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INCREASED URINARY IMIDAZOLEPROPIONIC ACID, *N*-ACETYLHISTAMINE AND OTHER IMIDAZOLE COMPOUNDS IN PATIENTS WITH INTESTINAL DISORDERS

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SUMMARY

In 26 out of a large group of patients with gastrointestinal disorders abnormal urinary imidazole excretion patterns were found. Most frequently excessive or increased amounts of imidazolepropionic acid (ImPA) occurred, and as next *N*-acetylhistamine was excreted in excess. In a number of cases the latter was accompanied by a substance identified as *N*-propionylhistamine. It is suggested that these excretory products are bacterial metabolites of histidine, if not absorbed in the intestinal lumen.

All 26 patients excreted increased amounts of bacterial metabolites of tyrosine and/or phenylalanine as well: *p*-OH-phenylacetic and/or *p*-OH-benzoic acids and phenylacetic and/or benzoic acids respectively.

Many patients showed increased urinary 4-amino-5-imidazolecarboxamide, its riboside and an unknown related compound X, especially in a later (recovery) phase when imidazolepropionic acid and *N*-acetylhistamine already decreased. It is thought that these metabolites are not of bacterial origin.

INTRODUCTION

We have reported on urinary waste metabolites of tyrosine and phenylalanine in patients with malabsorptive diseases[1,2]. Arguments were given for the formation of these metabolites by the intestinal bacteria from unabsorbed amino acids. These findings prompted us to investigate whether in the urine of our patients with chemical symptoms of tyrosine and phenylalanine malabsorption[2], abnormal amounts of bacterial histidine metabolites were present. Therefore we analysed our patients for urinary imidazoles by two-dimensional paper chromatography, as has been done before for the detection of histidinaemia[3]. Urinary analyses of imidazoles in a large group of mainly mentally retarded or neurologically disturbed patients served as controls. In the malabsorptive group of patients some showed a strikingly elevated concentration of imidazolepropionic acid (ImPA). In normals this compound is either absent or is

excreted only in amounts as small as 1 mg/24 h[4]. In a limited number of patients a compound identified as *N*-acetylhistamine (NAH) was discovered. *N*-acetylhistamine was often accompanied by a small amount of a substance with the paper chromatographic properties of *N*-propionylhistamine (NPH). Sporadically a trace of a third diazo-positive substance in the position of *N*-butyrylhistamine (NBH) was seen.

Apart from the above mentioned compounds paper chromatograms showed increased concentrations of 4-amino-5-imidazolecarboxamide (AICA) together with a smaller amount of a substance chromatographically corresponding to its riboside (AICAR) and with a still smaller quantity of an unidentified related compound (X). Elevated excretions of AICA were found by other authors in connection with folate and/or vitamin B_{12} deficiency[5-7] and with disturbed purine metabolism in patients with the Lesch–Nyhan syndrome[8]. As far as we know increased urinary excretion of these substances has not been directly related to intestinal disease before.

When paper chromatography revealed obviously elevated concentrations of ImPA (above 36 μmoles (1 mg)/l, quantitative column chromatographic determination was performed by the method described previously[9]. Also NAH, when obviously increased, was determined quantitatively. The other compounds were estimated by visual inspection of the two-dimensional chromatograms.

In this paper excretory values for these imidazole metabolites are given; their relationship is discussed. Their occurrence has also been correlated with increased concentrations of bacterial tyrosine and phenylalanine metabolites. Evidence is given for the bacterial origin of ImPA, NAH and NPH.

MATERIALS AND METHODS

Materials

ImPA and NAH (Calbiochem); AICA·HCl, AICAR (Sigma); AICA-Ribotide = AICARP (Boehringer, Mannheim).

N-propionylhistamine (NPH) was prepared by heating 5 mmoles of histamine and 5 mmoles of propionic acid anhydride for 3 h at 130°. Then 2 ml ethyl alcohol were added followed by 8 ml ethyl ether. After crystallization at −20° in a refrigerator, NPH was recrystallized from a large volume of benzene. Melting point NPH: 124.4–124.8°.

A. Procedures for qualitative analyses

(1) Urinary imidazoles. For analysis of urinary imidazoles by two-dimensional paper chromatography the method described elsewhere was used[3]. For this, imidazoles were isolated from the urine by a Dowex-50 procedure, yielding a ten-fold concentrated aqueous solution of the imidazoles; 0.05 ml corresponding to 0.5 ml urine was applied on the paper chromatogram. Paper chromatography: first solvent was isopropanol–ammonia 5% (4:1, v/v), second solvent *n*-butanol–acetic acid–water (4:1:1, v/v/v). Detection: diazotized sulfanilic acid in 10% aqueous Na_2CO_3 solution. Colour development: ImPA, NAH and NPH: red, AICA: blue, AICAR: grey-purple, AICARP (not identical with X): purple. See Fig. 1, 1a, b

(2) Faecal imidazoles. Faecal imidazoles were isolated by means of a modified acetone extraction procedure as described by Seakins *et al.*[10] for amino acids: 10 g of faeces were homogenized (magnetic stirring) in 50 ml acetone–water (1:1, v/v).

