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AMINO ACID CLEARANCES IN CYSTINURIA

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Cystinuria is a hereditary disorder in which there is a grossly excessive excretion of cystine in the urine. Owing to the low solubility of this amino acid the urine is often super-saturated in terms of cystine and crystals are likely to be deposited, then to grow and aggregate to form calculi in the urinary tract.

It was found by means of paper chromatographic methods (Dent & Rose, 1949) that there is also an excessive excretion of the basic amino acids lysine and arginine and, in a later paper (Dent & Rose, 1951), perhaps also of ornithine. This was conclusively confirmed by Stein (1951), who carried out quantitative analyses for amino acids by the highly specific method of ion-exchange column chromatography and found that in cases of cystinuria the urine contained an increased excretion of ornithine as well as of lysine and arginine. It was suggested (Dent & Rose, 1949, 1951) that the increased cystine excretion was not due to any failure to metabolize cystine in the body, as previously thought by all workers who had studied the condition, but was due to a low 'renal threshold' for cystine. These authors, and Fowler, Harris & Warren (1952), found that the cystine concentration in plasma was normal, but later Dent, Senior & Walshe (1954), by using a quantitative polarographic method for cystine, found the plasma cystine concentration lower in cystinuric patients than in normal subjects. As an increase in renal tubular secretion of cystine is inherently unlikely, the reason for the increased excretion of cystine in the urine would appear to be the partial or complete inability of the renal tubule to reabsorb this amino acid from the glomerular filtrate. In fact, Dent *et al.* (1954) found that the rate of clearance of cystine in cystinuric patients was about the same as the rate of glomerular filtration, the clearance of cystine and inulin being in one case almost identical. As there are many other conditions in which an increased excretion of cystine may occur in the urine, the latter authors proposed that the term 'cystinuria', when used to indicate a medical diagnosis, should be restricted to the highly specific disorder in which cystine stone formation may occur and they then gave

further criteria for defining the condition. We use the term in this sense exclusively in this paper.

Harris & Warren (1953) studied in some detail the inheritance of cystinuria. They showed that cystinuric subjects could be either homozygous for a true recessive gene or else homozygous for an incompletely recessive gene. In the latter case the heterozygotes, while clinically normal, could be detected by the constant excretion in the urine of slightly increased quantities of cystine and lysine.

The present work was undertaken to obtain clearance values of all the amino acids excreted in the urine of normal and cystinuric subjects and of subjects known to be heterozygous for the two types of cystinuric gene.

METHODS

Selection of patients. Four normal individuals were taken as controls. Three subjects were heterozygotes for the cystinuria gene, two of these being of the incompletely recessive form. Four homozygous cystinuric subjects were studied, three of these being of the incompletely recessive form. Only one cystinuric subject had had operations for the removal of stones (Table 1). She continued to have a normal blood urea and no symptoms of renal damage. We attempted to study patients without stones as far as possible since one patient had colic during collection and the samples had to be abandoned. A further difficulty arises when stones are present because the urine flow has to be maintained at a fairly high rate during the 3 hr collection period. Hence cystine stones, if present, may dissolve and further increase cystine output. This probably happened with A. P. (jr.), as his cystine clearance was unexpectedly high and he was admitted to hospital with renal colic a few months after the collection was taken, although at the time we believed him to be free from stones.

Procedure. After a light breakfast, patients were given water to drink at 9 a.m., and after voiding and rejecting the urine at 10 a.m., collection was started and continued until 1 p.m. Meanwhile blood samples (15 ml.) were taken at 10.30, 11.30 a.m. and 12.30 p.m., and transferred to heparinized tubes. After centrifuging, a 5 ml. portion was taken from each tube and mixed to form a composite sample. In the case of normal subjects urine was collected between 11 a.m. and 1 p.m., and a blood sample (30 ml.) was taken at 12 noon. The plasma (15 ml.) was deproteinized with picric acid (Hamilton & Van Slyke, 1943) and the excess picric acid was removed by passing the solution through a small column of Dowed 2-X 8 (200–400 mesh) resin in the chloride form (Stein & Moore, 1954). The amino acid analyses were carried out on $150 \times 0{\cdot}9$ cm columns of Dowex 50-X 4 (200–400 mesh) resin, using the Moore & Stein (1954) method. 8–10 ml. of deproteinized plasma and 10–20 ml. of urine, in both cases acidified to pH 2·0, were used for the plasma and urine analyses respectively. In addition, cystine was determined by polarograph (Reed, 1942; Fowler *et al.* 1952) on samples of deproteinized plasma and urine. Samples were used for two-way paper chromatograms (Dent, 1946) and electrophoretic strips (Harris & Warren, 1954).

