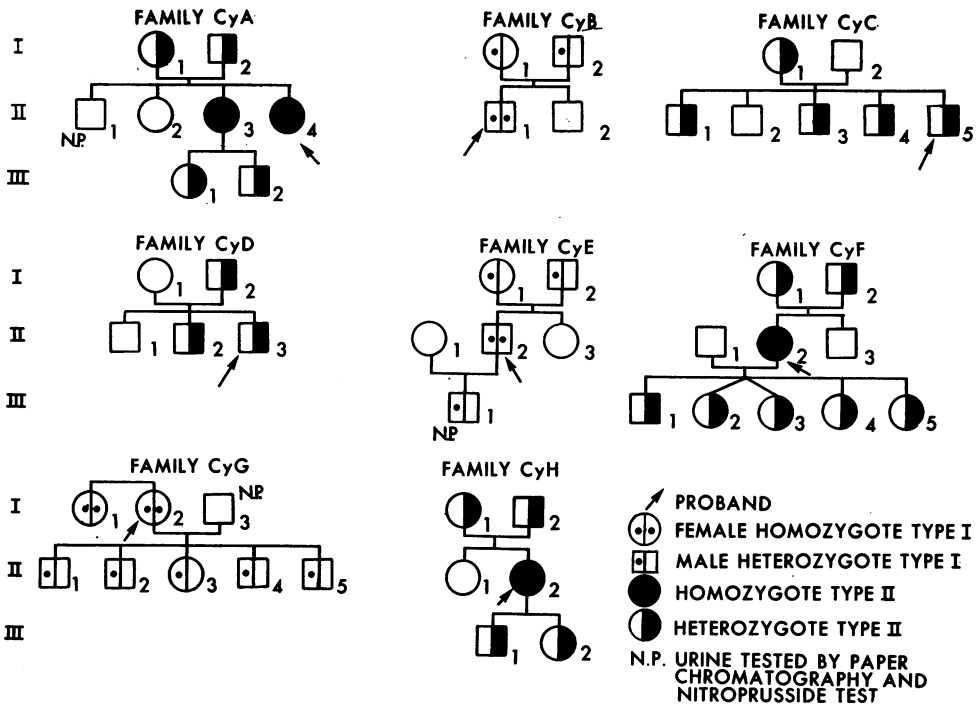

FIGURE 1 Pedigrees of eight typical cystinuria families (CyA-CyH).

II/II) when both parents were +/II. In family CyG, the proband and her sister (CyG, I-1) were assigned to the I/I group because all five of the former's children (CyG, II-1 through II-5) were phenotypically normal, i.e., presumably +/I. Families CyC and CyD are included among the eight typical families since the abnormal cystinuric trait was present not only in the probands, who were +/II, but also in the sibs of the probands and in one of each pair of parents.

In Table I we show the numbers of individuals of the various cystinuria genotypes in the eight typical families. Altogether we have 4 I/I and 4 II/II homozygous abnormals, 9 heterozygotes +/I, and 23 heterozygotes +/II. Seven individuals were homozygous normals (genotype +/+) and two were unclassifiable.

Atypical families. We have also studied 23 subjects from two additional families in which each proband had a phenotypically normal and a phenotypically abnormal parent. The pedigrees of these families, CyI and CyJ, are shown in Fig. 2.

Proband CyI, a 19 yr old white female, spontaneously passed a cystine calculus on two different occasions. Proband CyJ, a 15 yr old white female, had a severe form of cystinuria; on five different occasions since the age of 4 yr she had been operated on for removal of kidney or bladder stones and had spontaneously passed

TABLE I

Classification of the Members of Eight Typical Families, CyA–CyH

	Total	Homozygotes		Heterozygotes		Homozygous normals
		Type I (I/I)	Type II (II/II)	Type I (+/I)	Type II (+/II)	(+/+)
Probands	8	3	3		2	
Parents	14			4	8	2
Sibs of patients	13	1	1	?	4	5
Children of patients	12			5	7	
Other relatives	2				2	
Number of family members	49	4	4	9?	23	7

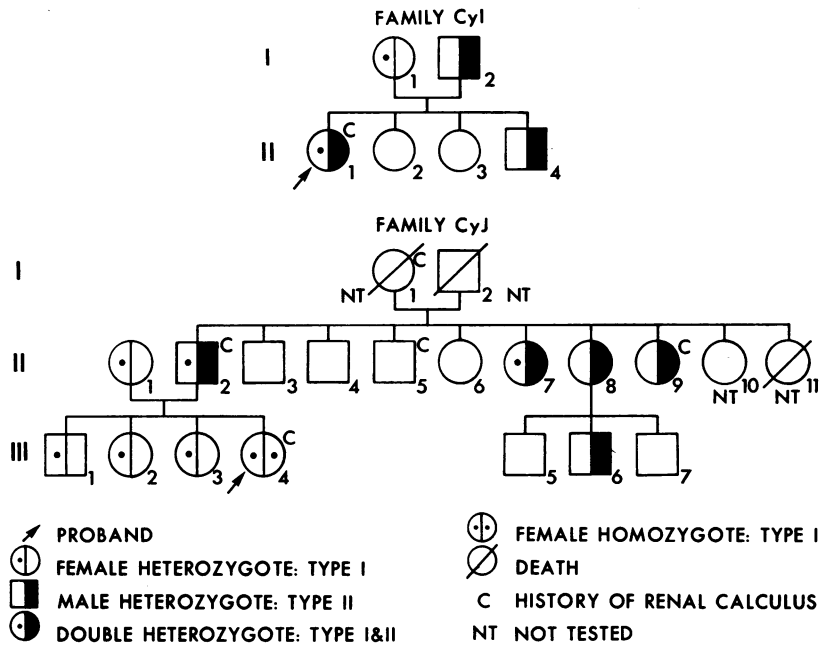

FIGURE 2 Pedigrees of two atypical cystinuria families (CyI and CyJ).

