THE PATHOGENESIS OF CYSTINURIA. II. POLAROGRAPHIC STUDIES OF THE METABOLISM OF SULPHUR-CONTAINING AMINO-ACIDS

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The preceding paper (1) in this series describes some paper chromatographic and microbiological analyses of plasma and urine during acute experiments in which loading doses of cystine, cysteine, and methionine were given by mouth to normal and cystinuric subjects. Cysteine alone caused large rises in plasma cystine levels and urine outputs in both normal and cystinuric subjects. In all cases, urine output varied directly with plasma cystine levels, but in the cystinuric subjects the urine output was very much greater for any given plasma level than in the normal subject. The results were taken as strong evidence in favour of the renal theory of cystinuria, namely that the high cystine output of a cystinuric patient is due not to an innate inability to metabolize cystine or other sulphur-containing amino-acids but to a "low renal threshold" for cystine, presumably from defective tubular reabsorption. The methods of analysis used though highly specific qualitatively were not, however, considered sufficiently accurate quantitatively to enable renal clearances to be calculated.

In this paper we have repeated in greater numbers experiments of the type described in the preceding paper (1) using an almost identical procedure for timing and collecting samples of plasma and urine. The cystine determinations, however, were done exclusively by polarography. This method, depending upon entirely different principles, is likely to be specific in the circumstances. There is no convincing evidence for the presence in these fluids (after deproteinization when necessary) of appreciable quantities of other substances which might interfere with the analysis owing to their containing disulphide (—S—S—) or thiol (—SH) groups.

In addition we have been able to study during these experiments the cystine clearances of normal and cystinuric subjects and in one case also of an "incomplete cystinuric" subject. A discordant series of cystine feeding experiments was shown to be due to the inadvertent use of partially racemized cystine, an important possibility of error which appears to have been overlooked by earlier workers. This led to a further study of the metabolism of DL-cystine. We have also investigated in a preliminary manner the possibility that there exists at some point in the kidney tubule a common mechanism for the reabsorption of cystine, lysine, arginine, and ornithine.

MATERIALS AND METHODS

Twenty-one observations were made on nine normal adult subjects, 10 observations on six cystinuric adults and one cystinuric child and two observations on one "incomplete cystinuric" adult (see later). Relevant clinical details of the cystinuric subjects are given in Table I. In all other respects they also conformed to the definition of the disease that Dent and Rose (2) have proposed. S. L. and A. L. were identical twins. Further details of cases J. H., S. L., A. L., and G. S. (under the same initials) may be found in the paper by the latter (2). The subject described as having "incomplete" cystinuria was healthy and symptom free; he excreted between 120 and 180 mg. per 24 hr. of cystine (2 to 3 times normal). He was believed to be one of the heterozygous forms of the condition (3).

The amino-acids fed (dissolved in or washed down with water) in the various experiments were: L-cystine (5.0 g.): L-cysteine hydrochloride (6.0 g.): DL-methionine (6.25 g.): DL-cystine[3] (5.0 g.)—this was prepared by the method of Hoffman and Gortner (4); a mixture of L-arginine hydrochloride, L-lysine hydrochloride and DL-ornithine hydrochloride (5.0 g. of each); glycine (15.0 g.). The hydrochlorides were neutralized with $NaHCO_3$ immediately before feeding.

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[3] We use the term DL-cystine with the knowledge that it is impossible at the moment to go further into the matter of differences in behaviour between racemic and meso-cystine.

The procedure in each experiment and the schedule of specimen collections was exactly the same as that described in the preceding paper in this journal (1), namely blood at 0, 0.5, 1, 2, 4, and 6 hr. intervals after feeding amino-acid; urine at 0, 1, 3, 5, and 7 hr. intervals. The subjects however were not all maintained recumbent. The rate of urine production was about 1 to 2 ml. per min. (It was fortunate that larger diureses were not attempted, as we have some recent results [unpublished] which indicate that the cystine "clearances" rise considerably with a high diuresis in a patient with stones as if dissolution of the stones was occurring. This could have produced considerable artefacts in acute experiments of the type done here.)

In addition to the amino-acid experiments, control studies following the same schedule but without amino-acid ingestion were carried out in three normal and one cystinuric subject.

In one cystinuric patient simultaneous inulin (5) and endogenous cystine clearances were carried out. Short collection periods were used with a marked diuresis. A cystinuric subject (J. H.) with no radiological evidence of stone formation was therefore chosen for this experiment.

The cystine was determined by polarography (6, 7) using a Tinsley ink recorder. In each urine analysis 1 ml. of the diluted urine was added to 3.9 ml. of buffer made up from equal volumes of 0.125 N ammonia and 0.125 M ammonium chloride and containing 4.0 g. per 100 ml. of sodium sulphite to avoid interference by oxygen. Immediately before the analysis, 0.1 ml. of M/10 cobaltous chloride was added. The plasma (0.5 ml.) was first dialyzed against distilled water (2.5 ml.) for 3.5 hr. at 4° C. in the type of apparatus described by Hamilton and Archibald (8). One ml. of the dialysate was analyzed as in the urine method. All determinations were done in duplicate. Our standard curve gave a straight line relationship between wave height and quantity of cystine in the cell between the range of 1 to 10 μg. of cystine. Recovery of cystine added to plasma or urine was $\pm$ 10 per cent. The polarograph, like most other methods of analysis, determines cystine and cysteine together, but it is most unlikely that cysteine exists as such in either plasma or urine. In plasma the high oxygen tension and pH would favour its rapid oxidation to cystine. Moreover a direct chemical method of determination has shown that no cysteine can be detected in normal plasma (9). This still leaves open the possibility that some cysteine is present during the peak rise of blood level after feeding cysteine (see later). As indicated in the preceding paper (1) and confirmed again here the urines at these periods give only very weak direct cyanide nitro-prusside reactions for cysteine, so cysteine was probably also absent from the blood. We have assumed here that the polarographic method is specific for cystine/cysteine. We note however that the recent method of Moore and Stein (10) for amino-acid assay while agreeing closely with the polarographic cystine determinations in plasma, and in cystinuric urines (11), gives consistently lower results with normal urine (12). We have confirmed the discrepancy in this laboratory using both methods of analysis on the same specimens of urine (D. F. Evered—unpublished). The matter remains *sub judice* for the time being, but does not affect the conclusions reached here which depend on changes in urine output rather than on absolute figures.