Then 100 ml of acetone were added and stirring was continued for 5 min. After filtration and washing the filter with another 150 ml acetone, the filtrate was evaporated to dryness in a vacuum rotator. The residue was dissolved in 25 ml water. This solution was treated as described for urine. An abnormal and a normal excretory pattern can be seen in Fig. 1c and 1d respectively.

B. Procedures for quantitative analysis

(1) Urinary imidazoles. Quantitative determination of urinary imidazoles was done by automated column chromatography as described earlier[9]. ImPA coincides with carnosine, therefore ImPA was estimated after acid hydrolysis. When NAH was clearly present on the paper chromatogram, a double column chromatographic analysis was performed: first without hydrolysis for the determination of NAH and then, after acid hydrolysis, for the determination of ImPA. Hydrolysis was done in 6 *N* HCl at 100° for 24 h. Alkaline hydrolysis cannot be used because of losses. After hydrolysis HCl was evaporated under vacuum at 40° in a Rotovapor. The residue, obtained from 2 ml urine, was dissolved in 5 ml water. The urine as well as the hydrolysate were extracted twice with ethyl acetate to remove interfering phenolic substances. An amount corresponding to 0.2 ml urine was applied to the column. Gradient elution was performed as described in[9].

(2) Faecal imidazoles. In the aqueous solution, obtained after acetone extraction of faeces as described under A (2), imidazoles were determined according to B (2) without hydrolysis.

(3) Urinary and faecal phenyl and phenolic acids. Methods as described before[1,2] were used.

Retention time values and recoveries

As described earlier[9], for ImPA a RT value of 1.16 relative to histidine was found. A mean recovery of 102% resulted; range 96–106; $n = 5$.

NAH is eluted later than ImPA from the column: RT value, relative to histidine 1.32. Recovery of NAH: mean value 98%, range 91–101%, $n = 5$.

NPH decomposed on the column probably due to the high temperature in combination with the low pH of the first buffer. Because in the patients examined NPH was increased only in a few cases, no attemps were made for a quantitative determination of this substance. A rough estimation of NPH was done by visual comparison of the spot on the paper chromatogram with standards.

Also AICA and its derivatives were not registered due to a rapid decomposition of their diazo-coupling products, caused by the high Na_2CO_3 concentration used. Visual evaluation of quickly fading spots on the paper chromatogram was performed (+, ++, +++).

Identification of urinary NPH and NAH

NAH and NPH were isolated from a number of two-dimensional paper chromatograms by cutting out the respective spots and eluting these cuttings with ethanol. After evaporation of the ethanol, the residue was acid hydrolysed. The formation of free histamine could be demonstrated by two-dimensional paper chromatography. Gas chromatography of extracted organic acids proved the presence of acetic and propionic acid respectively.

RESULTS

A. Evidence for the intestinal origin of ImPA and NAH

Patient E. v. G. with severely impaired intestinal amino acid absorption described previously[1] exhibited a strikingly increased urinary ImPA (see Fig. 1a and 1b). In the faeces of the same day ImPA was also high (see Fig. 1c and 1d). This is considered to plead for the intestinal origin of ImPA.

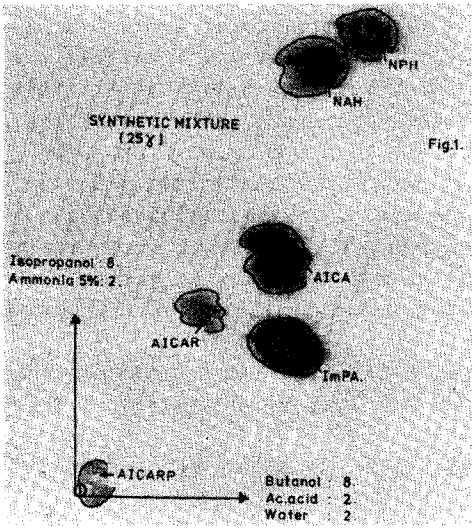

Fig. 1. Positions of imidazolepropionic acid (ImPA), *N*-acetylhistamine (NAH), *N*-propionyl histamine (NPH), 4-amino-5-imidazolecarboxamide (AICA), its riboside (AICAR) and its ribotide (AICARP) on the paper chromatogram.