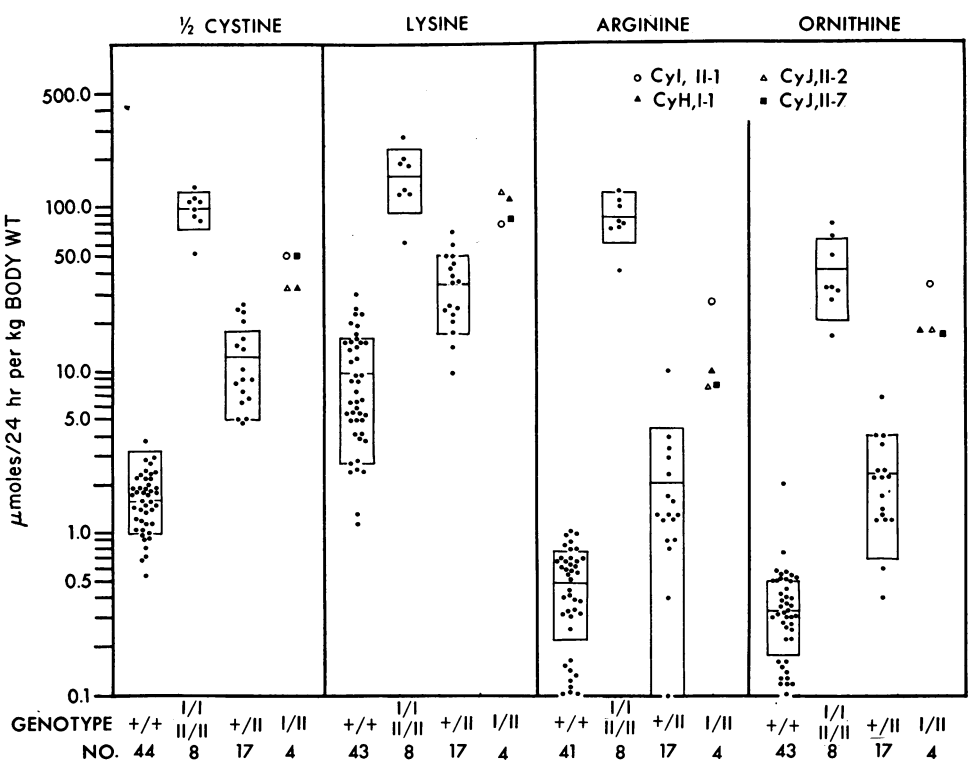

FIGURE 3 Quantitative 24 hr urinary excretion of dibasic amino acids in the various cystinuria genotypes and controls. Black dots represent individual values; horizontal lines denote means, and the boxes denote the range of ±1 SD. Note the logarithmic scale on the ordinate.

cystine calculi on many occasions. Her father was also a stone-former. All the subjects studied in these two families were over 12 yr of age except for CyI, II-4, who was 4 yr old.

Renal handling of amino acids

Typical families (CyA—CyG). The results of 24 hr urinary excretion of the four dibasic amino acids in the controls and in the various cystinuric genotypes encountered in these families are shown in Fig. 3.

Both I/I and II/II homozygous abnormals excrete gross excesses of the four amino acids. There was no significant difference between the mean excretions of I/I and II/II homozygotes, therefore these two groups are combined. Assuming normal distribution, there was no significant difference between controls and +/I heterozygotes. However, in individuals of genotype +/II, the urinary excretion of the four amino acids is significantly higher than in the controls (for cystine, lysine, ornithine $P < 0.01$, and for argine $P < 0.05$). Of the four amino acids, cystine permits clear-cut separation of genotypes +/II and +/+ since there is no overlap between the urinary cystine excretion of +/II and +/+ subjects. Excretion of lysine, arginine, and ornithine show some overlap. Lysine excretion appears to be the least discriminatory.

In one exceptional individual, CyH, I-1, the mother of a stone-former proband, the 24 hr urinary excretion of cystine, lysine, arginine, and ornithine was higher than the upper 99% confidence limits of 17 other +/II individuals, yet far lower than the range of homozygous abnormals. For this reason we did not include her among the +/II genotype group. We shall discuss this person's findings and genotype later.

The 24 hr urinary excretion of amino acids other than cystine, lysine, arginine, and ornithine was normal in every investigated members of the eight typical families.

The endogenous renal clearances of the four amino acids in the various genotypes in our eight typical families are shown in Fig. 4. The means and the range of the percentage of the filtered load reabsorbed are shown in Table III. In the controls, the clearances of the four amino acids are very low, the means ranging from 0.19 to 1.2 ml/min per 1.73 m^2 (Fig. 4), and over 99% of the filtered load is reabsorbed (Table III). These results are similar to those obtained by others in normal individuals (*22, 23*). The clearances of the four amino acids in homozygous abnormal I/I or II/II individuals are vastly increased. There is again no significant difference between the I/I and II/II genotypes and therefore these two groups were combined. Of our eight homozygous abnormal patients, five (two I/I and three

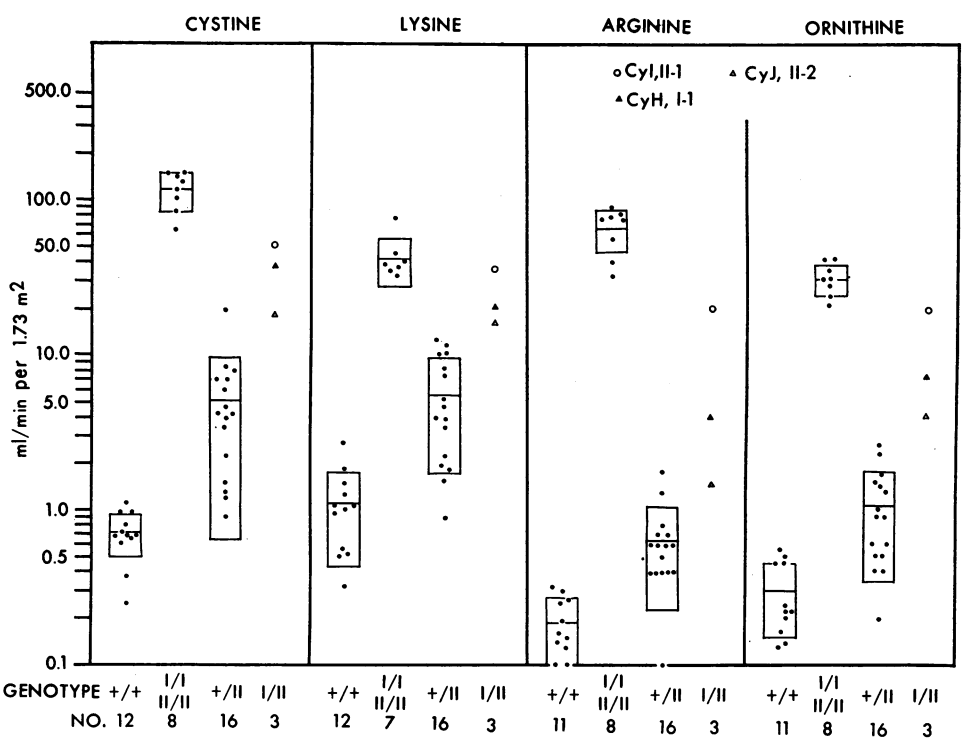

FIGURE 4 Endogenous renal clearance of dibasic amino acids in the various cystinuria genotypes and controls. The black dots represent individual values, horizontal lines denote means, and the boxes denote the range of ±1 SD. Note the logarithmic scale on the ordinate.