TABLE I

Clinical data in cystinuric subjects *

Patient	Sex	Age in years	Surface area in *sq. meters*	Blood pressure, *mm. Hg*	Blood urea, *mg./100 ml.*	Cystine excretion, *mg./24 hours*	Cystine lysine pattern of amino-acids in urine (by paper chromatography)	Nephrectomy	Renal calculi
G. M.	F	8	0.87	105/75	48	480	Yes	None. Right kidney non-functional	Bladder stone removed 18 months earlier
J. H.	M	27	1.92	130/80	36	1,026	Yes	None	None
A. La.	F	29	1.44	118/80	27	756	Yes	None	Bladder stone removed one month earlier
S. L.	M	38	1.80	130/85	30	1,075	Yes	Right nephrectomy 12 years earlier	Stones removed left kidney 7 years earlier
A. L.	M	38	1.68	160/100	90	645	Yes	None	Bilateral renal and ureteric stones
G. S.	M	39	1.76	175/115	30	1,212	Yes	None	Bilateral renal calculi
P. G.	M	40	1.87	155/115	36	840	Yes	None	Stones present left kidney, removed from right kidney

* All stones removed surgically were shown to be of cystine on analysis.

TABLE II

Control observations (no amino-acid fed) on normals and on cystinuric patients

Subject	Cystine estimation	Fasting	Time in hours ½	1	2	3	4	5	6	7
B. S.	Plasma, *μg./ml.*	12.8	12.8	12.6	11.7		12.0		12.0	
Normal	Urine, *μg./min.*	26.9 ←	33.0	→ : ←	26.2	→ : ←	24.0	→ : ←	24.0	→ :
	Clearance, *ml./min.*	←	2.6	→ : ←	2.15	→ : ←	2.0	→ : ←	2.0	→ :
C. G.	Plasma, *μg./ml.*	11.4	11.7	12.0	10.8		11.7		10.8	
Normal	Urine, *μg./min.*	27.4 ←	31.0	→ : ←	24.0	→ : ←	23.0	→ : ←	21.0	→ :
	Clearance, *ml./min.*	←	2.65	→ : ←	2.25	→ : ←	2.0	→ : ←	2.0	→ :
J. M. W.	Plasma, *μg./ml.*	13.8	11.4	12.0	14.4		14.2		9.0	
Normal	Urine, *μg./min.*	36.5 ←	43.0	→ : ←	32.0	→ : ←	32.0	→ : ←	30.0	→ :
	Clearance, *ml./min.*	←	3.45	→ : ←	2.2	→ : ←	2.25	→ : ←	3.3	→ :
A. La.	Plasma, *μg./ml.*	3.8	5.5	6.0	6.0		4.8		7.2	
Cystinuric	Urine, *μg./min.*	540.0 ←	532.0	→ : ←	512.0	→ : ←	510.0	→ : ←	469.0	→ :
	Clearance, *ml./min.*	←	97.0	→ : ←	85.0	→ : ←	104.0	→ : ←	65.0	→ :

RESULTS

1. *Control studies on normal and cystinuric subjects*

These results are given in Table II. Although there were fluctuations in both blood levels and urine outputs over the seven-hour fasting period these were of small degree. Even though the plasma levels in the cystinuric patient were lower than in the normal subjects, she excreted almost twenty times more cystine. The renal clearance of cystine was between 2 and 4 ml. per min. in the normals and about 90 ml. per min. in the cystinuric patient.

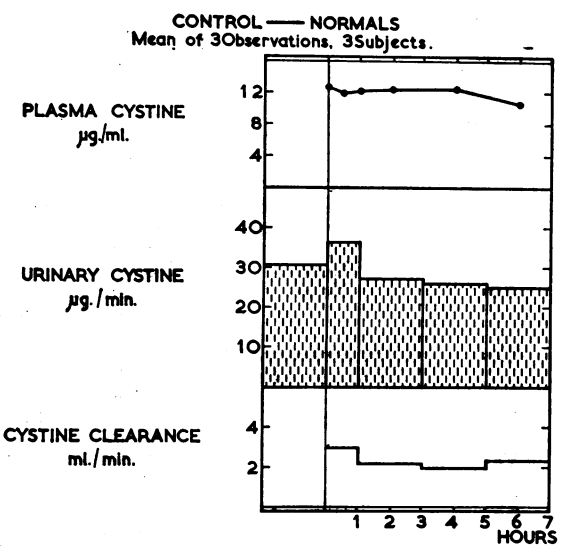

FIG. 1.

The mean results for the three normal subjects are shown in Figure 1. The diagram showing the results obtained in the cystinuric subject is to be found in Figure 7. In these and all subsequent figures and tables basal clearances have not been calculated owing to the fact that the first blood specimens obtained (0 hr.) corresponded in time to the end rather than to the mid-point of the fasting urine collection.