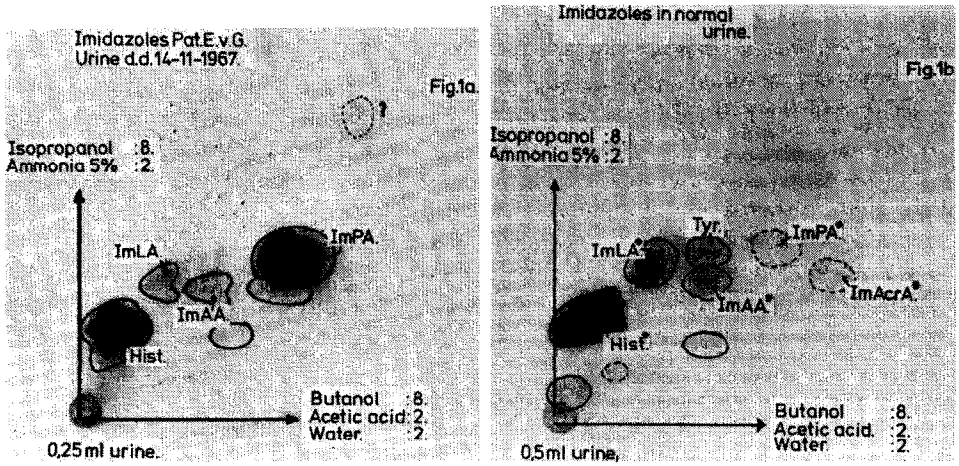

Fig. 1a. Urinary imidazoles in patient E. v. G. Large amount of imidazolepropionic acid (ImPA); normal amounts of imidazolelactic (ImLA) and acetic (ImAA) acids.

Fig. 1b. Normal excretory pattern of imidazoles. Normal amounts of imidazolelactic (ImLA) and acetic (ImAA) acids; only trace amounts of imidazolepropionic (ImPA) and acrylic (ImAcrA) acids.

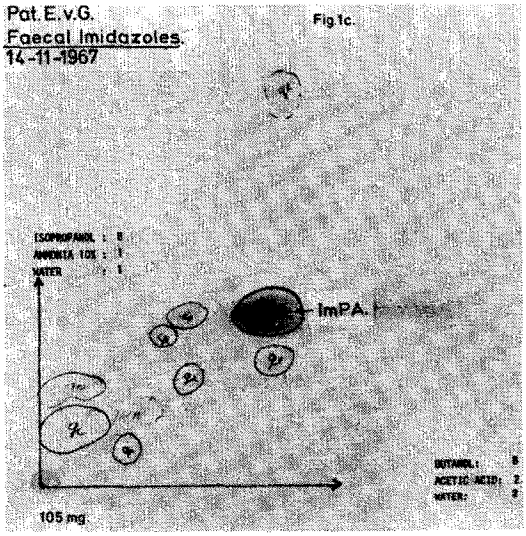

Fig. 1c. Faecal imidazoles in patient E. v. G. Strongly increased amount of imidazolepropionic acid (ImPA).

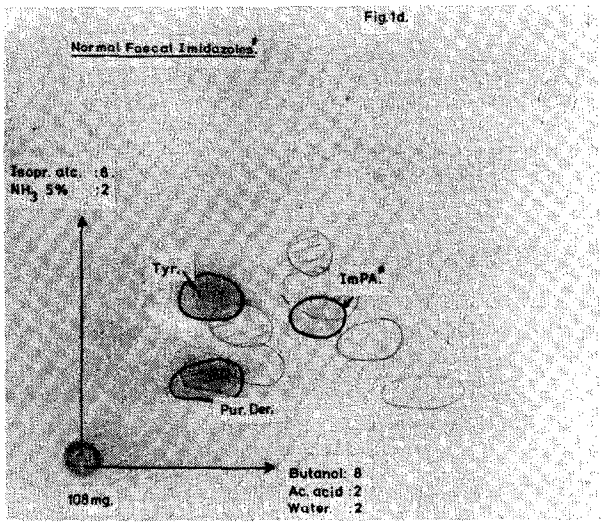

Fig. 1d. Imidazoles in normal faeces. Only a trace of imidazolepropionic acid (ImPA) is present.

The excretion of ImPA corresponded well with the degree of tyrosine and phenylalanine malabsorption described before[1], as can be seen from Table I. This is another argument for the intestinal origin of ImPA. In Fig. 2 the excretory levels of ImPA and the sum of bacterial phenylalanine and tyrosine metabolites were plotted. Urinary ImPA roughly correlated with the aromatic acids. Only three faecal analyses were performed. When urinary ImPA, phenylalanine and tyrosine metabolites were most elevated, also faecal values were found to be high.

