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Review

Investigation of polyamine metabolism by high-performance liquid chromatographic and gas chromatographic profiling methods

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Abstract

Measurements of polyamines, polyamine conjugates and their metabolites in tissues, cells and extracellular fluids are used in biochemistry, (micro)biology, oncology and parasitology. Decarboxylation of ornithine yields putrescine. Aminopropylation of putrescine yields spermidine, and aminopropylation of spermidine yields spermine. Spermidine and spermine are retroconverted to putrescine and spermidine, respectively, by initial N-acetylation and subsequent polyamine oxidation. The intermediate N-acetylputrescine, N^1-acetylspermidine and N^8-acetylspermidine are the major urinary N-acetylpolyamines. Polyamines and N-acetylpolyamines are terminally degraded to non-α-amino acid metabolites by oxidative deamination and aldehyde dehydrogenation. Chromatography with on-line detection is the most commonly applied profiling method for polyamines, N-acetylpolyamines and their non-α-amino acid metabolites. Cation-exchange and reversed-phase high-performance liquid chromatography require pre- or post-column derivatisation, followed by UV-Vis spectrophotometric or fluorimetric detection. Isolation and derivatisation precedes gas chromatography with flame-ionisation, nitrogen–phosphorus, electron-capture or mass spectrometric detection. High-performance liquid chromatography and gas chromatography of polyamines are not competitive techniques, but rather supplementary.

Contents

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List of abbreviations and trivial names used

ACISOGA	N-acetylisoputreanine-γ-lactam [N-(3-aminopropyl)pyrrolidin-2-one]
Cad	Cadaverine (1,5-diaminopentane)
DAP	1,3-Diaminopropane
ECD	Electron-capture detection
FID	Flame-ionisation detection
GABA	γ-Aminobutyric acid
GC	Gas chromatography
GI	Gastrointestinal tract
HPLC	High-performance liquid chromatography
ISOGA	Isoputreanine-γ-lactam [N-(3-aminopropyl)pyrrolidin-2-one]
Isoputr	Isoputreanine [N-(3-aminopropyl)-4-aminobutyric acid]
MS	Mass spectrometry
n-α-AA	Non-α-amino acid
NacDAP	N-Acetyl-1,3-diaminopropane
NacIsoputr	N-Acetylisoputreanine [N-(3-acetamidopropyl)-4-aminobutyric acid]
NacPA	N-Acetylpolyamines
N^1acSd	N^1-Acetylspermidine
N^8acSd	N^8-Acetylspermidine
N^1acSp	N^1-Acetylspermine
N^1,N^{12}diacSp	N^1,N^{12}-Diacetylspermine
OPA/ME	*o*-Phthalaldehyde/2-mercaptoethanol
PA	Polyamine
Pu	Putrescine (1,4-diaminobutane)
Putreanine	(4-Aminobutyl)-3-aminopropionic acid
Sd	Spermidine [N-(3-aminopropyl)-1,4-diaminobutane; $H_2N^1(CH_2)_3NH(CH_2)_4N^8H_2$]
SID	Stable isotope dilution
Sp	Spermine [N,N′-bis(3-aminopropyl)-1,4-diaminobutane; $H_2N^1(CH_2)_3NH(CH_2)_4NH(CH_2)_3N^{12}H_2$]
Spermic acid-1	N-(3-Aminopropyl)-N′-(2-carboxyethyl)-1,4-diaminobutane
Spermic acid-2	N,N′-Bis(2-carboxyethyl)-1,4-diaminobutane
Spermidic acid-2	N-(2-Carboxyethyl)-4-aminobutyric acid
δ-Val	δ-Aminovaleric acid

1. Introduction

The polyamines (PAs) putrescine (Pu), spermidine (Sd) and spermine (Sp) are straight-chain aliphatic amines that occur in all human and animal cells. At physiological pH they are positively charged. Consequently they have a tendency to form ionogenic bonds with negatively charged cellular constituents, like nucleic acids, proteins and phospholipids. The physiological functions of the PAs are as yet unclear. Since the finding of increased extracellular PA levels in cancer patients [1] numerous investigators studied their potential usefulness as tumour markers [2]. Like many of these, extracellular PAs and their metabolites proved aspecific and rather unsensitive for diagnostic purposes, but nevertheless useful for estimation of therapy efficacy, and establishment of disease progression and recurrence in selected cases. Experiments with highly specific PA synthesis inhibitors showed that PAs are essential for proliferation. PA depletion is therefore intensively studied as treatment modalities for cancer and parasitic diseases, notably those caused by trypanosomas [3].