2. *The feeding of l-cystine to normal and cystinuric subjects*

The results are given in Table III and Figures 2 and 3. In the normal subjects there was a small rise in the plasma concentration of cystine and a correspondingly small increase in the urine output; in the cystinuric subject there was a similar but relatively smaller rise. These variations appear to be of doubtful significance as slight fluctuations of this kind also occurred in the control fasting experiments.

That there is no greater increase in cystine plasma level following its ingestion may be due to its prior destruction in the gut by bacteria. The experiment was therefore repeated in two normal subjects after 4.0 g. of aureomycin had been taken in divided doses over the preceding 48 hours. The results were very similar (Table III, expts. 4 and 5). Cystine is extremely insoluble in water at near neutrality and as a result may be absorbed

very slowly from the gut. In an attempt to facilitate absorption 5.0 Gm. of L-cystine was ground in a ball mill with 'Tween 80' as the dispersing agent. The fine emulsion of cystine was then fed to a normal subject and again there was no convincing alteration in the results (Table III, expt. 7).

Although the plasma levels of cystine are lower in the cystinuric than in the normal subjects, they are of the same order of magnitude. The clearance estimations in the case of the normal subjects gave a figure varying betwen 3 and 5 ml. per minute. In the cystinuric patients the much higher clearance figures showed similar variations. These could be explained by the fact that only a single mid-point plasma cystine estimation was done for each one or two-hour clearance period.

The low cystine clearance of G. M. relative to G. S. and A. L. is presumably related to her smaller size.

3. *Feeding of methionine to normal and cystinuric subjects*

The results are shown in Table IV and Figures 4 and 5. In the case of the normal subjects there was a slight increase in both the plasma level and urinary output of cystine. This change was simi-

TABLE III

5.0 g. l-cystine; normals and cystinuric patients

Subject	Cystine estimation	Fasting	Time in hours ½	1	2	3	4	5	6	7
B. S.	Plasma, *μg./ml.*	12.6	13.8	14.4	13.0		12.0		12.0	
Normal	Urine, *μg./min.*	23.0 ←	56.0 → : ←		64.0 → : ←		55.0 → : ←		44.0 → :	
	Clearance, *ml./min.*	←	4.1 → : ←		4.9 → : ←		4.6 → : ←		3.7 → :	
J. M. W.	Plasma, *μg./ml.*	13.0	12.6	12.4	14.3		14.4		10.2	
Normal	Urine, *μg./min.*	36.0 ←	39.0 → : ←		42.0 → : ←		41.0 → : ←		35.0 → :	
	Clearance, *ml./min.*	←	3.1 → : ←		2.9 → : ←		2.9 → : ←		3.4 → :	
B. S.	Plasma, *μg./ml.*	7.8	9.6	13.2	12.6		13.4		11.4	
Normal	Urine, *μg./min.*	30.0 ←	50.0 → : ←		43.0 → : ←		46.0 → : ←		42.0 → :	
	Clearance, *ml./min.*	←	5.2 → : ←		3.4 → : ←		3.4 → : ←		3.7 → :	
J. M. W.	Plasma, *μg./ml.*	11.5	15.6	12.6	13.2		15.2		13.2	
Normal*	Urine, *μg./min.*	36.0 ←	34.0 → : ←		31.0 → : ←		42.0 → : ←		41.0 → :	
	Clearance, *ml./min.*	←	2.2 → : ←		2.3 → : ←		2.8 → : ←		3.1 → :	
B. S.	Plasma, *μg./ml.*	10.8	10.7	16.2	14.2		14.4		12.6	:
Normal*	Urine, *μg./min.*	40.0 ←	49.0 → : ←		53.0 → : ←		42.0 → : ←		43.0 → :	
	Clearance, *ml./min.*	←	4.6 → : ←		3.7 → : ←		2.9 → : ←		3.4 → :	
C. H.	Plasma, *μg./ml.*	9.0	10.8	9.0	11.0		9.0		9.0	
Normal	Urine, *μg./min.*	27.0 ←	21.0 → : ←		29.0 → : ←		27.0 → : ←		29.0 → :	
	Clearance, *ml./min.*	←	1.9 → : ←		2.6 → : ←		3.0 → : ←		3.2 → :	
B. S.	Plasma, *μg./ml.*	11.1	16.9	15.0	14.5		10.3		10.6	
Normal†	Urine, *μg./min.*	32.0 ←	47.0 → : ←		66.0 → : ←		46.0 → : ←		44.0 → :	
	Clearance, *ml./min.*	←	2.8 → : ←		4.5 → : ←		4.5 → : ←		4.2 → :	
G. M.	Plasma, *μg./ml.*	7.2	9.0	7.5	7.2		5.4		4.8	:
Cystinuric‡	Urine, *μg./min.*	400.0 ←	450.0 → : ←		450.0 → : ←		354.0 → : ←		246.0 → :	
	Clearance, *ml./min.*	←	51.0 → : ←		63.0 → : ←		66.0 → : ←		51.0 → :	
G. S.	Plasma, *μg./ml.*	7.8	10.2	8.1	7.2		7.0		6.0	
Cystinuric	Urine, *μg./min.*	842.0 ←	983.0 → : ←		771.0 → : ←		771.0 → : ←		754.0 → :	
	Clearance, *ml./min.*	←	96.0 → : ←		104.0 → : ←		110.0 → : ←		126.0 → :	
A. La.	Plasma, *μg./ml.*	4.4	7.0	5.4	5.4		6.0		4.4	
Cystinuric	Urine, *μg./min.*	540.0 ←	580.0 → : ←		660.0 → : ←		628.0 → : ←		511.0 → :	
	Clearance, *ml./min.*	←	83.0 → : ←		122.0 → : ←		105.0 → : ←		116.0 → :	

* Experiments carried out after the subjects had been on aureomycin 0.5 g. 6-hourly for 48 hours.
† Cystine used in this experiment had been ground in a ball mill with 'Tween 80' as a dispersing agent.
‡ 2.5 g. of l-cystine only given as surface area of patient was only 0.87 sq. meters.