The highest excretions of ImPA and tyrosine and phenylalanine metabolites

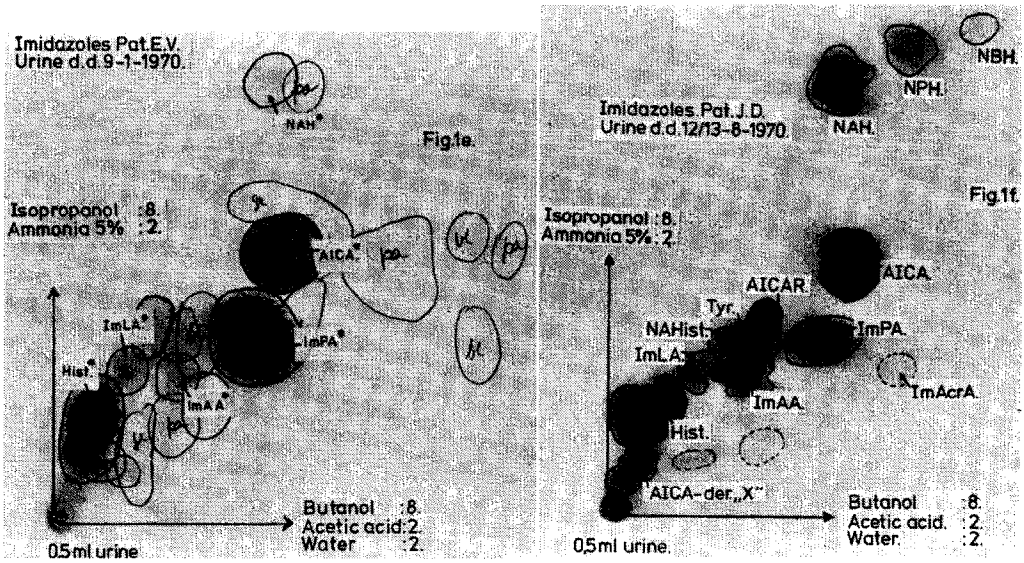

Fig. 1e. Urinary imidazoles in patient E. V. An excessive amount of imidazolepropionic acid (ImPA); small amounts of *N*-acetylhistamine (NAH) and 4-amino-5-imidazolecarboxamide (AICA); normal amounts of imidazolelactic (ImLA) and acetic (ImAA) acids. AICA was pencil-coloured because of rapid fading of its diazo-coupling product.

Fig. 1f. Urinary imidazoles in patient J. D. Large amounts of imidazolepropionic acid (ImPA) and *N*-acetylhistamine (NAH); increased amounts of *N*-propionylhistamine (NPH) and 4-amino-5-imidazolecarboxamide (AICA), together with its riboside (AICAR) and an unknown related compound (AICA-der. X); normal amounts of imidazolelactic (ImLA) and acetic (ImAA) acids; trace amounts of "*N*-butyrylhistamine" (NBH), imidazoleacrylic acid (ImAcrA) and *N*-acetylhistidine (NAHist.) The spots of AICA and AICAR were pencil-coloured because of rapid fading of their diazo-coupling products.

were found in periods of grave clinical condition (13/14.11.1967 and 28/29.12.1967) while all excretions were much lower when the patient improved clinically (9.4.1970).

Elevated excretions of ImPA were also found in other patients with gastrointestinal disorders, listed in Table II. An excessive value of 3882 μmoles (544 mg)/g creatinine was observed in E.V., a girl with coeliac disease. In the mentally retarded twins A.V. and M.V., who were treated for iron deficiency, both with increased urinary ImPA, gastrointestinal disease was not clinically manifest, although some bacterial tyrosine and phenylalanine metabolites were elevated in their urines.

Clinical improvement of the patients resulted in a significant decrease of urinary ImPA, together with urinary phenylalanine and tyrosine metabolites, which is in accordance with our assumption of the intestinal origin of these compounds.

As can be seen from Table II, urinary *N*-acetylhistamine was clearly present in 16 out of the 25 patients. In patient L.E. a value of 1626 μmoles (249 mg)/g creatinine was found. The highest excretory levels of NAH occurred in patients with coeliac disease and in those with an intestinal resection. Generally the excretion of NAH was not parallel to the excretion of ImPA, although in 10 cases both these compounds were increased. Six patients had increased NAH only. Clinical improvement resulted in the disappearance of NAH. This compound was found only in the urine of patients with gastrointestinal disorders.

TABLE I

COINCIDENCE OF ABNORMAL EXCRETIONS OF IMIDAZOLEPROPIONIC ACID, BACTERIAL METABOLITES OF TYROSINE AND OF PHENYLALANINE IN THE URINE OF PATIENT E. v. G.

Also the occurrence of AICA, AICAR and related unknown substance X is tabulated.