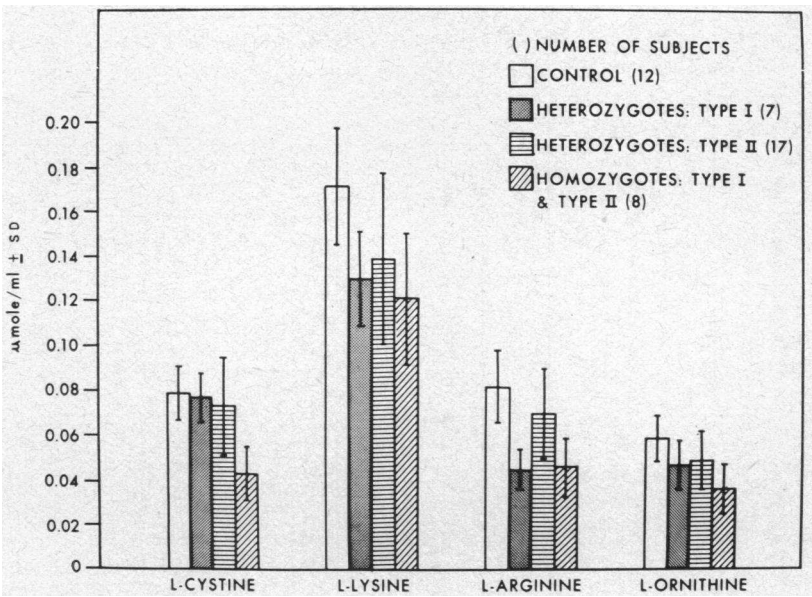

FIGURE 5 Fasting plasma concentration of dibasic amino acids (cystine expressed as ½ cystine) in the various cystinuria genotypes and controls. The height of the columns denote the means, the vertical bars the range of ±1 SD.

II/II) had a cystine clearance which exceeded the glomerular filtration rate as measured by endogenous creatinine clearances. This indicates tubular secretion of cystine. It is noteworthy that two I/I type sisters in family CyG are discordant for tubular secretion.

In +/I heterozygotes the endogenous renal clearances of amino acids were not significantly different from those of the controls.

Of the 16 +/II individuals, only 1 had a cystine clearance value in the normal range, the other 15 having elevated values. The clearances of lysine, arginine, and ornithine in the +/II individuals were also distinctly elevated, but there was considerable overlap between the two groups.

The renal clearances of other amino acids in the subjects studied in the eight typical families were all normal.

Plasma concentration of amino acids. The venous plasma concentrations of cystine, lysine, arginine, and ornithine in the various groups are shown in Fig. 5. It has been reported previously (24, 25) that the plasma level of these four amino acids in cystinuria homozygotes is lower than in normals. Our results are similar and show a significant difference ($P < 0.01$) between the mean plasma level of these four amino acids in normals and in homozygous abnormals. The mean plasma concentrations of lysine, arginine, and ornithine (but not cystine) in both the +/I and the +/II heterozygotes are also lower than in the normals. The plasma arginine level in the +/I genotypes was particularly low and, in fact, similar to that seen in the homozygous abnormals. In this connection it is remarkable that despite the low plasma levels of arginine in our group of seven +/I heterozygotes, the mean of the endogenous clearance of arginine (which was measured in six of these seven) is elevated: 0.45 ml/min per 1.73 m^2 versus 0.17 ml/min per 1.73 m^2 in controls. However, as noted earlier and perhaps because of small numbers, this difference is not significant. The plasma concentration of all other amino acids was normal in both type of heterozygotes and in homozygotes.

Atypical families (CyI and CyJ). The pedigrees of the two atypical families are shown in Fig. 2. In Table II, the 24-hr urinary excretions of the four dibasic amino acids are shown in those members of these families whose ½ cystine excretion was higher than 3.4 μmoles/kg per 24 hr, that is higher than the upper 99% confidence limit found in controls. The members of these two families who had a 24 hr urinary ½ cystine excretion lower than 3.4 μmoles/kg, also had normal urinary excretion of lysine, arginine, and ornithine.

In the first of these atypical families (CyI), the proband's father (CyI, I-2) and brother (CyI, II-4) had results well within the range of +/II individuals. Her mother (CyI, I-1) and her two sisters (CyI, II-2 and 3) had normal 24 hr urinary amino acid excretion. Proband CyI, in three 24-hr urinary collections excreted the four amino acids in amounts midway between +/II heterozygotes and homozygous abnormals (see also

TABLE II

Mean Values of 24 Hr Urinary Excretion of Dibasic Amino Acids in the Phenotypically Abnormal Persons of Families CyI and CyJ, in Cystinuria Genotypes, and in Controls

Subjects	No.	½ Cystine	Lysine	Arginine	Ornithine
			μmoles of amino acid/24 hr per kg body wt		
CyI, I-2		21.5	47.3	1.9	3.6
II-1		50.5	77.9	27.0	32.6
II-4		7.5	29.0	1.3	1.1
CyJ, II-2		32.7	126.0	7.8	17.5
II-7		49.8	85.0	8.1	16.9
II-8		11.7	25.8	1.2	1.7
II-9		12.3	22.8	1.0	1.4
III-4		65.1	115.0	66.1	22.4
III-6		4.2	24.8	1.1	0.6
Heterozygotes* +/II	17	12.0±7.0 (4.7–26)	34.0±17 (9.6–71)	2.06 ±2.28 (0.1–10)	2.30 ±1.61 (0.4–6.8)
Homozygotes* I/I & II/II	8	99.2±24.2 (52.8–134)	158±64 (61.3–273)	85.3±25.6 (40.9–126)	41.4±21.0 (16.0–78.0)
Controls* +/+	44	1.68 ±0.67 (0.5–3.7)	9.72 ±7.0 (1.13–29.6)	0.48 ±0.27 (0.1–1.0)	0.33 ±0.16 (0.1–0.75)

* The means ±1 SD is shown and the range in parentheses.