TABLE IV

6.25 g. dl-methionine; normals and cystinuric patients

Subject	Cystine estimation	Fasting	Time in hours ½	1	2	3	4	5	6	7
B. S.	Plasma, *μg./ml.*	9.0	14.4	7.2	10.0		10.2		7.5	:
Normal	Urine, *μg./min.*	19.4 ←	37.5 →	: ←	34.5 →	: ←	26.5 →	: ←	25.5 →	:
	Clearance, *ml./min.*	←	2.6 →	: ←	3.45 →	: ←	2.55 →	: ←	3.4 →	:
J. M. W.	Plasma, *μg./ml.*	9.0	11.4	10.2	9.0		7.8		9.0	
Normal	Urine, *μg./min.*	20.5 ←	33.7 →	: ←	30.0 →	: ←	29.5 →	: ←	25.0 →	:
	Clearance, *ml./min.*	←	3.0 →	: ←	3.3 →	: ←	3.8 →	: ←	2.8 →	:
G. P.	Plasma, *μg./ml.*	5.6	7.2	6.6	10.2		7.2		8.4	
Normal	Urine, *μl./min.*	32.9 ←	43.0 →	: ←	36.5 →	: ←	32.0 →	: ←	27.0 →	:
	Clearance, *ml./min.*	←	6.0 →	: ←	3.6 →	: ←	4.5 →	: ←	3.2 →	:
D. F. E.	Plasma, *μg./ml.*	9.0	8.6	13.2	11.4		10.2		15.6	
Normal	Urine, *μl./min.*	19.4 ←	32.0 →	: ←	35.0 →	: ←	27.0 →	: ←	31.5 →	:
	Clearance, *ml./min.*	←	3.7 →	: ←	3.1 →	: ←	2.6 →	: ←	2.0 →	:
A. L.	Plasma, *μg./ml.*	7.0	9.0	9.6	9.5		11.4		9.7	:
Cystinuric	Urine, *μg./min.*	300.0 ←	270.0 →	: ←	400.0 →	: ←	333.0 →	: ←	366.0 →	:
	Clearance, *ml./min.*	←	30.0 →	: ←	42.0 →	: ←	29.0 →	: ←	38.0 →	:
S. L.	Plasma, *μg./ml.*	5.4	8.9	7.8	7.6		9.5		9.0	
Cystinuric	Urine, *μg./min.*	700.0 ←	733.0 →	: ←	802.0 →	: ←	750.0 →	: ←	825.0 →	:
	Clearance, *ml./min.*	←	82.0 →	: ←	106.0 →	: ←	79.0 →	: ←	92.0 →	:
P. G.	Plasma, *μg./ml.*	7.7	7.2	7.8	6.5		11.0		7.2	
Cystinuric	Urine, *μg./min.*	720.0 ←	648.0 →	: ←	594.0 →	: ←	724.0 →	: ←	706.0 →	:
	Clearance, *ml./min.*	←	90.0 →	: ←	88.0 →	: ←	65.0 →	: ←	98.0 →	:

lar to that found after feeding cystine. The cystinuric subjects showed only small and inconstant variations.

The results in Table IV show that A. L., who had severe impaired renal function (blood urea 90 mg. per 100 ml.), excreted rather less than half the amount of cystine than did S. L. his identical twin. A. L. subsequently died in uremia and showed a progressive diminution of cystine output which paralleled the degree of renal failure.

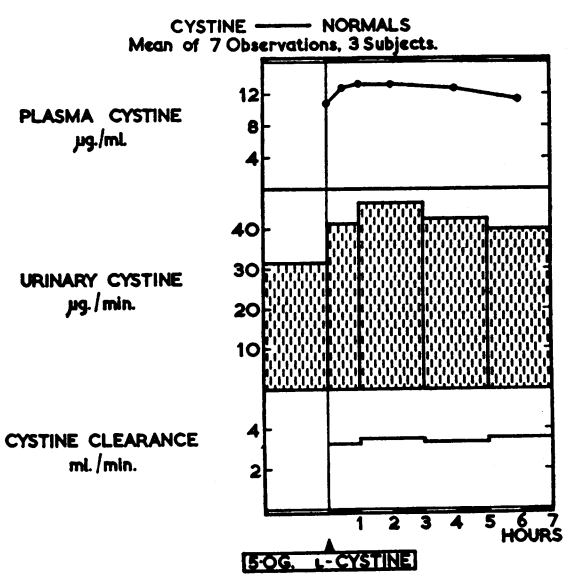

FIG. 2.

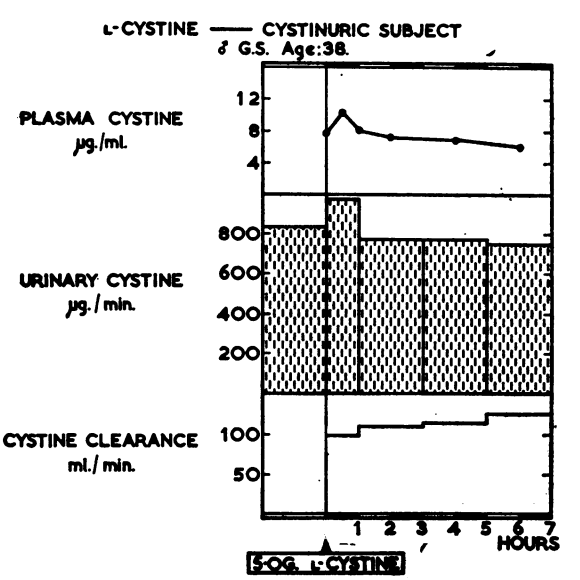

FIG. 3.