PA contents of cells and body fluids are in the 0.05–500 μmol/10^{12} cells and 0.05–15 μmol/l ranges, respectively. Their analyses have been performed by virtually all available techniques,

including thin-layer and paper chromatography, electrophoresis, liquid chromatography in amino acid analyzers, immunochemical methods, enzymatic methods, high-performance liquid chromatography (HPLC) and gas chromatography (GC). For PA quantification in tissues, cells and body fluids, most investigators nowadays employ HPLC. PAs, N-acetylpolyamines (NacPAs) and their non-α-amino acid (n-α-AA) metabolites do not contain functional groups that allow sensitive and selective on-line HPLC detection without derivatisation. Pre- or post-column derivatisation, followed by spectrophotometric and fluorimetric detection are, therefore, commonly applied. GC, although less popular and usually more time-consuming than HPLC, may offer advantages for the analysis of NacPAs and notably n-α-AA metabolites. Since the topic has been excellently reviewed by Seiler [4] up to 1986 and recently by Fujita et al. [5], this contribution mainly concentrates on HPLC and GC methods that have recently become available and particularly those that allow profiling of PAs, NacPAs and their terminal n-α-AA metabolites.

2. Exogenous sources, synthesis, retroconversion and terminal degradation

PAs derive from the diet, synthesis by bacteria in the gastrointestinal tract (GI), de novo synthesis and retroconversion. Mammalian cells synthesize Pu by decarboxylation of ornithine (ornithine decarboxylase). Ornithine is available from the extracellular compartment or formed from arginine by deimination (arginase). Bacteria may also produce Pu by initial decarboxylation of arginine to agmatine (arginine decarboxylase), followed by agmatine deimination to Pu (agmatinase). Pu is converted to Sd by attachment of an aminopropyl moiety (spermidine synthase; Fig. 1). Attachment of a second aminopropyl moiety to the primary nitrogen of the diaminobutane-part of Sd (its N^8-position)

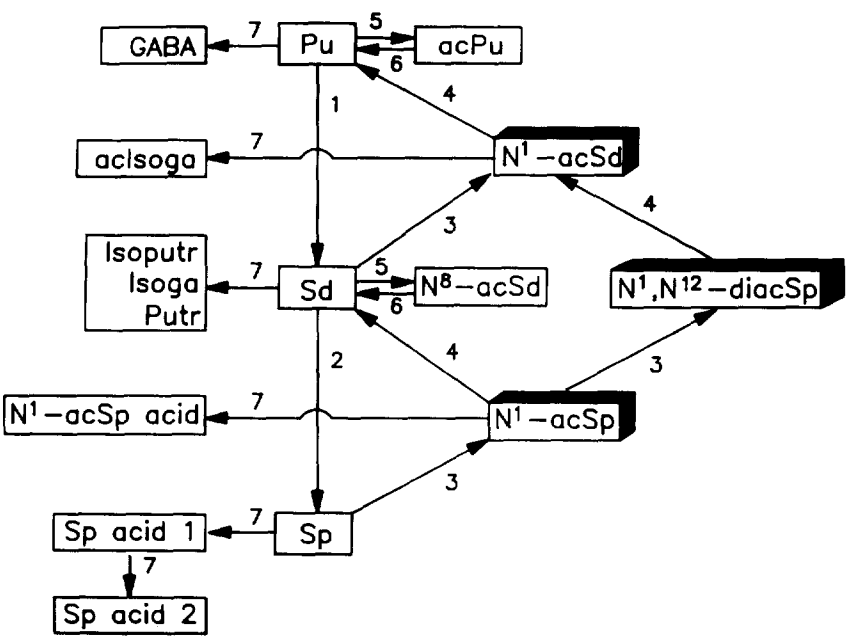

Fig. 1. Polyamine biosynthesis, retroconversion and terminal degradation. 1, spermidine synthase; 2, spermine synthase; 3, cytosolic spermidine/spermine N^1-acetyltransferase; 4, polyamine oxidase; 5, nuclear acetyltransferase; 6, acetylhydrolase; 7, copper-containing amine oxidase/aldehyde dehydrogenase.

yields Sp (spermine synthase). Both aminopropyl moieties derive from decarboxylated S-adenosylmethionine. The latter is produced from S-adenosylmethionine by decarboxylation (S-adenosylmethionine decarboxylase). Bacteria in the GI decarboxylate a number of amino acids to biogenic amines for non-biosynthetic purposes. These so called biodegradative pathways yield e.g. histamine from histidine, Pu from ornithine (biodegradative ornithine decarboxylase) and cadaverine (Cad) from lysine (lysine decarboxylase). PAs from bacterial and dietary origin become partly absorbed in the GI and may subsequently be transported into cells by carrier-mediated mechanisms.

Retroconversion [6] of Sd to Pu and Sp to Sd takes place by initial N^1-acetylation of Sd to N^1-acetylspermidine (N^1acSd) and Sp to N^1-acetylspermine (N^1acSp). N^{12}-Acetylation of N^1acSp yields N^1,N^{12}-diacetylspermine (N^1,N^{12}diacSp). The reactions are catalysed by cytosolic spermidine/spermine N^1-acetyltransferase, which uses acetyl-CoA as cosubstrate. Polyamine oxidase releases acetamidopropyl moieties from N^1acSd, N^1acSp and N^1,N^{12}diacSp to yield Pu, Sd and N^1acSd, respectively. Nuclear acetyltransferase converts Pu to N-acetylputrescine (NacPu) and Sd to N^8-acetylspermidine (N^8acSd). These may subsequently be retroconverted to Pu and Sd, respectively, by acetylhydrolase.