Fig. 3). Her urinary excretion of cystine, arginine, and lysine is clearly higher than that of 17 +/II individuals, but lower than that of 8 homozygous abnormals except for a single lysine value in a homozygote. Her excretion of ornithine is within the homozygote range.

In the second atypical family, the proband (CyJ, III-4), who presented with severe clinical cystinuria, had mean 24 hr urinary excretion of the four dibasic amino acids within the range of eight homozygous abnormals. However, her father (CyJ, I-2) had rather anomalous findings. His urinary excretion of cystine was higher than in +/II heterozygotes but lower than in homozygous abnormals (Table II, Fig. 3). His excretion of lysine and ornithine was low in the range of homozygous abnormals while his arginine excretion was close to the upper limit of the range in +/II individuals. His sister (CyJ, II-7), had almost identical findings. Two other sisters of this man (CyJ, II-8 and 9) had urinary dibasic amino acid excretions within the +/II range, and so did the son of one of these (CyJ, III-6). Four additional sibs of the proband's father (CyJ, II-3, 4, 5, and 6) and the two remaining sons of CyJ, II-8, had normal 24-hr urinary amino acid excretions.

As mentioned earlier, a member of one of our eight typical families (CyH, I-1) had 24-hr urinary dibasic amino acid excretions higher than the 99% confidence limits for 17 +/II individuals and lower than in homozygous abnormals. This 60 yr old woman has never had a urinary calculus or suggestive symptoms yet her 24-hr dibasic amino acid excretions (Fig. 3) fall within the range of the unusual individuals in our two atypical families (CyI, II-1, CyJ, II-2 and 7).

The quantitative 24 hr urinary excretion of the amino acids other than cystine, lysine, arginine, and ornithine was normal in the 23 members investigated in the 2 atypical families except in proband CyJ. Her urinary excretion of methionine (0.182 mmoles/24 hr, the mean of four separate 24-hour urinary collections) was increased over that found in our control group and in data published elsewhere (26).

The endogenous renal clearances of the dibasic amino acids expressed in terms of per cent reabsorption of the filtered load in members of our two atypical families are shown in Table III. Values are shown only for those members who had an abnormal 24 hr urinary excretion of cystine.

In proband CyI, II-1, the percentage of tubular reabsorption of these four amino acids was approximately midway between that of 16 +/II heterozygotes and 8 homozygous abnormals. Her clearances expressed in terms of ml/min per 1.73 m^2 are shown in Fig. 4 and the same conclusion can be drawn. Her father (CyI, I-2) and her brother (CyI, II-4) had a reabsorption of dibasic amino acids similar to those of +/II phenotypes (Table III). However, the renal clearances of her mother (CyI, I-1) and her two sisters (CyI, II-2 and 3) were normal.

In proband CyJ, III-4 tubular reabsorption was similar to that of homozygous abnormals of cystinuria. She had tubular secretion not only of cystine but also of arginine. Tubular secretion of arginine was observed only once previously by Frimpter, Horwith, Furth, Fellows, and Thompson (25) in an individual of presumed I/I genotype. The father of the proband (CyJ, II-2) who had anomalous 24-hr urinary excretions also had anomalous clearances. His results were much lower than in heterozygotes +/II, but higher than those of eight homozygous abnormals (Table III). His values expressed in terms of ml/min per 1.73 m^2 are shown in Fig. 4 and the same conclusion is evident. The sister of this man (CyJ, II-7), whose 24 hr urinary excretions were similar to his, was not available for clearance studies. The mother of the proband (CyJ, II-1) had normal amino acid clearances. Other members of this family (CyJ, II-8 and III-6), whose 24 hr urinary excretion pattern conformed to those of +/II genotypes, had clearances also within the range of +/II genotypes.

The endogenous renal clearances of the amino acids other than cystine, lysine, arginine, and ornithine were normal, except in 1 of the 21 investigated members of these 2 families. Proband CyJ, who had a high urinary excretion of methionine, also had a high methionine renal clearance of 15 ml/min per 1.73 m^2 with an 84% reabsorption of the filtered methionine. Her plasma methionine level was normal.

Intestinal handling of amino acids

The results of in vitro intestinal transport studies with labeled cystine, lysine, or arginine are shown in Fig. 5. Jejunal mucosa from five normal subjects accumulated these amino acids to a much higher concentration than that in the incubation medium, with mean distribution ratios of 6.12 for cystine and 7.4 for lysine. The ratio for cystine is used here for comparison with previously published studies in spite of the fact that cystine, after being transported into the cell, is reduced to cysteine (27, 28). In homozygotes I/I, the concentrative transport mechanism for cystine and lysine into the epithelial cell of the jejunum is barely present; the mean distribution ratio is 1.7 for both cystine and lysine. The same is true for II/II homozygotes in whom the mean distribution ratios were 1.6 for cystine and 1.9 for lysine.

In two +/II individuals investigated, the mean distribution ratios were 5.1 for cystine and 4.6 for lysine. These ratios are lower than the means of our control group.

The uptake of arginine was not studied in controls. Only one mucosal segment of one +/II individual, and one from each of three homozygotes (two II/II and one I/I) were studied. Therefore, we are not in a position to know whether the difference between the distribution ratios of 2.3 in homozygotes II/II, and 1.1 in a homozygote I/I is real.

TABLE III

Endogenous Renal Clearances of Dibasic Amino Acids of the Phenotypically Abnormal Subjects of Families CyI and CyJ, Cystinuria Genotypes, and Controls

Subjects	No.	Per cent of filtered amino acid reabsorbed			
		Cystine	Lysine	Arginine	Ornithine
CyI, I-2		89.0	91.3	98.9	98.3
II-1		59.1	72.7	84.9	85.7
II-4		98.8	98.0	99.8	99.7
CyJ, II-2		84.0	85.5	98.7	96.3
II-7,		not tested			
II-8		86.0	85.0	99.3	97.7
II-9,		not tested			
III-4		−30.5*	47.5	−12.6*	69.3
III-6		98.7	98.6	99.6	99.7
Heterozygotes‡ (+/II)	16	96.3 (87.7–99.3)	96.1 (92.0–98.8)	99.5 (98.8–99.8)	99.2 (98.3–99.8)
Homozygotes‡ (I/I & II/II)	8	(§ −33.1)	58.7 (26.0–69.8)	37.5 (25.9–68.4)	70.9 (57.6–77.7)
Controls‡ +/+	12	99.4 (99.0–99.8)	99.1 (98.3–99.8)	99.9 (99.6–100)	99.7 (99.4–100)

* Negative values presumably mean tubular secretion.
‡ The means are shown, and in brackets the range.
§ Tubular secretion.