RESULTS

Apart from stone formation, the most distinctive feature of cystinuria is the high excretion of cystine, lysine, ornithine and arginine in the urine. The output of these amino acids for the cystinuric patients and for normal subjects is shown in Table 1. The average total excretion of these substances in the cystinuric urine is 3·95 g/day, which agrees closely with 3·73 g reported by Stein (1951).

The urinary excretion rate, the concentration in plasma and the calculated clearance values of cystine, lysine, ornithine and arginine are summarized in Table 2. The analyses of four normal urines and three plasmas are in good agreement with other published work: in fact, two of our normal subjects had been studied previously in this laboratory by Evered (1956), and our repeat determinations agree well with his figures. The increased excretion of cystine, lysine, ornithine and arginine in the urine of the homozygous cystinuric subjects is similar to that found by Stein (1951) and by Harris, Mittwoch, Robson & Warren (1955), the latter using microbiological methods for assay. There was no appreciable difference between the incompletely and fully

TABLE 1. Excretion of cystine, lysine, ornithine and arginine in cystinuric patients and normal individuals

Subject	Sex	Age	Cystinuric genotype	Excretion			
				Cystine (mg/24 hr)	Lysine (mg/24 hr)	Ornithine (mg/24 hr)	Arginine (mg/24 hr)
C.G.*	F.	20	Homozygote	636	939	190	650
A.M.	F.	40	Homozygote	677	603	250	498
P.H.S.	M.	37	Homozygote	1150	2580	622	1550
A.P. (jr.)	M.	18	Homozygote	1780	1570	573	1550
A.P. (sr.)	M.	40	Heterozygote (recessive)	18·6	5·6	1·85	27
A.W.	M.	24	Heterozygote (incomplete recessive)	82	183	26	13
M.M.	F.	38	Heterozygote (incomplete recessive)	187	302	2	6
V.K.A.	F.	23	Normal	14·5	46	—	12·2
R.G.W.	M.	47	Normal	10·7	68	—	—
G.A.R.	M.	29	Normal	12·6	296	—	—
J.A.	M.	34	Normal	17·5	17	—	33

* Subject C.G. had had an operation for removal of stones.

TABLE 2. Cystine, ornithine, lysine and arginine clearances in normal and cystinuric subjects

	Normal			Homozygous				Heterozygous		
				Incomplete recessive			Recessive	Incomplete recessive		
Cystine										
Urine (μg/min)	7·3	8·8	12·1	442	472	805	1230	12·7	56·7	128
Plasma (mg/100 ml.)	1·38	1·42	1·43	0·97	0·54	0·71	0·60	1·18	0·93	1·21
Clearance (ml./min)	0·53	0·40	0·84	46	88	112	206	1·1	6·1	10·5
Ornithine										
Urine (μg/min)	Not detectable			132	172	430	398	1·2	18	1·3
Plasma (mg/100 ml.)	0·7	1·30	0·99	0·98	0·53	0·93	0·74	0·81	0·78	0·38
Clearance (ml./min)	—	—	—	13	33	46	54	0·15	2·3	0·35
Lysine										
Urine (μg/min)	46	206	117	653	418	1780	1090	4·0	126·4	210
Plasma (mg/100 ml.)	2·43	4·93	3·18	0·81	1·57	2·66	1·65	0·97	2·11	1·28
Clearance (ml./min)	1·9	4·2	3·7	81	27	67	66	0·42	6·0	16
Arginine										
Urine (μg/min)	Tr.	Tr.	23	452	344	1080	1074	17·8	9·0	44
Plasma (mg/100 ml.)	2·28	1·68	1·63	1·50	1·0	1·12	1·35	1·15	1·5	0·93
Clearance (ml./min)	—	—	1·4	30	35	96	80	1·6	0·6	0·47

recessive forms. Quantitative studies of the excretion of these amino acids in the heterozygous types have not been reported before and, as we expected from previous paper chromatographic studies, the incompletely recessive heterozygotes excreted slightly increased quantities of cystine and lysine, whilst the completely recessive type excreted the normal amounts.

Full analysis of amino acids in the plasma from cystinuric subjects have not been reported before, but Stein (1951) stated that the plasma cystine concentration in these cases was below normal. Our results confirm this finding and show further that plasma concentrations of lysine and arginine tend also to be below normal.

TABLE 3. Clearance of other amino acids in normal (3) and cystinuric (7) subjects

	Plasma (mg/100 ml.)		Urine (μg/min)		Clearance (ml./min)	
	Average	Range	Average	Range	Average	Range
Taurine	1·12	0·4 –1·87	57	2·3–143	8	0·2 –20
Aspartic acid	0·14	0·06–0·29	Trace	—	—	—
Threonine	1·69	0·86–2·87	23	6·0–59·0	2·7	0·7 –3·5
Serine	1·25	0·89–1·88	44	23·6–81·0	3·4	1·5 –5·6
Glutamine + asparagine	3·47	0·52–6·32	37	17·0–70·0	1·4	0·6 –3·3
Proline	3·23	1·37–5·57	Trace	—	—	—
Glutamic acid	1·15	0·54–1·93	15	2·2–65	1·4	0·17–4·0
Glycine	1·87	1·42–2·79	128	28·0–310	7	1·8 –19·0
Alanine	4·07	2·52–5·53	29	8·0–51	0·6	0·21–1·2
Valine	2·98	1·93–3·69	6	1·3–11·8	0·23	0·04–0·47
Methionine	0·43	0·13–0·73	8	4·0–14·5	2·3	0·2 –3·9
Isoleucine	0·98	0·69–1·44	11	4·2–31·6	1·1	0·34–2·5
Leucine	1·84	1·33–2·39	12	3·5–39·8	0·8	0·27–2·2
Tyrosine	1·15	0·80–1·78	20	7·0–40·5	1·9	0·7 –3·8
Phenylalanine	1·04	0·87–1·35	11	6·4–26·0	1·1	0·54–1·9
Histidine	1·69	0·82–2·59	94	30·0–204	7	1·4 –15·0

The calculated plasma clearances of cystine, lysine, ornithine and arginine are all high in the homozygous cystinurics. A possible explanation has already been given for the abnormally high cystine clearance (206 ml./min) for A.P. (jr.). In the heterozygous subjects of the incompletely recessive type only the cystine and lysine clearances are moderately raised. The fully recessive heterozygote A.P. (sr.) would appear to have completely normal clearances and, in fact, his lysine clearance is well below normal, owing to an extremely low excretion of this amino acid. Clearly more subjects of this type must be studied, but we did not have the opportunity on this occasion. The general problem of detecting heterozygotes in so-called recessive conditions constitutes one of the most interesting current problems in genetics. The clearances of the other amino acids in cystinuric subjects are not significantly different from those of normal persons and the two have been grouped together to give the values shown in Table 3.

DISCUSSION

These results provide further evidence that homozygous cystinuric patients have a highly specific and gross defect of renal tubular reabsorption not only of cystine, but also of lysine, ornithine and arginine. Dent *et al.* (1954) established that, in the one case studied, the cystine clearance was the same as the inulin clearance. Certainly, in three out of four cases the cystine clearance was of the same order and it seems likely that they were unable to reabsorb any cystine at all in the renal tubules. The clearances for the other three amino acids involved were, in general, somewhat lower, so that one must assume that at least a proportion of these compounds was reabsorbed. No other known amino acids are concerned; in particular, we did not find any decrease in taurine or increase in isoleucine excretion as originally reported by Stein (1951).

These facts strongly support the hypothesis of other workers (Dent & Rose, 1951; Harris *et al.* 1955) that the four amino acids involved probably share, somewhere in the renal tubule, a common pathway of reabsorption. We have no data to confirm or deny the theory of Dent & Rose (1951) that the reason for this association between the four amino acids concerns some subtle gross structural resemblance between them. Presumably this could be further studied by measuring the clearances of various synthetic compounds of similar type using the methods described herein for the naturally occurring substances in urine and plasma. As already stated, the average daily excretion of the four amino acids in excess amounts to nearly 4 g/day. It is of some interest that this large loss of important dietary constituents does not appear to lead to any nutritional deficiencies.

SUMMARY

1. Timed plasma and urine specimens have been obtained from normal persons and from homozygous and heterozygous cystinuric subjects. They were analysed quantitatively for all the amino acids present by the Moore & Stein (1954) method.

2. The cystine clearances in cystinuric subjects were of the order to be expected from their glomerular filtration rates. The other three amino acids, lysine, arginine and ornithine, had also grossly raised clearances but they were slightly lower than those for cystine.

3. Incompletely recessive heterozygotes showed moderately raised clearances for cystine and lysine only. A completely recessive heterozygote showed normal clearances for all the amino acids.

4. Our results are consistent with the theory that in cystinuria a highly specific gross defect of renal tubular reabsorption is present for cystine, lysine, ornithine and arginine.

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Addendum. Whilst revising this paper for publication an article appeared by Doolan, Harper, Hutchin & Alpen (1957) on 'The renal clearance of lysine in cystinuria'. These authors agree with our findings: that the lysine clearance is very high, that the plasma concentration of lysine and arginine is lower than normal and that the renal tubular reabsorption of the other amino acids with the exception of cystine, lysine and arginine is normal in cystinuric subjects. This agreement is particularly pleasing since the analyses of the amino acids were obtained by micro-biological methods.

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