Date of sampling	*μmole/g cr.*		*mmole/g cr.*				*AICA*	*AICAR*	*X*
	ImPA	*NAH*	*BA*	*PAA*	*p-OHBA*	*p-OHPAA*			
6/7.8.1967	264	13*	1.84	10.81	3.89	3.35	+	+	np
27/28.9.1967	614	13*	4.94	10.47	1.33	9.87	+	+	np
14/15.10.1967	742	26*	3.50	20.46	7.84	10.96	p	np	np
16/17.10.1967	564	np	1.20	16.12	9.25	6.96	p	p	np
13/14.11.1967	2676	np	2.87	50.60	18.29	5.40	np	np	np
28/29.12.1967	2462	np	59.55	66.31	0.68	5.51	3+	+	+
9.4.1967	171	np	0.78	2.42	0.15	0.78	np	np	np
Upper normal limits	<36	np	1.66	1.73	0.30	0.54	np	np	np

* = estimated by visual inspection of paper chromatograms.
p = present on paper chromatogram.
np = not present on paper chromatogram.
+ = increased as estimated by visual inspection of paper chromatograms.
ImPA = imidazolepropionic acid
p-OHPAA = *p*-hydroxyphenylacetic acid
p-OHBA = *p*-hydroxybenzoic acid
PAA = phenylacetic acid
BA = benzoic acid
AICA = 4-amino-5-imidazolecarboxamide
AICAR = 4-amino-5-imidazolecarboxamide riboside

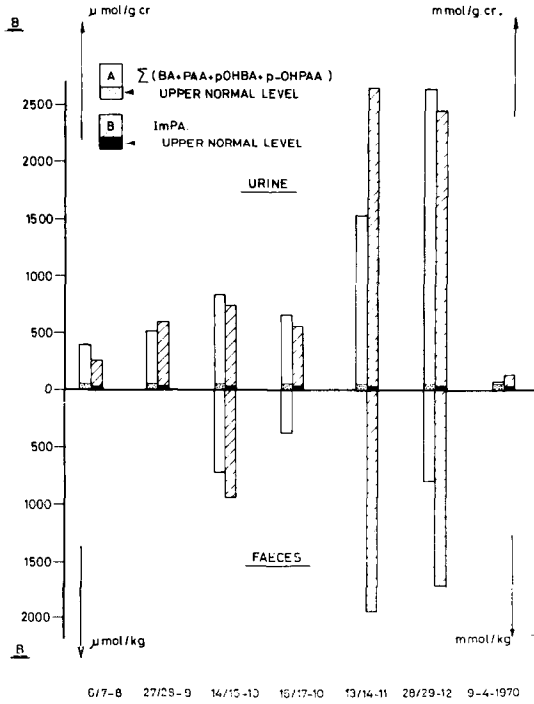

Fig. 2. Excretory levels of ImPA and bacterial aromatic acids, derived from phenylalanine and tyrosine (BA+PAA+*p*-OHBA+*p*-OHPAA) in urine and faeces of patient E. v. G.

TABLE II

EXCRETORY LEVELS OF IMIDAZOLEPROPIONIC ACID, *N*-ACETYLHISTAMINE, *N*-PROPIONYLHISTAMINE, MAIN METABOLITES OF PHENYLALANINE AND TYROSINE IN URINES OF PATIENTS WITH GASTROINTESTINAL DISORDERS

The occurrence of AICA, AICAR and an unknown related substance X as estimated by visual inspection of paper chromatograms is tabulated.

Patients	*Sex*	*Age*	*Date of urine sampling*	*μmole/g creatinine*			*AICA*	*AICAR*	*X*	*mmole/g creatinine*			
				ImPA	*NAH*	*NPH*				*BA*	*p-AA*	*p-OHBA*	*p-OHPAA*
L.P.	f	11 m	11/12.2.1970	29*	157	np	+	p	p	0.98	1.23	—	3.60
coeliac dis.			26/27.2.1970	43*	255	np	++	p	p	1.45	5.27	0.30	4.06
E.V.	f	1j5m	9.1.1970	3882	13*	np	+	np	np	6.14	16.89	0.83	5.59
coeliac dis.			2.2.1970	150	np	np	++	p	p	2.41	3.16	0.20	0.37
			3.2.1970	260	np	np	++	p	p	3.44	5.73	0.27	0.48
			8.2.1970	114	np	np	+++	p	—	2.95	4.28	0.32	0.58
J.D.	m	1j1m	12/13.8.1970	350	372	126*	++	p	p	5.13	14.85	0.51	0.45
coeliac dis.			6/7.10.1970	21*	np	np	p	np	np	2.19	5.31	0.35	0.45
L.E.	f	1j2m	15/16.9.1970	29	353	30*	++	p	p	3.79	0.58	0.35	3.53
coeliac dis.			22.9.1970	36*	1626	np	+	p	p	—			
I.G.	f	1j5m	24.6.1970	300	np	np	np	np	np	1.98	2.80	—	1.47
coeliac dis.			16/17.10.1970	100*	46*	p	p	np	np	0.66	0.85	0.30	0.43
			18/19.10.1970	257*	72*	p	np	np	np	1.79	3.75	0.62	0.78
T. M.	f	1j3m	8.9.1970	221	13*	np	++	p	p	2.37	0.79	0.39	1.22
coeliac dis.													
J.S.	f	4j7m	15/16.10.1970	75	np	np	+	p	p	3.30	5.46	0.57	1.08
coeliac dis.													
J.v.D.	m	2j2m	25.6.1968	278	98	p	p	np	np	1.95	3.78	0.14	2.32
cystic fibrosis													
P.vd E.	m	6j3m	28.1.1971	43*	59*	p	np	np	np	2.30	2.97	0.56	2.62
cystic fibrosis													
L.S.	m	10j3m	16/17.10.1970	157	np	np	p	np	np	0.78	1.20	0.22	0.56
cystic fibrosis													
B.A.	m	8m	23/24.12.1970	271	13*	np	+	np	np	0.51	1.10	0.13	1.11
gastroint. sympt.													
A.v.G.	f	10j	16/17.9.1970	157	np	np	+	p	p	3.34	4.61	0.16	0.26
gastroint. sympt.													
A.J.K.	m	13j4m	25.3.1970	164*	np	np	+	p	np	2.15	3.87	0.13	0.87
gastroint. sympt.													