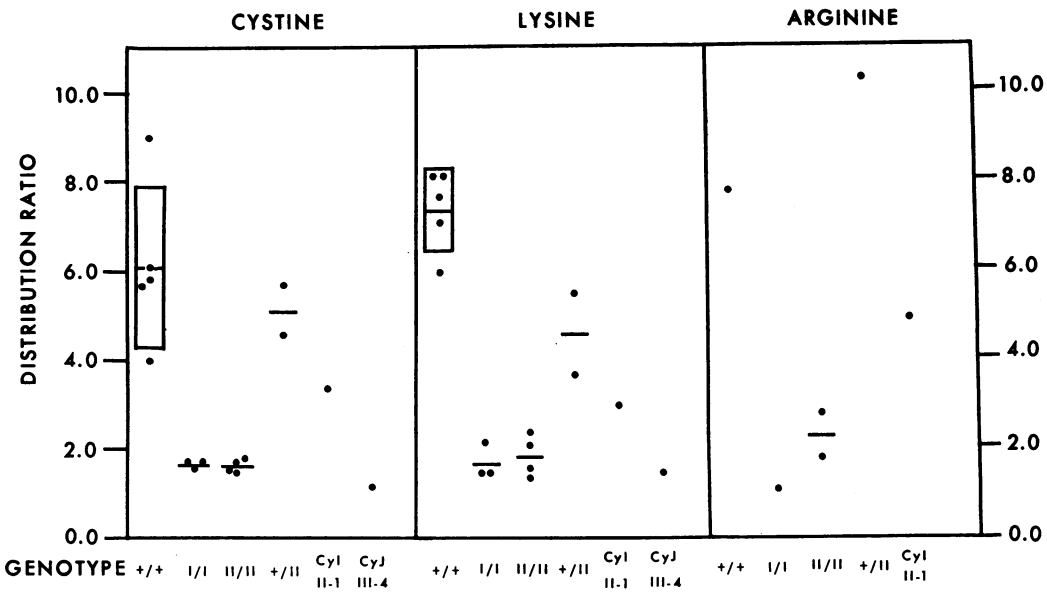

FIGURE 6 Results of in vitro intestinal transport studies in controls, in various cystinuria genotypes and in probands CyI and CyJ. Distribution ratio is the product of the intracellular concentration of labeled amino acid after 45 min incubation and the concentration of same in the medium. Black dots represent values of individuals (means of two parallel measurements unless noted in the text). Horizontal lines denote the means of groups, and the boxes the range of ±1 SD.

In our two atypical families we have studied the probands (CyI, II-1 and CyJ, III-4). Proband CyI's jejunal mucosa accumulated cystine, lysine, and arginine well in excess of their concentrations in the incubation medium, yet there was impairment of the active transport mechanism when her results are compared with those in normal controls (Fig. 5). Here again, as for the quantitative urinary excretion and the endogenous clearances of these amino acids, proband CyI's results fell midway between those of seven homozygous abnormals and two +/II heterozygotes. In the studies with arginine the number of homozygous abnormals and +/II individuals was lower, but the same conclusion still holds.

Proband CyJ, III-4's intestinal mucosal uptake-studies with cystine and lysine yielded results which fell within the range of homozygous abnormals. Her ability to concentrate cystine and lysine in excess of the concentrations in the incubation medium was almost completely lost. Her father (CyJ, II-2), whose renal handling of dibasic amino acids was similar to proband CyI's, had a subtotal gastrectomy and for this reason was not studied by this technique.

Fig. 6 shows the results of oral-cystine loading tests. In homozygous abnormal I/I and II/II genotypes there was no rise in the plasma levels of cystine, whereas in one +/II heterozygote a significant rise was seen. The rise in the cystine level was highest in the two normals.

Proband CyI did show a slight increase in her plasma cystine concentration which was lower than that of one +/II heterozygote but definitely higher than we and others have found in homozygous abnormals of genotype I/I and II/II. This provides in vivo confirmation of her in vitro studies with jejunal mucosal segments.

Proband CyJ showed no rise in plasma cystine concentration after the oral load thus behaving like homozygotes type I/I and II/II.

Serum-uric acid levels and quantitative 24-hr urinary excretion of uric acid, calcium, inorganic phosphate, and oxalate

Because of the history of renal stones in five members of family CyJ (see Fig. 2), some of whom were definitely not homozygous cystinurics, investigation was carried out to uncover other possible reasons for familial nephrolithiasis in this and some of the other kindreds.

Of the 23 subjects investigated in families CyJ and CyI, the serum uric acid level, measured in all but two, was found to be elevated only in proband CyJ. Her serum uric acid level was 7.6, 6.5, and 7.5 mg per 100 ml on three different occasions measured within a period of 1 yr. The normal range for females with the method used in this study is 1.5–6.5 mg per 100 ml (17).

Two members of family CyJ, the mother and paternal aunt of the proband, had a 24 hr urinary uric acid excretion of over 750 mg. The proband's mother (CyJ, II-1)

had an excretion of 792 mg/24 hr with a normal blood level of 5.1 mg per 100 ml. The paternal aunt CyJ, II-7, had a urinary excretion of 969 mg/24 hr, her serum uric acid level was not measured. 24 hr urinary excretion of uric acid was also measured in members of families CyA, CyF, and CyG and was found to be normal in all.

An association of cystinuria, hyperuricaemia, and methioninuria, which we found in proband CyJ, has been reported once before (29). Considering the results obtained in the other members of the family, this does not appear to be genetically significant.