Biosynthesis and retroconversion are together referred to as interconversion, as opposed to terminal degradation [6]. The latter produces metabolites that can not be reused for PA synthesis. Terminal degradation comprises oxidative deamination of primary amino groups of PA and NacPA by copper-containing amine oxidases (diamine oxidase and spermine oxidase). The intermediate aldehydes that derive from these reactions are oxidized to n-α-AA by aldehyde dehydrogenase. In this manner Pu is converted to γ-aminobutyric acid (GABA); Cad to δ-aminovaleric acid (δ-Val); Sd to isoputreanine (Isoputr), putreanine (Putr) and spermidic acid-2; N^1acSd to N-acetylisoputreanine (NacIsoputr); Sp to spermic acid-1 and spermic acid-2; and N^1acSp to N^1acetylspermic acid. GABA, Isoputr and NacIsoputr may cyclizise to their γ-lactams [i.e. pyrrolidin-2-one, Isoputreanine-γ-lactam (ISOGA) and N-acetylisoputreanine-γ-lactam (ACISOGA), respectively]. Alternatively, the intermediate aldehyde of GABA may form an internal Schiff base to form Δ^1-pyrroline. Δ^1-Pyrroline may be oxidized to pyrrolidin-2-one and oxidative deamination of GABA produces succinic acid. Shunting of the latter into the citric acid cycle finally produces carbon dioxide, water and urea. As yet poorly delineated PA degradation in mammals involves production of 1,3-diaminopropane (DAP), N-acetyl-1,3-diaminopropane (NacDAP), β-alanine, 2-hydroxyputrescine and the alcoholic counterparts of the n-α-AAs.

Mean urinary PA, NacPA and n-α-AA levels of healthy adults (in mmol/mol creatinine) may illustrate the importance of polyamine metabolism in the human body (Table 1). PAs, NacPAs and n-α-AAs that contain the same "ground structure" are grouped. β-Ala and GABA are not unique metabolites of polyamines. Data in Table 1 show that NacPu and GABA are the major excretory forms that contain the Pu ground structure, whereas Sd is mainly excreted as ACISOGA, Isoputr/ISOGA, N^1acSd and N^8acSd.

3. HPLC of polyamines

Early liquid chromatographic methods for PAs in tissues, cells and physiological fluids made use of modified amino acid analyzers with post-column ninhydrin/colorimetric detection [7] or, the 6–10 times more sensitive, fluorimetric detection following post-column derivatisation with *o*-phthalaldehyde/2-mercaptoethanol (OPA/ME, [8]). With advancing developments in HPLC, measurements of PAs with thin-layer chromatography, electrophoresis and cation-exchange chromatography in amino acid analyzers became gradually replaced by cation-exchange, ion-pair reversed-phase and reversed-phase HPLC, either with pre- or post-column derivatisation. From these, especially fluorogenic reagents became popular because of their superior sensitivity

Table 1
Mean levels of polyamines, N-acetylpolyamines and their non-α-amino acid metabolites in urine of healthy adults

Analyte	Level (mmol/mol creat)	Reference
1,3-Diaminopropane	0.16	45
N-Acetyl-1,3-diaminopropane	0.04	45
β-Alanine[a,b]	6.33	44
Putrescine	0.35	45
N-Acetylputrescine	1.18	45
γ-Aminobutyric acid[a,b]	3.20	44
Cadaverine	0.40	45
N-Acetylcadaverine	0.16	45
δ-Aminovaleric acid[b]	1.10	44
Spermidine	0.05	45
N^1-Acetylspermidine	0.39	45
N^8-Acetylspermidine	0.30	45
Isoputreanine + Isoputreanine-γ-lactam	0.64	45
N-Acetylisoputreanine-γ-lactam	0.65	49
Putreanine	0.24	45
Spermidic acid-2[b]	0.39	44
Spermine	0.08	45
N^1-Acetylspermine	0.02	45
Spermic acid-1[b]	0.10	42

Data (in mmol/mol creatinine) were obtained by capillary gas chromatography with nitrogen–phosphorus detection [43,45,46] and stable-isotope dilution mass fragmentography [50].
[a] Not a unique polyamine metabolite.
[b] Levels in acid-hydrolysed urine (percentage conjugate unknown).

compared with those that introduce UV-Vis absorbing moieties.

Pre-column derivatisation with 5-dimethylaminonaphtalene-1-sulphonylchloride (dansyl chloride) in aqueous solution, followed by derivative purification and reversed-phase HPLC/fluorimetry is a widely employed method for PA in tissues and body fluids [9]. