GAS CHROMATOGRAPHIC ANALYSIS OF URINARY TYROSINE AND PHENYLALANINE METABOLITES IN PATIENTS WITH GASTRO-INTESTINAL DISORDERS

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SUMMARY

Main urinary bacterial metabolites of phenylalanine (total benzoic and phenylacetic acids) and of tyrosine (total *p*-hydroxybenzoic acid and *p*-hydroxyphenylacetic acid) were determined by gas chromatography in controls and patients with cystic fibrosis of the pancreas, coeliac disease, intestinal resection and unclassified enteritis. In various patients, especially in the untreated coeliacs, high amounts of one or more of the abovementioned metabolites were found. In this paper results in controls and patients are presented and discussed.

INTRODUCTION

In a preceding paper[1] we described an abnormal urinary excretion of typical phenolic and phenyl acids in a patient with severely impaired intestinal amino acid resorption. Presumably the greater part of these metabolites are bacterial degradation products of non-absorbed tyrosine and phenylalanine. From tyrosine are thought to originate *p*-hydroxyphenylacetic acid, *p*-hydroxybenzoic acid (excreted as *p*-hydroxyhippuric acid), *p*-hydroxyphenylpropionic acid and *p*-hydroxyphenylacrylic acid; from phenylalanine mainly benzoic and phenylacetic acids (both conjugated) are formed. Small amounts of *p*-hydroxyphenylpropionic, *p*-hydroxyphenylacetic, *p*-hydroxybenzoic, phenylacetic and benzoic acids were found in the patient's faeces, but the faecal excretion was small compared with the urinary excretion. In the above mentioned paper only one patient has been described. It would be desirable to examine other patients with an impaired intestinal resorption. Here, we describe the urinary excretion of bacterial phenylalanine and tyrosine metabolites in patients with cystic fibrosis, coeliac disease, resection of the small intestine and unclassified diarrhoea.

METHODS

The urine samples were hydrolysed with 11 *N* NaOH. Extraction of phenolic and phenyl acids and trimethyl silylation were performed as described earlier[1].

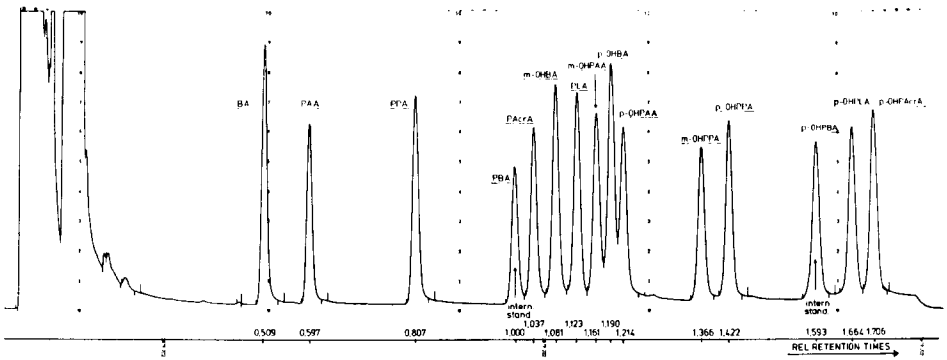

Fig. 1. Gas chromatogram of the trimethylsilyl derivatives of some phenyl and phenolic acids and their retention times relative to phenylbutyric acid (internal standard). BA: benzoic acid; PAA: phenylacetic acid; PPA: phenylpropionic acid; PBA: phenylbutyric acid; PAcrA: phenylacrylic acid and PLA: phenyllactic acid.

Gas-chromatographic separation was improved by using a high performance 5% SE-52 chromosorb W AW DMCS; 100–120 mesh. Temperatures: oven 100–220° (2°/min), injection port 190°; detector 220°. Gas flows N_2: 27 ml/min; H_2: 28 ml/min; air: 450 ml/min. Other parameters remained unchanged.

Methodical results

A chromatogram is shown in Fig. 1. At present a better separation of *p*-hydroxybenzoic from *p*-hydroxyphenylacetic acid can be obtained than previously[1]. Rt values relative to phenylbutyric acid (internal standard), are given.