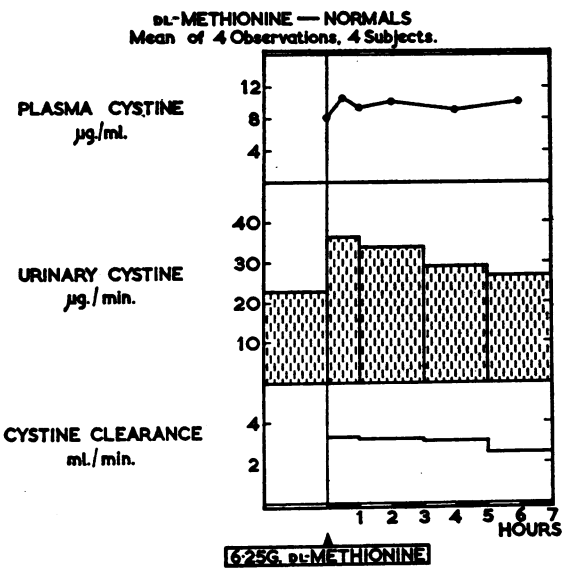

FIG. 4.

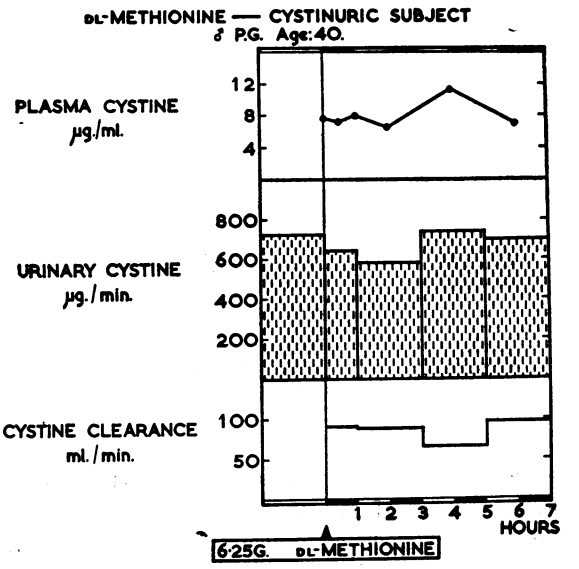

FIG. 5.

4. *Feeding of l-cysteine to normal and cystinuric subjects*

The results are shown in Table V and Figures 6 and 7.

Cysteine produced a very marked increase in the plasma concentration of cystine and a sharp rise in the urinary output. This was seen in both normal and cystinuric subjects. The normal subjects showed an increase in the cystine clearance, which rose in one subject from the basal level of 2 to 4 ml. per min. to a peak of 8.9 ml. per min. The maximum reabsorptive capacity of the tubules for cystine was therefore not exceeded despite a threefold increase in the plasma level. Both cystinuric patients, in spite of the even larger rise in plasma level, did not show a rise in clear-

TABLE V

Cysteine HCl 6.0 g.; normals and cystinuric patients

Subject	Cystine estimation	Fasting	Time in hours ½	1	2	3	4	5	6	7
J. M. W.	Plasma, *μg./ml.*	7.2	14.4	15.0	24.0		14.8		9.6	
Normal	Urine, *μg./min.*	31.0 ←	73.0 → : ←		130.0 → : ←		105.0 → : ←		57.0 → :	
	Clearance, *ml./min.*	←	5.0 → : ←		5.4 → : ←		7.1 → : ←		5.9 → :	
B. S.	Plasma, *μg./ml.*	8.4	25.2	27.0	22.2		19.8 :		10.8	
Normal	Urine, *μg./min.*	32.5 ←	73.0 → : ←		129.0 → : ←		110.0 → : ←		52.5 → :	
	Clearance, *ml./min.*	←	2.9 → : ←		5.8 → : ←		5.5 → : ←		4.9 → :	
T. G.	Plasma, *μg./ml.*	11.4	27.2	27.0	26.2		13.8		13.8	
Normal	Urine, *μg./min.*	28.4 ←	67.5 → : ←		135.0 → : ←		176.0 → : ←		65.6 → :	
	Clearance, *ml./min.*	←	2.5 → : ←		5.1 → : ←		5.5 → : ←		4.8 → :	
G. F.	Plasma, *μg./ml.*	12.0	—	20.4	20.4		15.0		12.0	
Normal	Urine, *μg./min.*	29.8 ←	85.0 → : ←		155.0 → : ←		133.0 → : ←		78.0 → :	
	Clearance, *ml./min.*		—	←	7.6 → : ←		8.9 → : ←		6.5 → :	
A. La.	Plasma, *μg./ml.*	7.4	36.0	38.4	37.0		25.8		12.8	
Cystinuric	Urine, *μg./min.*	580.0 ←	3067.0 → : ←		5000.0 → : ←		2533.0 → : ←		1725.0 → :	
	Clearance, *ml./min.*	←	85.0 → : ←		135.0 → : ←		98.0 → : ←		135.0 → :	
A. L.	Plasma, *μg./ml.*	10.2	25.2	42.0	43.0		24.6		15.6	
Cystinuric	Urine, *μg./min.*	297.0 ←	696.0 → : ←		1108.0 → : ←		785.0 → : ←		475.0 → :	
	Clearance, *ml./min.*	←	27.5 → : ←		26.0 → : ←		32.0 → : ←		30.5 → :	