M.M. gastroint. sympt.	f	7m	24.4.1970	364	189	54	+	np	np	0.92	1.67	0.30	1.99
G.W. gastroint. sympt.	m	2j11m	10/11.11.1969	143	np	np	p	np	np	7.53	0.68	0.26	0.18
H.T.d.M. gastroint. sympt.	m	3m	1/2.3.1970	237	21	p	++	+	np	1.54	3.80	0.25	3.10
			22.3.1970	295	165	p	p	np	np	2.66	6.38	0.01	0.53
			22/23.3.1970	149	40	p	np	np	np	2.02	2.50	0.01	0.18
			8/9.7.1970	80	np	np	np	np	np	2.75	2.62	0.41	0.57
B. v.d. V. gastroint. sympt.	m	7m	26.1.1971	257	7*	np	p	np	np	2.45	1.42	0.32	1.37
A.B. gastroint. sympt.	m	9m	16.3.1970	236*	np	np	np	np	np	1.51	1.70	0.17	0.82
B.B. int. resection	m	2j3m	1/2.9.1970	14*	287	np	p	np	np	1.72	0.43	0.35	0.27
K.v.I. int. resection	m	ad.	23.9.1970	7*	52	p	p	np	np	3.68	1.92	0.12	0.20
V.	m	ad.	18.9.1970	21*	46	p	np	np	np	3.13	1.27	0.18	2.28
M. O.-B. int. resection	f	ad.	29.8.1969	7*	7*	np	np	np	np	3.49	7.83	0.17	1.05
			13/14.1.1970	14	86	30	p	np	np	7.42	5.09	0.50	0.76
A.J. mental ret.	f	9m	5.1.1970	264	np	np	p	np	np	3.01	4.66	0.41	1.06
A. v.d. V. mental ret.	m	1j3m	5.2.1970	1527	np	np	np	np	np	7.14	1.79	0.74	1.43
M. v.d. V. mental ret.	m	1j3m	5.2.1970	221	np	np	np	np	np	3.42	0.51	0.17	0.26
Upper normal limits				<36	np	np	p	np	np	1.66	1.73	0.30	0.54

* = estimated by visual inspection of paper chromatograms.
p = present on paper chromatogram.
np = not present on paper chromatogram.
+ = increased as estimated by visual inspection of paper chromatograms.
NPH = *N*-propionylhistamine
Other abbreviations see note to Table I.

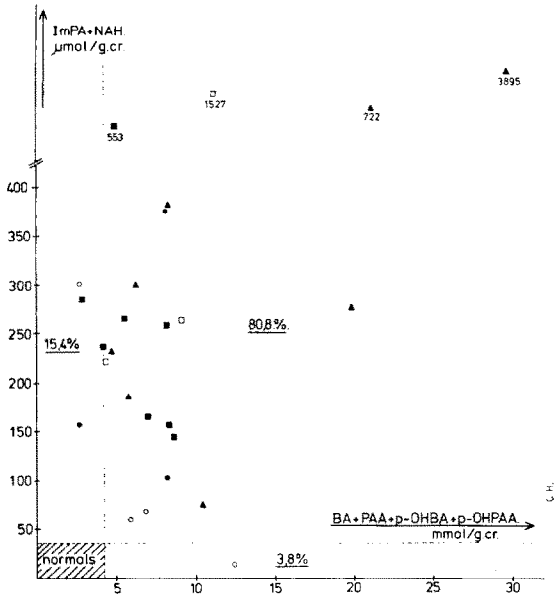

Fig. 3. Coincidence of increased amounts of the imidazole compounds (ImPA + NAH) and the bacterial aromatic acids, derived from phenylalanine and tyrosine (BA + PAA + *p*-OHBA + *p*-OHPAA) in urines of patients with gastrointestinal disorders (▲: coeliac disease; ●: cystic fibrosis; ○: intestinal resection; ■: gastrointestinal symptoms; □: mental retardation).