Table I shows the reproducibility of the complete urinary analysis and in Table II recoveries are given. Only the reproducibility of *p*-hydroxyphenylacrylic acid is out of the range.

RESULTS OF URINARY ANALYSIS

The Fig. 2, 3, 4, and 5 show the excretion of benzoic, phenylacetic, *p*-hydroxybenzoic and *p*-hydroxyphenylacetic acids in 27 controls and 53 patients with gastro-

TABLE I

REPRODUCIBILITIES OF PHENYL- AND PHENOLIC ACIDS RELATED TO BACTERIAL METABOLISM OF PHENYLALANINE AND TYROSINE

Compounds	*Urine + addition I (N = 5)*		
	Mean	*Standard deviation*	
	mg/l	*mg/l*	%
Benzoic acid	940	27	2.9
Phenylacetic acid	481	16	3.4
Phenylpropionic acid	840	16	1.9
Phenylacrylic acid	883	34	3.9
p-hydroxyphenylbenzoic acid	720	12	1.7
p-hydroxyphenylacetic acid	1475	35	2.4
p-hydroxyphenylpropionic acid	879	28	3.2
p-hydroxyphenylacrylic acid	990	123	12.4

TABLE II

RECOVERIES OF PHENYL- AND PHENOLIC ACIDS RELATED TO BACTERIAL METABOLISM OF PHENYLALANINE AND TYROSINE

Compounds	*Urine + addition I (N = 5) Mean: mg/l*	*Urine + addition II (N = 4) Mean: mg/l*		*Recovery of addition II (%)*	
	Found	*Found*	*Calculated*	*Mean*	*Range*
Benzoic acid	940	1398	1334	105	100–110
Phenylacetic acid	481	861	902	95	88–102
Phenylpropionic acid	840	1620	1662	97	94–100
Phenylacrylic acid	883	1694	1654	102	93–111
p-hydroxyphenylbenzoic acid	720	1560	1477	106	99–113
p-hydroxyphenylacetic acid	1475	2400	2287	105	97–113
p-hydroxyphenylpropionic acid	879	1704	1659	103	97–109
p-hydroxyphenylacrylic acid	990	2120	2150	99	73–125

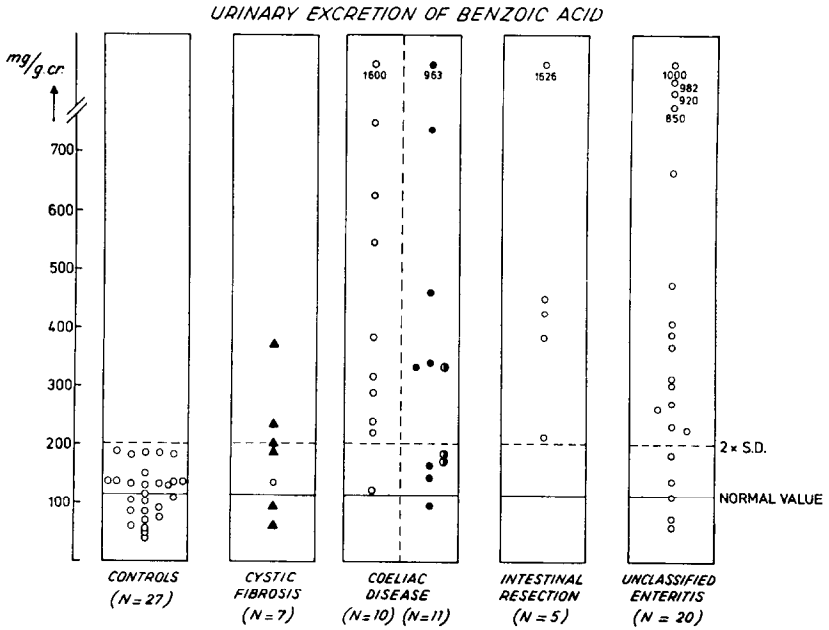

Fig. 2. Total benzoic acid excretion in patients with various gastrointestinal disorders: untreated (○); after treatment during a period $> \frac{1}{2}$ year (●); $< \frac{1}{2}$ year (◑); pancreatine substituted (▲).

intestinal disorders; the first two metabolites originate from phenylalanine and the latter two from tyrosine. In order to exclude urinary phenolic acids of exogenous origin as much as possible, the 27 controls (18 children aged 0–7 years and 9 adults) were given a special diet, with normal protein content, but no vegetables, fruits, coffee, tea, spices and beverages. The mean values and standard deviations of the above mentioned metabolites, expressed as mg/g creatinine, were as follows: total benzoic acid: 115 ± 44; total phenylacetic acid: 116 ± 60; total *p*-hydroxybenzoic acid: 19 ± 11 and *p*-hydroxyphenylacetic acid: 36 ± 23. The mean value of *p*-hydroxyphenylacetic acid equalled that of phenylacetic acid, whereas the mean value of *p*-hydroxyphenylacetic acid was nearly twice as large as that of *p*-hydroxybenzoic acid. From a total

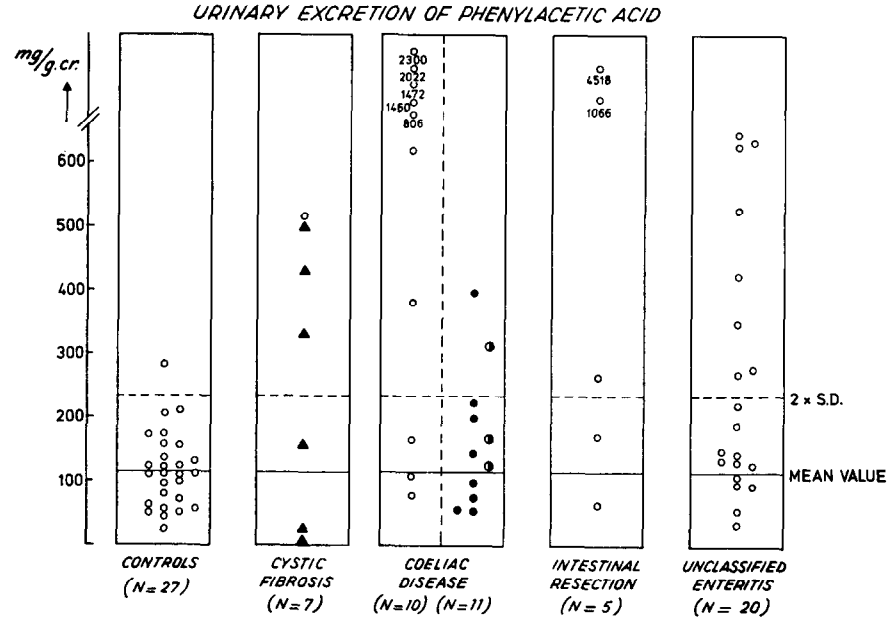

Fig. 3. Total phenylacetic acid excretion in patients with various gastrointestinal disorders: untreated (○); after treatment during a period $> \frac{1}{2}$ year (●); $< \frac{1}{2}$ year (◑); pancreatine substituted (▲).

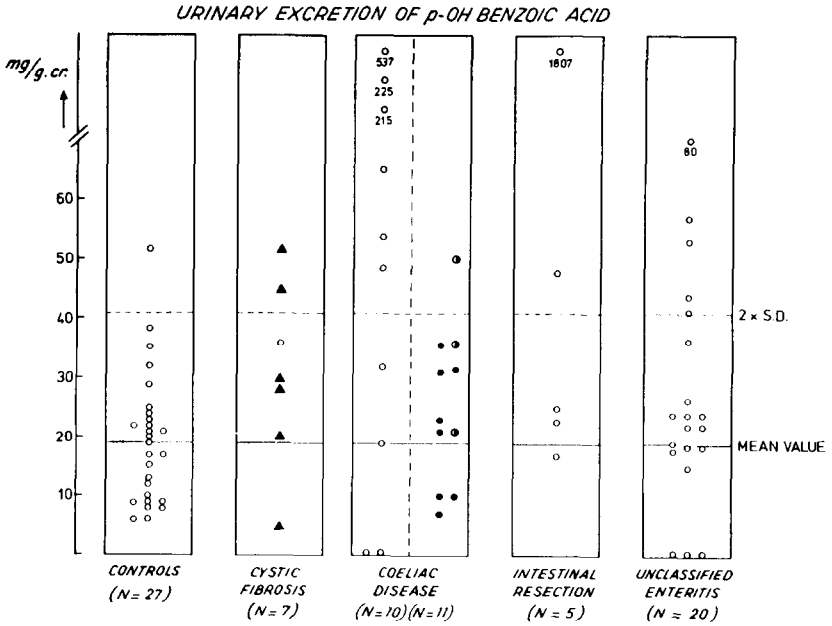

Fig. 4. Total *p*-hydroxybenzoic acid excretion in patients with various gastrointestinal disorders: untreated (○); after treatment during a period $> \frac{1}{2}$ year (●); $< \frac{1}{2}$ year (◑); pancreatine substituted (▲).

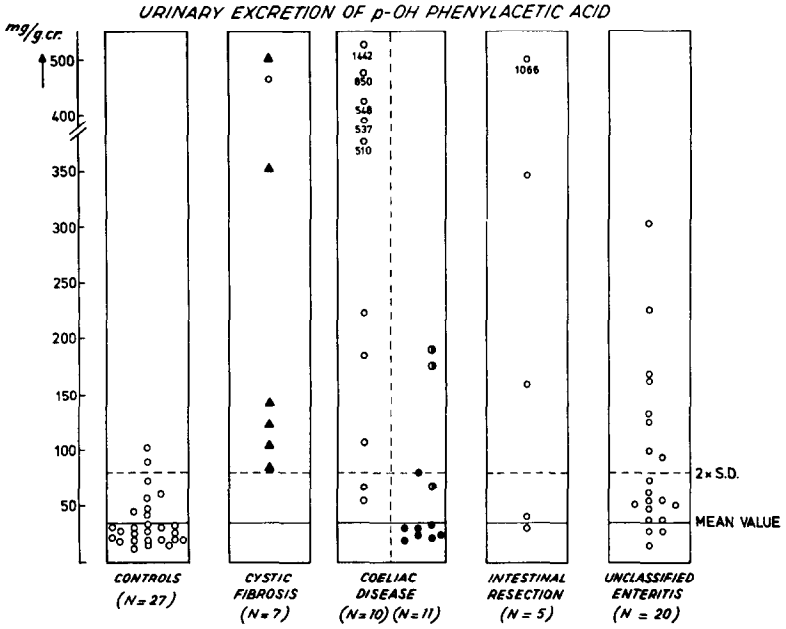

Fig. 5. Excretion of *p*-hydroxyphenylacetic acid in patients with various gastrointestinal disorders: untreated (○); after treatment during a period $> \frac{1}{2}$ year (●); $< \frac{1}{2}$ year (◑); pancreatine substituted (▲).

of 53 patients, untreated and treated, 37 showed an increased benzoic acid excretion; 24, 15 and 28 had elevated phenylacetic acid, *p*-hydroxybenzoic acid and *p*-hydroxyphenyl acid excretions respectively.

In 7 patients with cystic fibrosis, 6 receiving pancreatine substitution therapy, *p*-hydroxyphenylacetic acid was increased while in 4 out of 7 the phenylacetic acid excretion was elevated. Only in 2 out of 7 patients an increased excretion of benzoic and *p*-hydroxybenzoic acids was observed.

Within the group of patients with coeliac disease, benzoic acid was increased in 9 out of 10 untreated cases and in 6 out of 11 patients given a gluten-free diet for some time. For phenylacetic acid these numbers were 7 out of 10 and 2 out of 11, for *p*-hydroxybenzoic acid 6 and 1 and for *p*-hydroxyphenylacetic acid 8 and 2 in untreated and treated patients respectively. In untreated patients very high excretions of phenylacetic acid were found up to 2300 mg/g creatinine, even exceeding benzoic acid.