TABLE VI

Racemic ('dl') cystine 5.0 g. normals and cystinuric patient

Subject	Cystine estimation	Fasting	Time in hours ½	1	2	3	4	5	6	7
J. M. W.	Plasma, *μg./ml.*	9.0	10.2	13.8	12.6		13.8		12.2	
Normal	Urine, *μg./min.*	26.0	← 213.0 →	:	← 394.0 →	:	← 304.0 →	:	← 285.0 →	:
	Clearance, *ml./min.*		← 21.0 →	:	← 31.0 →	:	← 22.0 →	:	← 23.0 →	:
C. P. H.	Plasma, *μg./ml.*	14.4	13.2	20.2	21.6		18.6		11.4	
Normal	Urine, *μg./min.*	39.0	← 297.0 →	:	← 545.0 →	:	← 330.0 →	:	← 280.0 →	:
	Clearance, *ml./min.*		← 22.0 →	:	← 25.0 →	:	← 18.2 →	:	← 24.5 →	:
J. H.	Plasma, *μg./ml.*	7.0	7.3	9.0	7.2		6.0		8.1	
Cystinuric	Urine, *μg./min.*	720.0	← 720.0 →	:	← 720.0 →	:	← 730.0 →	:	← 740.0 →	:
	Clearance, *ml./min.*		← 99.0 →	:	← 100.0 →		← 121.0 →	:	← 93.0 →	:

ance. As in the methionine experiments, the lower output of A. L. is attributed to his renal failure. In each cystinuric subject the clearance figures were similar to those obtained previously on the same subject when different amino-acids were fed (A. La. Table II, expt. 4; A. L. Table IV, expt. 5).

5. *Feeding of racemic DL-cystine to normal subjects and to one cystinuric subject*

The results appear in Table VI and Figure 8.

In the case of the two normal subjects there was a small rise in the plasma level of cystine. As the percentage rise was exceeded by one normal subject (B. S.) after feeding L-cystine it is probably of no special significance. In spite of these small changes in plasma cystine the cystine output in the urine increased ten-fold. The clearance values therefore showed a like increase. By contrast the results in the cystinuric subject were essentially the same as those seen when L-cystine was fed to other cystinuric patients (Table III), both the output and the clearance remaining virtually constant.

6. *Feeding of L-arginine HCl, L-lysine HCl and Dl-ornithine HCl to a normal subject and to an "incomplete cystinuric" subject*

The normal control (Table VII a and b) showed slight variations in the blood and urine levels of a degree comparable to that obtained when water

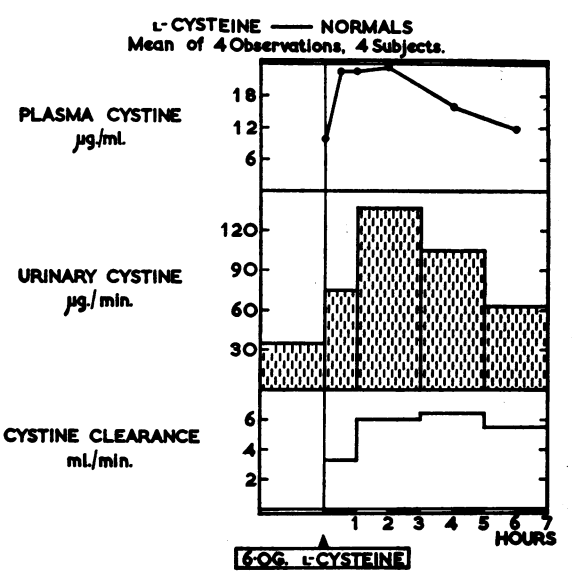

FIG. 6.

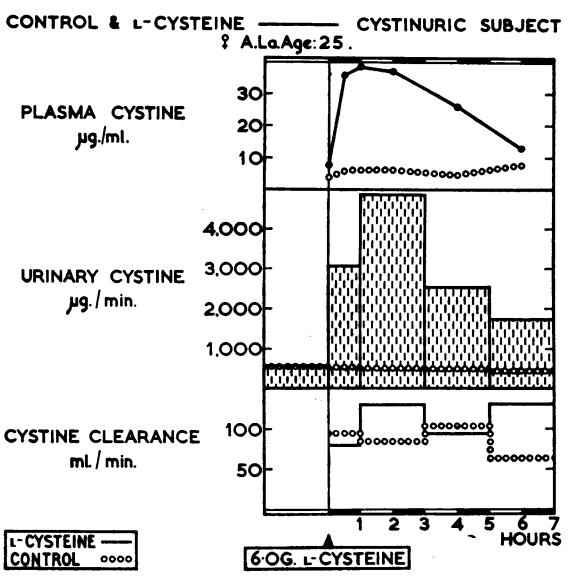

FIG. 7.