In the five members of family CyJ who had renal stones the urinary excretion of calcium, magnesium, and inorganic phosphate was also determined. Of these five stone-formers, only the proband had her stones analyzed. The other four persons with a history of renal calculus were the following: CyJ, I-1, the proband's paternal grandmother, who was dead at the time of this study; CyJ, II-2, the proband's father, and CyJ, II-9, the proband's paternal aunt, who both had an abnormal urinary excretion of cystine, lysine, arginine, and ornithine; and CyJ, II-5 the proband's paternal uncle, who had a normal urinary excretion of these four amino acids. Magnesium and phosphorus excretion was within normal limits in every subject, but the calcium excretion was particularly high in two. The normal range of calcium excretion is considered to be between 100 and 300 mg/24 hr for males and from 100 to 250 mg/24 hr for females (30). CyJ, II-9, a +/II individual with a history of renal stones, had an excretion of 480 mg/24 hr of calcium, while CyJ, III-7, who had normal urinary excretion of amino acids and no history of calculus formation, excreted 380 mg/24 hr. CyJ, II-5, who had passed five calculi spontaneously, had a normal calcium excretion of 162 mg/24 hr, though earlier investigation of this family member in another hospital showed values up to 300 mg/24 hr. The proband and her father had a urinary calcium excretion lower than 120 mg/24 hr.

The high incidence of renal calculi in family CyJ combined with high urinary excretion of calcium found in three subjects could indicate genetic predisposition for the formation of calcium renal calculi (31) independent of cystinuria in this family.

Urinary oxalate was found to be normal in those subjects of family CyJ who had either abnormal excretion of cystine, lysine, arginine, and ornithine or a history of calculus.

DISCUSSION

The findings in 49 members of our 8 typical families with cystinuria conform to the genetic patterns for this disease previously described by Harris, Rosenberg, and their coworkers. Their conclusions were based mainly on amino acid measurements in random urine specimens and the values were expressed in relation to creatinine excretion. Most of their findings are confirmed here on the basis of the method exclusively used in this study, i.e., measurements of amino acids by column chromatography in quantitative 24-hr urine collections.

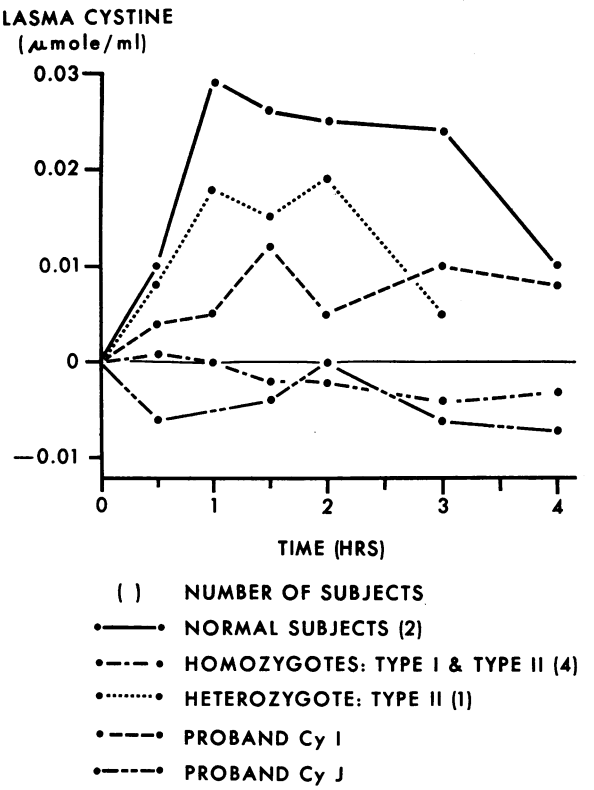

FIGURE 7 Changes in plasma levels of cystine (expressed as ½ cystine) in response to an oral cystine load of 0.5 mmoles per kg body weight in controls, in various cystinuria genotypes and in probands CyI and CyJ. Black dots represent the means of differences between the fasting cystine value and the one observed at various times after the oral ingestion of the cystine load.

In homozygous cystinuric subjects cystine, lysine, arginine, and ornithine are excreted in great excess (1, 2); their excretion by both types of homozygotes is in the same range. As reported by Harris, type +/I heterozygotes have normal excretion of amino acids, while those of genotype +/II have abnormal amounts of urinary cystine, lysine, arginine, and ornithine though the amounts excreted are much lower than in homozygotes (1, 2). The quantitative 24 hr urinary excretion of cystine, lysine, arginine, and ornithine, as well as the clearances of these four amino acids in 17 +/II individuals compared with controls, indicate that cystine is the most reliable marker for differentiation of heterozygotes +/II from normal. This finding is at variance with a report from Crawhall, Saunders, and Thompson,

who suggested that arginine may be more reliable in this respect (32).

The relatively small difference between +/II heterozygotes and normals, in terms of quantitative 24 hr urinary excretion and renal clearances of these four amino acids, and the range of variation within each group make it difficult to insert between these two a third group: the heterozygotes type III of Rosenberg (6). The values of the urinary excretion of cystine, lysine, arginine, and ornithine in heterozygoes type III were found by Rosenberg to fall in an intermediate range, statistically different from those of the normals and heterozygotes type II.

Of the eight homozygous cystinuric patients investigated by us, five showed evidence of tubular secretion of cystine. This was also observed by others (25, 33). In our patients, tubular secretion of cystine does not appear to be restricted to either of the two genetic types, since we encountered it in both types of homozygotes. It would be difficult to explain it on genetic grounds since in the two homozygote type I sisters in family CyG, one had tubular reabsorption of cystine while the other secreted it. Presumably, these two sisters have inherited the same type of disease although the presence of diffrent modifying genes cannot be ruled out. Dissolution of cystine calculi and high rates of urine flow during clearance studies have been proposed as mechanisms explaining a cystine clearance greater than the glomerular filtration. None of our "secretors" had stones at the time of clearance studies. Only proband CyF had radiological and clinical evidence of calculi and her cystine clearance was slightly lower than her glomerular filtration rate. On this basis we do not feel that stone dissolution is a factor. Our clearance measurements were performed at a relatively high urinary flow rate. However, Frimpter et al. (25) and also Crawhall and Segal (27) have reported that tubular secretion of cystine is not explained by high rates of urine flow.