Varying quantities of these metabolites were found in 5 patients with intestinal resection. The excretion of phenylacetic acid in the urine of one patient with a subtotal intestinal resection was exceptionally high: 4518 mg/g of creatinine. In this patient the excretion of the other compounds were also excessive: 1626, 1870 and 1066 mg/g creatinine for benzoic acid, *p*-hydroxybenzoic acid and *p*-hydroxyphenylacetic acid respectively.

In patients with unclassified enteritis, phenylalanine metabolites were more frequently increased than tyrosine metabolites. In 15 out of 20 patients benzoic acid and in 8 out of 20 phenylacetic acid was abnormal, the excretory level of benzoic acid being highest.

In all patients, the intermediate metabolites of tyrosine: *p*-hydroxyphenylacrylic acid and *p*-hydroxyphenylpropionic acid were occasionally present, but only in very small amounts compared with the main metabolites. The same can be said of the corresponding phenylalanine metabolites: phenylacrylic and phenylpropionic acids.

In Table III, all 53 patients were classified according to the number of increased main metabolites of tyrosine and phenylalanine. Only 3 patients, treated for coeliac disease, were completely normal. Of the patients with most abnormal excretions (4 main metabolites increased) 3 belonged to the group of untreated coeliac patients, 1 to the intestinal resection group and 2 had unclassified enteritis. From the table it can be concluded that in the group of coeliac patients treatment resulted in an improvement of the intestinal resorption of phenylalanine and tyrosine.

TABLE III

CLASSIFICATION OF THE PATIENTS ACCORDING TO THE NUMBER OF INCREASED MAIN METABOLITES OF PHENYLALANINE AND TYROSINE

Increased = > mean + 2 S.D. of the normal population.

Main metabolites increased		*Number of patients*					
Phenylalanine	*Tyrosine*	*Cystic fibrosis*	*Coeliac disease untr.*	*tr.*	*Intestinal resection*	*Unclassified enteritis*	*Total*
2	2	–	3	–	1	2	6
1	2	1	2	–	–	–	3
2	1	2	3	–	1	2	8
1	1	1	–	2	2	2	7
2	0	–	1	1	1	3	6
0	2	1*	–	–	–	–	1
1	0	–	–	4	–	7	11
0	1	2	1	1	–	4	8
0	0	–	–	3	–	–	3

* Pancreatine substituted.

DISCUSSION

In a preceding paper[1] we described the urinary excretion pattern of phenyl- and phenolic acids in a patient with a severely impaired amino acid absorption. Very high excretions of benzoic, phenylacetic, *p*-hydroxybenzoic and *p*-hydroxyphenylacetic acids were found. These metabolites probably originate from bacterial degradation of phenylalanine and tyrosine in the intestinal lumen. In order to confirm our concepts, we examined the excretion of increased amounts of the above mentioned main tyrosine and phenylalanine metabolites in various patients with gastrointestinal disorders. As expected, the excretory abnormalities appeared to be present in many of the patients investigated. A matter of importance is the definition of the normal ranges. We considered it necessary to establish firmly the variation of the excretions in a large group of healthy individuals. Data given by other authors and obtained by various methods, are widely divergent. In our control group benzoic acid amounted to 115 ± 44 mg/g creatinine. This is much lower than the date of Stein *et al.*[2]: 680–1700 mg/24 h, of Williams *et al.*[3]: 450 mg/g creatinine with a range of 218–506 and of Hoffman[4]: 340 mg/g creatinine. The range found by Sunderman *et al.*[5] was 68–680 mg/24 h. All the values mentioned were calculated from those given for hippuric acid.

In our controls we found 116 ± 60 mg/g creatinine for the excretion of phenyl-

acetic acid, which is also lower than found by Stein[2]: 129–258 mg/24 h (calculated from phenylacetylglutamine) but higher than found by Vavich *et al.*[6]: 60 ± 20 mg/g creatinine.

For *p*-hydroxybenzoic acid values found in a normal population are not available in the literature. In our control group 19 ± 11 mg/g creatinine was found.