TABLE VII

Effect of amino-acid loading on cystine clearance

Subject	Cystine estimation	Fasting	Time in hours ½	1	2	3	4	5	6	7
(a) Arginine HCl 5.0 g., lysine HCl 5.0 g., and ornithine HCl 5.0 g.: normal and "incomplete cystinuric" subject										
J. M. W. Normal	Plasma, *μg./ml.*	9.0	9.6	8.4	10.8		9.2		9.0	
	Urine, *μg./min.*	25.0 ←	38.5 →	: ←	29.0 →	: ←	27.0 →	: ←	32.0 →	:
	Clearance, *ml./min.*	←	4.0 →	: ←	2.7 →	: ←	2.9 →	: ←	3.6 →	:
A. W. "Incomplete cystinuric"	Plasma, *μg./ml.*	12.0	11.9	12.0	13.8		13.2		12.1	
	Urine, *μg./min.*	110.0 ←	168.0 →	: ←	150.0 →	: ←	92.0 →	: ←	96.0 →	:
	Clearance, *ml./min.*	←	14.0 →	: ←	11.0 →	: ←	7.0 →	: ←	7.9 →	:
(b) Glycine 15.0 g.; "incomplete cystinuric" subject										
A. W. "Incomplete cystinuric"	Plasma, *μg./ml.*	10.8	10.6	11.6	12.0		10.8		10.7	
	Urine, *μg./min.*	85.0 ←	85.0 →	: ←	86.0 →	: ←	77.0 →	: ←	77.5 →	:
	Clearance, *ml./min.*	←	8.0 →	: ←	7.2 →	: ←	7.1 →	: ←	7.0 →	:

only was taken by the same subject in an earlier experiment (Table II, expt. 3). It is noted that the clearance values showed no alteration. When fed arginine, lysine, and ornithine the "incomplete cystinuric" subject showed a slight (50 per cent) rise in the excretion of cystine, and an even smaller change in the plasma level. To see whether this small increase of excretion was simply a result of the large amino-acid intake, 15.0 Gm. of glycine were fed to the same subject. The plasma cystine level remained constant as did the urinary excretion.

7. *Simultaneous cystine and inulin clearances; one cystinuric subject*

Three twenty-minute clearances were performed. The results presented in Table VIII show that the clearance values for cystine and for inulin are virtually identical.

8. *Fasting plasma cystine levels in normal and cystinuric subjects*

Fifty plasma cystine levels were measured on 17 fasting normal subjects, and 31 on 9 fasting cystinuric subjects. Cystinuric subjects having marked renal damage were excluded as we have some evidence (unpublished) that the plasma cystine level tends to rise slightly as the output falls with the onset of uremia.

TABLE VIII

	Period 1 *ml./min.*	Period 2 *ml./min.*	Period 3 *ml./min.*
Cystine clearance	95	99	95
Inulin clearance	94	102	104

The normal subjects had a mean fasting plasma cystine level of 10.64 μg. per ml., S.D. 1.87, and the cystinuric subjects a mean of 5.88 μg. per ml., S.D. 1.5. This difference is statistically significant at the 0.1 per cent level.

DISCUSSION

In both normal and cystinuric subjects, L-cysteine alone, of the three sulphur-containing amino-

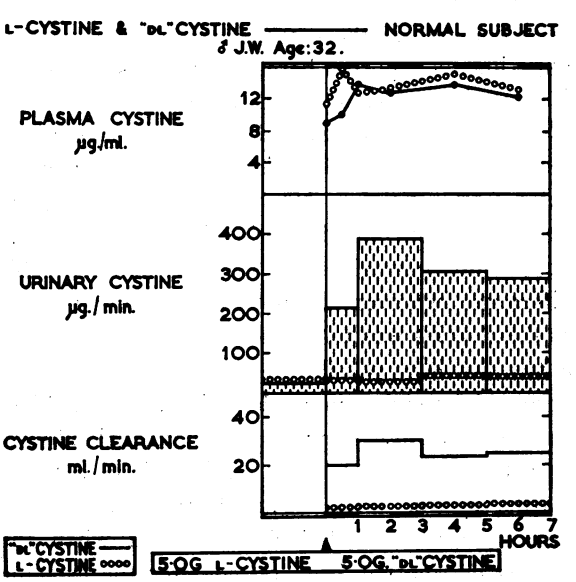

FIG. 8.

acids, produced a large increase in plasma level and urinary output of cystine. (Somewhat higher peak plasma levels were obtained in cystinuric patients). This is presumably because cysteine, being very soluble, can be rapidly absorbed and converted into cystine. Cystine is very insoluble and therefore may be only slowly absorbed. Methionine though readily absorbed (1) is only slowly and in part converted into cystine. In every case there were similar trends between the changes in normal and cystinuric subjects. These results are very similar to those obtained by quite different methods of analysis by Dent, Heathcote, and Joron (1) and serve to reinforce strongly their conclusion that the excessive cystine excretion in cystinuria is the result of a lowered renal threshold for cystine and is not due to a disorder of intermediary metabolism.

Our results after feeding methionine to cystinuric patients are different from those reported by previous workers (13, 14) who consistently found an increase in cystine output. It is possible that the different conditions of our experiments were responsible. In some experiments (unpublished) in which the urine collection was made, as in their series, over a 24 hr. period we have observed increases of 200 to 250 mg. over the basal output. This may have been due to the longer duration of urine collection or to the concomitant ingestion of food.

Further support for the renal hypothesis has been obtained in the more accurately quantitative experiments reported here. In the first place the fasting plasma cystine levels of cystinuric subjects (5.88 μg. per ml., S.D. 1.5) were significantly lower than in normal subjects (10.64 μg. per ml. S.D. 1.5). Fowler, Harris, and Warren (15) found a similar trend, cystinuric subjects av. 6.9 μg. per ml., normal subjects 8.2 μg. per ml. This difference however was not considered significant as their series was too small. In the second place it has now been possible to calculate cystine clearances. The endogenous clearance is about thirty times greater in cystinuric than in normal subjects even though the cystinuric subjects have a lower plasma cystine level. The clearance in normal subjects remains relatively low even when the plasma cystine has been raised after the cysteine feedings. The cystine clearances in cystinuric subjects are of the order to be expected of the glomerular filtration rates especially when allowance is made for the small surface area of G. M. and impaired renal function of A. L. These clearances did not rise as they did in the normal patients when the blood level of cystine was raised after taking cysteine. In the cystinuric J. H. the simultaneous inulin and endogenous cystine clearances were virtually identical over three separate clearance periods. It seems almost certain then that in this patient at least the kidney tubule was unable to reabsorb any of the cystine passing into the glomerular ultrafiltrate.