N-Propionylhistamine only occurred when NAH was present, the excretory level always being much lower than that of NAH. The third substance, *N*-butyrylhistamine possibly only occurred in 2 out of the 25 patients in trace amounts. From the above mentioned data, it seems likely that NAH and NPH are also bacterial metabolites. As urinary imidazole analysis was retrospective, no faeces of high excretion for direct analysis of NAH and NPH was available. Only in the faeces of E.v.G. (14/15.10) (whose urine contained a trace of NAH), the presence of a very small amount of NAH could be demonstrated by paper chromatography.

B. Occurrence of AICA and accompanying substances in relation to ImPA and NAH

These substances were not present in all patients listed in Table II. Especially in the patients with coeliac disease (except I.G.) concentrations of AICA, as estimated by visual inspection of the chromatograms, were at times strikingly elevated. The same could be observed in only one other patient, T.M. with gastrointestinal symptoms of unknown cause.

If present, AICA always was the predominant excretory product. AICAR and the related unknown substance (X) never appeared without AICA. The excretion of AICA, AICAR and X seemed to follow an other course than that of ImPA and NAH and NPH. In E.v.G. at maximal ImPA, high concentrations were seen on 28/29.12 whereas on 13/14.11 the substances were absent. In E.V. at maximal ImPA on 9.1.1970, AICA was lower than later on, when ImPA was moderately increased. In L.E. the maximal excretion of AICA did not coincide with a maximal excretion of ImPA and NAH.

Excretion of urinary ImPA and NAH in relation to bacterial metabolites of tyrosine and phenylalanine

In order to test the coincidence of abnormal excretions of ImPA, NAH with increased bacterial tyrosine and phenylalanine metabolites, all first analysed urine samples of the 25 patients in Table II and also of E.v.G. were considered. In Fig. 3, the sum of ImPA and NAH was plotted against the sum of benzoic acid+phenylacetic acid+*p*-hydroxybenzoic acid+*p*-hydroxyphenylacetic acid (BA+PAA+*p*-OHBA+*p*-OHPAA). It can be seen that 21 out of the 26 patients (80.8%) fell in the abnormal ranges; 4 patients (B.B., L.S., B.A., A.B.) had increased ImPA+NAH but normal aromatic acids and in 1 patient (M.O.-B.) with increased aromatic acids ImPA was normal, NAH, however, was present (about 7 μmoles (1.1 mg)/g creatinine).

The upper normal values were defined as follows. For ImPA the level of 5 mg or 36 μmoles/g creatinine was based on visual estimations of this compound on paper chromatograms in a large series of patients without gastrointestinal disorders; NAH is normally not present on paper chromatograms. For the aromatic acids the maximal excretions calculated from the normals given before[2] were used.

In all the 26 patients with abnormal ImPA and/or NAH excretions, at least one parameter BA+PAA or *p*-OHBA+*p*-OHPAA was abnormal, indicating the occurrence of bacterial phenylalanine and/or tyrosine metabolism in the intestine of these patients together with bacterial histidine metabolism.

DISCUSSION

Up till now the bacterial origin of ImPA in human urine has not been established clearly. Auerbach *et al.*[11] found a small amount of ImPA in the urine of a patient with histidinaemia after intravenous administration of urocanic acid. This suggests, but does not prove, that its endogenous formation is possible. Other investigators who found ImPA in the urine of patient after oral loading with histidine[12] or with urocanic acid[13] also supposed that it is formed endogenously. The same opinion, based on the results from loading experiments in rats, was given by others[4,14].

Although its endogenous formation may well be possible, we suggest that urinary ImPA can also originate from bacterial histidine metabolism in the gut. For many years it has been known that bacteria can produce ImPA from histidine[15,16]. We demonstrated its presence in the faeces of patients E.v.G. and the obvious relation between the excessive urinary excretion of ImPA and bacterial metabolites of aromatic amino acids in our patients with gastrointestinal disorders. In our patients with excessive ImPA excretion urinary urocanic acid was not elevated or it was even absent, quite different from the results in the loading experiments cited above.

Moreover, in one of our histidinaemic patients, J.v.H.[9], incapable to form urocanic acid endogenously due to histidine ammonia-lyase (E.C. 4.3.1.3) deficiency ImPA could be detected in relatively large amounts in the faeces.

Another urinary imidazole derivative, found in excess in some of our patients, is *N*-acetylhistamine. *N*-Acetylhistamine has been described as a normal urinary constituent[17–23], but the normal excretory level is very low, less than 1 mg/24 h, depending on dietary factors. Ingestion of large amounts of meat results in a significant increase[19,24]. *N*-Acetylhistamine was found to be a metabolically inert compound, which after subcutaneous administration in dogs can be recovered almost

quantitatively from the urine[24]. Many speculations were made about its origin. Our investigations in patients with gastrointestinal disorders obviously suggest that either its precursor histamine or *N*-acetylhistamine itself is a product of bacterial activity in the intestinal tract. Both suggestions are sustained by observations of several authors. Orally administered histamine resulted in a significantly increased excretion of *N*-acetylhistamine[19,25,26], whereas histamine, administered by slow intravenous infusion, resulted in urinary excretion of mainly free histamine[25]. Moreover, Sjaastad[26] demonstrated that histamine, administered by injection in several parts of the intestinal tract, resulted in a significant increase of urinary *N*-acetylhistamine, whereas the urinary excretion of free histamine hardly altered at all. In addition he found that the urinary excretion of *N*-acetylhistamine significantly decreased when humans were loaded orally with histamine after administration of phthalylsulphathiazole as antibacterial therapy during 5 days. The results of these experiments point to a direct formation of *N*-acetylhistamine by the intestinal bacteria, as already suggested by Urbach[27]. This theory was confirmed by the results of experiments, in which faecal specimens of patients with Hartnup disease were incubated with L-histidine. This resulted in the formation of large amounts of free histamine, but also of small quantities of conjugated (not specified) histamine[28].

It is not likely that the large amount of histamine, precursor of the urinary *N*-acetylhistamine in our patients, was formed endogenously. Our patients did not show the typical symptoms of histamine overproduction. On the contrary, histamine could well have been formed *via* decarboxylation of histidine by intestinal bacteria[29-33]. The site of the acetylation of histamine is still open to speculation. The following possibilities may be considered: the intestinal bacteria[26-28], the intestinal wall, which has been shown to contain arylamine acetyltransferase (E.C. 2.3.1.5)[34], the liver and other organs[34].

It seems plausible to consider the propionylation of histamine as a process parallel to acetylation, but proceeding at a lower level. Other acylations may occur as minor reactions. The above mentioned possibilities for the site of acetylation may also hold for the formation of the other acylated histamines.

There seems to be no correlation between urinary *N*-acetylhistidine and *N*-acetylhistamine, the former being elevated in histidinaemia, the latter only in patients with gastrointestinal disorders.

About the origin of IACA and related compounds we can only speculate. The excretion of AICA is not parallel to ImPA and NAH, suggesting that AICA is not of bacterial origin. An increase of its excretion was seen when ImPA and NAH already diminished, which might indicate a relationship with recovery of the intestinal mucosa. Increased endogenous production and urinary excretion of IACA is concluded to be connected with a deranged purine synthesis, caused by endogenous folate antagonists as amethopterine[5,35], vitamin B_{12} deficiency and folate deficiency[5-7,36] and in the Lesch–Nyhan syndrome[8]. In rats, however, McGeer *et al.*[37] demonstrated that a high excretion of AICA is found when folic acid and vitamin B_{12} deficiencies are present, but that the high AICA excretion is not associated with any block in AICA conversion and therefore in purine biosynthesis. An increased retention of intraperitoneally administered [2-^{14}C]AICA in the vitamin B_{12}- and folic acid-deficient group was found, compatible with an enlarged body pool of AICA derivatives, resulting from a feedback breakdown of purine nucleotides. Such a process has been shown to

occur in bacteria[38] and in mammalian tissue *in vitro*[39]. In coeliac patients, however, a strongly accelerated renewal of intestinal mucosa cells during the recovery phase is present[40], implicating a strongly increased *de novo* purine synthesis. In such conditions a relative or local shortage of folate or vitamin B_{12} may result in impairment of the purine synthesis at the level of folate–AICA transformylase.

In fact, low blood levels of folate are frequent in patients with coeliac disease[41]. At a [^{57}Co]vitamin B_{12} plasma level absorption test Armstrong and coll.[42] also showed that one out of 5 patients with coeliac disease in remission and 2 out of 2 patients having ileac resections, demonstrated malabsorption of vitamin B_{12}, which could not be corrected by intrinsic factor.

We believe to have collected sufficient data in order to justify the conclusion that strongly increased urinary ImPA and/or NAH levels indicate bacterial histidine metabolism in the intestine. Under these conditions also excessive urinary bacterial tyrosine and/or phenylalanine metabolites can be expected and the presence of a severe malabsorptive disease has to be considered. Excessive urinary AICA, in combination with ImPA and/or NAH is an additional argument for the existence of such a clinical condition. However, it must be stressed that amino acid malabsorption is not excluded when ImPA and NAH are absent. Abnormal excretions of bacterial tyrosine and/or phenylalanine metabolites frequently occur without ImPA and NAH being present. The excretory pattern resulting may depend on many factors, such as the severity of the intestinal damage limiting the amino acid transport, the composition of the bacterial flora and the composition of the intestinal contents as a function of diet and digestion.

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