For the excretion of *p*-hydroxyphenylacetic acid we found 36 ± 23 mg/g of creatinine in accordance with Thompsett[7]: 15–31 mg/24 h, Williams[3]: 19 mg/g creatinine with a range of 8–42, Ruge[8]: 24.5 mg/g creatinine, range 6.3–46.0, and Horning *et al.*[9]: 31.3 mg/24 h.

In the literature benzoic acid has hardly been correlated with bacterial metabolism of phenylalanine in the intestinal lumen. Only Young[10] pointed to such a relation.

In normals, benzoic acid may arise from an excess of dietary protein, phenylalanine and phenylalanine derived from proteins of (a) gastrointestinal secretory fluid (b) sloughed gastrointestinal mucosal cells or (c) intestinal bacteria undergoing lysis. Possibly, benzoic acid can also be formed by the intestinal bacteria from phenylalanine transported from the serosal to the mucosal side of the intestine and finally secreted into the intestinal lumen[11]. The same can be said of the origin of phenylacetic acid.

In the patients benzoic acid was elevated more frequently than phenylacetic acid but the excretory levels of the latter were higher than those of the former. In the treated coeliac patients significantly lower excretions of phenylacetic acid were found than in the untreated ones. However, this difference was less pronounced for benzoic acid. Also, a striking difference between the excretory level of *p*-hydroxyphenylacetic acid in treated and untreated coeliac patients existed. The same can be said of *p*-hydroxybenzoic acid. In all untreated coeliacs a more or less severe steatorrhea was present, whereas in the treated ones the steatorrhea was greatly improved or normalized. Because an increased *p*-hydroxyphenylacetic was also found by Boscott and Cooke[12] in patients with idiopathic steatorrhea, there may be a close relation between the occurrence of steatorrhea and the urinary excretion of abnormally high amounts of bacterial amino acid metabolites. Such a relation has already been demonstrated for urinary indican, a well-known bacterial metabolite of tryptophan[13].

All 7 patients with cystic fibrosis showed an increased *p*-hydroxyphenylacetic acid excretion, despite pancreatine therapy. In this disease *p*-hydroxyphenylacetic acid has been found increased by several authors[14–16]. With respect to the bacterial origin of *p*-hydroxyphenylacetic acid in cystic fibrosis of the pancreas Gjessing[15] demonstrated that this compound decreased in the urine, when neomycin was administered.

Contrary to *p*-hydroxyphenylacetic acid in most patients the *p*-hydroxybenzoic acid was not strikingly abnormal for both excretory level and frequency. This compound has been considered to be a product of baterial degradation of benzenoid structures different from phenylalanine and tyrosine[17]. Shaw[18] described a high urinary excretion of this compound after ingestion of coffee or bananas. In our patients the increased *p*-hydroxybenzoic acid excretion could not be explained by such a dietary source, because urine collection was started 3 days after a coffee- and fruit-free diet. Booth and coll.[19] found *p*-hydroxybenzoic acid as an ethereal sulfate in urines of rats. Contrary to our theory, they apparently believed that its formation does not depend on intestinal micro-organisms.

Convincing evidence for our concepts was obtained from the urinary analysis of a patient with a sub-total intestinal resection. Very high values were found for benzoic acid: 1626 mg/g creatinine, phenylacetic acid: 4518 mg/g creatinine, *p*-hydroxybenzoic acid: 1807 mg/g creatinine and *p*-hydroxyphenylacetic acid: 1066 mg/g creatinine. In this patient the excretion of *p*-hydroxybenzoic acid exceeded that of *p*-hydroxyphenylacetic acid. In most patients *p*-hydroxyphenylacetic acid excretion surpassed that of *p*-hydroxybenzoic acid. Possibly, the type of the excretion pattern is determined by the bacterial flora, present in the patient's intestinal lumen.

The pathways of bacterial amino acid catabolism were already discussed in a preceding paper[1]. They were deduced from the excretion pattern of the patient described, characterized by the occurrence of minor intermediate metabolites as *p*-hydroxyphenylpropionic, *p*-hydroxyphenylacrylic and phenylacrylic acids. These minor metabolites occurred only occasionally in the patients here described.

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