In the in vitro intestinal uptake studies and oral-cystine loading tests, there was no apparent difference between homozygotes I/I and II/II. Both types were unable to accumulate cystine and lysine in excess of their concentration in the incubation medium and neither showed a rise in plasma cystine concentration after an oral load. These results are comparable with those of Rosenberg (4) for I/I homozygotes, but different for II/II homozygotes. In his two II/II subjects, the distribution ratio of cystine was slightly greater than 2.0, indicating some retention of ability to accumulate this amino acid. Yet the same two individuals did not respond to an oral cystine load with significant increase in plasma levels. In our four II/II individuals, the in vitro intestinal uptake of cystine was not higher than in the three of genotype I/I. Thus we were unable to confirm that II/II homozygotes retain a reduced ability to accumulate cystine in the gut against a concentration gradient.

In the two atypical families, each of the probands have a phenotypically normal and a phenotypically abnormal parent. Though both probands have formed cystine stones in the past, the proband of family CyJ is clinically much more severely affected than the proband of family CyI. The difference in severity between these two probands is also evident when one considers the results of the four parameters of investigation used in this study. Proband CyJ has findings similar to those of eight homozygotes I/I and II/II, while the findings of proband CyI consistently fall midway between those of the +/II genotype and those of I/I and II/II.

Moreover, the disease affecting the two probands is not inherited from their phenotypically abnormal fathers only, because in both fathers the quantitative 24 hr excretion of cystine, lysine, arginine, and ornithine, and endogenous-renal clearances of these amino acids are much lower than those observed in their respective daughters. Therefore, it is reasonable to assume that the proband of each of these two families received a cystinuric allele from her mother as well as from her father. Since, by definition, in the recessive type of cystinuria (type I), heterozygotes are phenotypically normal, we consider these two mothers to be heterozygotes type I. If this is so, both probands are the products of a heterozygous +/I mother and of a phenotypically abnormal father.

To explain the difference in phenotype of probands CyI and CyJ, it is important to analyze closely the results obtained in the investigation of their fathers. The quantitative 24 hr urinary excretion and endogenous renal clearances of dibasic amino acids in proband CyI's father were within the range of heterozygotes +/II. Her brother (CyI, II-4) also excreted amino acids in amounts similar to those of the father and of heterozygotes +/II. It is concluded therefore that proband CyI is the product of a father of +/II genotype and a mother of +/I; and she is, in fact, a double heterozygote type I/II.

In the case of proband CyJ, the mother is phenotypically normal and one would therefore expect proband CyJ to be a homozygote I/I and her father to be +/I and phenotypically normal. However, the father is not only phenotypically abnormal (thus not +/I) but also a stone-former; since the age of 20 he has spontaneously passed four calculi. His urinary excretion of cystine, lysine, and ornithine is higher and outside the range of heterozygotes +/II, while his measurements of renal tubular reabsorption of the four amino acids are lower than the range of heterozygotes +/II. Consequently, proband CyJ's father does not appear to fit

the criteria for heterozygote +/II. Moreover, if he were a heterozygote +/II, his daughter, proband CyJ, should be similar to proband CyI, but she is not. Thus a more logical explanation of the findings in this pedigree is to consider proband CyJ's father to be a double hetreozygote type I/II similar to proband CyI, and proband CyJ herself to be a homozygote I/I. Indeed, the renal handling of cystine and the dibasic amino acids by CyJ's father is closely similar to that of proband CyI whom we consider to be I/II. If proband CyJ's father is of genotype I/II, some members of his sibship could also be I/II with test values similar to his, and others could be +/II heterozygotes. Family members with the characteristics of both these genotypes were observed. One sister, CyJ, II-7, was biochemically similar to proband CyJ's father and can also be considered as a double heterozygote type I/II. 2 sisters, CyJ, II-8 and CyJ, II-9, were classed as heterozygotes +/II, in accordance with our previous observations with 17 heterozygotes type II. A son (CyJ, III-6) of one of these (CyJ, II-8) was also a heterozygote +/II.

Another possible explanation of the results found in proband CyI and the father of proband CyJ might be that these two individuals are extreme examples of heterozygotes type II. This hypothesis is far less likely since in each family the proband is phenotypically and biochemically quite different from her father. That the probands received a mutant allele from their respective mothers would explaine the difference better.

If one carefully reviews the data presented by Harris and by Rosenberg, it seems probable that some of their probands were double heterozygotes of the I/II type. For example, in two of Harris' families (Cy14 and Cy20), the probands (stone-formers) had cystine excretion corresponding either to the lower range of the homozygous abnormal class or to the upper limit of the heterozygous type II class. Lysine and arginine values, however, corresponded more closely to those of heterozygous type II individuals than to those of homozygotes. Because data for cystine and lysine excretion in both parents of the probands were lacking, Harris drew no definite conclusion about the genetical situation in these families but considered the probands to be extreme heterozygotes who formed stones because of their high urinary cystine excretion (2). A further proband, in Harris' Cy8 family, considered by him to be a homozygous type I cystinuric, may also have been a double heterozygote type I/II, because in this family the proband's mother was completely normal, whereas the father, judged by Harris to be a heterozygote type I, had a slightly elevated urinary excretion of cystine and lysine (2). Harris encountered values of urinary excretion of cystine and lysine elevated by his criteria in 1.2% of 250 unselected, presumably normal, individuals (2), a percentage very close to the apparent incidence of heterozygotes type II of 1:200 found by Crawhall (32). It is quite probable that the father of Harris' proband was a heterozygote type II and the proband a double heterozygote type I/II. This is all the more likely since Harris found the urinary, cystine, lysine, and arginine excretion (expressed per gram of creatinine) of this proband to be, respectively, 338, 738, and 180 mg, considerably lower than the means of 456, 1074, and 769 mg for his 19 homozygotes type I (2).

Indeed there is one individual among the members of our eight typical families who could be classed as a double heterozygote type I/II. This is the 60 yr old mother (CyH, I-1) of a homozygous abnormal proband, in whom we have no evidence of nephrolithiasis, but whose 24 hr urinary excretion and renal clearances of the four amino acids involved in cystinuria were consistently above the upper 99% confidence limits of heterozygotes type II, and lower than the range in homozygous abnormals. Since we were not able to study either the sibs or the parents of this individual we merely consider the available evidence suggestive.

Rosenberg et al. described a third variant of cystinuria (4). While we have not encountered this type III cystinuria in our material, we have no reason to doubt its existence. We feel, nevertheless, that one or at the most two of Rosenberg's type III homozygotes may be I/II double heterozygotes for the following reasons. In two of the type III homozygotes of Rosenberg, the in vitro uptake of the labeled cystine, lysine, and arginine was quite similar to the results we have obtained in proband CyI whom we consider a I/II double heterozygote (4). Rosenberg states that the urinary dibasic amino acid excretions in the type III patients were within the range of type I and II homozygous abnormals yet one type III patient has lower cystine excretion when compared with his type I and II patients (4). Finally, two of the eight parents of type III patients from two different pedigrees were excreting normal quantities of cystine, but were considered to be heterozygotes type III because they had increased urinary content of lysine as measured semiquantitatively by high voltage electrophoresis (4). In a more recent publication, Rosenberg presents measurements of dibasic amino acid excretions by quantitative column chromatography in early morning urine specimens from seven parents of type III homozygous abnormals (6). Two of these parents had urinary cystine and arginine within or close to his control range. All lysine and ornithine values were higher than the controls. Considering the considerable overlap that we have shown to exist between the 24-hr urinary lysine and ornithine excretions in controls and heterozygotes type II, one wonders whether one or both of these parents could be classed as heterozy-

TABLE IV

Relative Frequency of Cystinuria Genotypes

Genotype	Frequency	
	One locus hypothesis	Two locus hypothesis
Normal	~1	~1
Heterozygote		
Type I	1/200	1/200
Type II*	1/200	1/200
Total	1/100	1/100
Homozygote		
Type I	1/160,000	1/160,000
Type II	1/160,000	1/160,000
Total	1/80,000	1/80,000
Double heterozygote type I/II	1/80,000	1/40,000
Ratio of I/II to I/I + II/II	1/1	2/1
Homozygote I & II and double heterozygotes combined	1/40,000	1/27,000

* For simplicity, type III is assumed to be combined with type II.

gotes type I. Since the family relationships in the material published by Rosenberg cannot be traced, we consider these arguments intriguing but tenuous.

Recently, Rosenberg reported four stone-forming cystinuric patients from three unrelated pedigrees whom he considered to be double-heterozygotes (5, 6). The genotype of one of these was I/II, the same as our four double heterozygotes, the others were II/III, I/III, and I/III), respectively. The quantitative excretion of cystine, lysine, arginine, and ornithine by these four subjects, as measured in first morning urine samples, was in the range noted for cystinuric homozygotes. The uptake of dibasic amino acids by jejunal mucosa from these four subjects yielded results similar to those of the homozygotes type I. However, there was a small but perceptible rise in plasma cystine after an oral load in two individuals with genotype I/III.

Comparison of our results with those of Rosenberg is difficult, since the methods of investigation were different. Despite the differences in methodology, the fact remains that in the families of three out of four of Rosenberg's double heterozygotes, one parent was phenotypically normal, while the other was phenotypically abnormal. According to his findings these double heterozygotes behave as cystinuric homozygotes. We, however, have found the clinical and biochemical manifestations of the disease in the four individuals whom we consider double heterozygotes type I/II to be different from those in homozygotes type I or type II. Further studies on more such patients and their families are needed to settle this discrepancy.

The important question of whether the various cystinuria genes are allelic or nonallelic cannot be decided on the basis of present knowledge, since the essential genetic data to decide between segregation or independent assortment are not at hand and are unlikely to be provided until a specific test is available for the detection of +/I heterozygotes. However, it is worth noting that whether the mutant genes are alleles or are interacting nonalleles, the frequency of double heterozygotes should be high as compared with homozygous abnormals.

The frequencies of the various cystinuria genes and genotypes are not accurately known. Crawhall et al. (32) found the frequency of "detectable" heterozygotes (types II and III combined?) to be 1/200, and estimated the frequency of undetectable (type I)heterozygotes to be the same. These figures can be used to derive estimates of the frequencies of homozygotes and double heterozygotes, assuming a single locus with multiple alleles and, alternatively, two loci each with at least one mutant allele (Table IV).

On the single-locus hypothesis, the frequency of the *I* allele is 1/400, and the frequency of I/I homozygotes 1/160,000. The relative frequencies of type II and type III homozygotes are not known. For the purpose of these calculations we shall combine these two types under the designation II. The frequency of II/II homozygotes would then also be 1/160,000, and the combined frequency of the two homozygous cystinuria genotypes 1/80,000. However, a fraction $2 \times (1/400) \times (1/400)$ or 1/80,000 would be double heterozygotes of the I/II genotype. In other words, if there are two rare mutant alleles of equal frequency, the proportion of double heterozygotes is the same as the combined frequency of the two homozygotes. Thus, the ratio between homozygous abnormals of both types combined and double heterozygotes would be 1 : 1.

The two-locus hypothesis would lead to an even higher proportion of double heterozygotes at each loci (individuals heterozygous at each of two loci), as compared with the proportion homozygous at either locus. Again, assuming the frequency of each type of heterozygote to be 1/200, each gene frequency is 1/400 and each homozygote frequency 1/160,000. The frequency of double heterozygotes on this model is the product of the two heterozygote frequencies, or 1/40,000, which is twice the frequency of the combined homozygotes.

Since there have been suggestions that the true frequency of type I cystinuria may actually be double that of type II (1, 2), we have repeated these calculations for a variety of reasonable gene frequency estimates and

have found that double heterozygotes invariably form a large proportion of the individuals who carry two mutant alleles.

Why is it then that the existence and characteristics of the double heterozygous state was not reported earlier except for four instances by Rosenberg (5, 6)? Perhaps these patients are less seriously affected, less prone to form cystine calculi and therefore escape medical attention. Or the reason may be that the usual investigation of a cystinuric stone-former consists only of analysis of the stone and qualitative assessment of the amino aciduria without quantitative measurement of 24 hr excretion of amino acids or genetic investigation. According to the data presented here the distinction of I/II double heterozygotes from homozygous abnormals and +/II heterozygotes requires quantitative measurement of excretion of the four dibasic amino acids in 24-hr urine collections, combined with measurements of the endogenous renal clearances of these amino acids. It is also important to study the parents, of whom one should be +/I and thus phenotypically normal and the other a heterozygote +/II.

Finally, the findings presented here concerning the nature of the abnormality in two double heterozygote type I/II stone-formers are of more than academic interest. In them, the tubular reabsorptive mechanisms are less severely affected. Since these individuals have a lower urinary cystine excretion and thus a lower urinary cystine concentration than the homozygotes I/I and II/II, they could be effectively treated by high fluid intake and urine alkalinization alone and thus may not require treatment with D-Penicillamine, which is costly and may have undesirable side effects.

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