There is a further argument in support of this that can be derived from the experiments in which DL-cystine was fed. In the normal subjects, though there were but minor changes in the plasma level a marked increase in the output of cystine occurred and the clearance values rose from a basal level of 2 to 4 ml. per min. to 25 to 30 ml. per min. Solubility studies (unpublished) on the urine of C. P. H. obtained after feeding DL-cystine, showed that the major part of the cystine present must have been in a form other than L-cystine. The normal tubule probably absorbs D-cystine less readily (if at all) than L-cystine. In the cystinuric on the other hand no change in cystine clearance occurred after feeding DL-cystine presumably because the tubule cannot reabsorb cystine in any isomeric form, all filtered cystine being excreted.

We would emphasize the importance of making sure that a given batch of cystine is exclusively in the L-form before using it for studies of the kind described here, since most of the cystine on the market is prepared by acid hydrolysis of protein, and some racemization must occur during this process, and will contaminate the final product when insufficiently recrystallized. Albanese (16) has determined cystine outputs after feeding L- and DL-cystine and claimed a large increase after DL-cystine but that no increased output occurred after L-cystine. We have recalculated his figures to express the cystine output in μg. per min. instead of as cystine output per urine sample (30 to 120 min.). The results showed a ten-fold increase after DL-cystine and fourfold increase after L-cystine. Possibly his L-cystine was partly racemized since the latter figure is considerably higher than we would expect and corresponds to our results

(unpublished) with one batch of cystine which was later shown by solubility studies to be partly racemized.

Other workers have shown that the normal kidney tubule distinguishes D- from L-amino-acids (17, 18). The latter suggested (18) on the basis of clearance studies that some D-amino-acid passively diffused through the tubule back into the blood stream. Our results do not suggest any passive diffusion of D-cystine in cystinuric subjects.

The changes in plasma level following the feeding experiments with DL-cystine are of interest. They were similar to those found in a few normal subjects after feeding L-cystine. Silber, Seeler, and Howe (17) using dogs determined plasma α-aminonitrogen levels after intravenous infusions of amino-acids where either 5 per cent or 25 per cent were in D-form. They found similar changes in levels in each case. These experiments suggest that the tissues do not appear to distinguish between D- and L-amino-acids in contrast to their very different handling by the renal tubule.

In cystinuria the urine contains large quantities of lysine, arginine, and ornithine as well as of cystine. Dent and Rose (2) suggested on the basis of similarities in their structure, that there might be a common mechanism responsible for their reabsorption by the tubule. If so it should be possible to demonstrate competition between these substances so that overloading the tubule with lysine, arginine, and ornithine should interfere with cystine reabsorption and therefore increase cystine output and clearance. This experiment was not attempted on a cystinuric subject because we now believe that these subjects have no cystine reabsorption at all. It was, therefore, carried out on a normal subject and on an "incomplete" cystinuric subject (A. W.), the latter being potentially more promising since there was already a decreased ability to reabsorb cystine. The normal subject showed no significant increase in cystine clearance after large oral doses of these three amino-acids. The incomplete cystinuric subject (Table VIIa) showed a small rise in clearance under the same conditions. Probably the normal subject gave a negative result because of the high reserve capacity of the tubule for cystine reabsorption. However the clearance rise of the incomplete cystinuric subject may be interpreted, it was probably not due to unspecific amino-acid competition as a large dose of glycine given to him on another occasion produced no change in cystine clearance (Table VIIb). The differences in clearance, however, were too small to prove conclusively the hypothesis of tubular competition.

In conclusion it can now be stated that stone formation in cystinuric subjects is the immediate result of the defect in cystine reabsorption which resides in the kidney, and contrasts therefore with the stone formation of, for example, hyperparathyroidism, where a primary cause of the hypercalcuria is known to be remote from the renal tract. The therapeutic implications arising from this are being investigated and form the subject of a further communication (19).

SUMMARY

1. Polarographic determinations of cystine in plasma and urine were carried out in normal and in cystinuric subjects before and after feeding cystine, cysteine, and methionine.

2. After feeding cysteine large rises in plasma cystine level occurred, these changes being similar in both normal subjects and in cystinuric subjects. The urine cystine output increased greatly in both groups, being at all plasma levels very much higher in cystinuric than in normal subjects. The results after feeding cystine and methionine were also similar in both normal and cystinuric subjects, except that lesser changes, if any, in cystine level or excretion occurred.

3. The endogenous cystine clearance in normal subjects was 2 to 4 ml. per min. In cystinuric subjects it was about 30 times higher. The cystine clearance was the same as the simultaneous inulin clearance in the one cystinuric patient investigated in this way.

4. Plasma cystine levels in cystinuric subjects are significantly lower than in normal subjects.

5. The kidney tubule in normal subjects appears to reabsorb racemic cystine less readily (possibly not at all) than L-cystine, in cystinuric subjects no difference in excretion was noted, neither apparently being reabsorbed.

6. It is concluded that cystinuria results from greatly diminished (or absent) cystine reabsorption by the renal tubule. No evidence has been

found for an innate disorder of intermediary metabolism of sulphur-containing amino-acids.

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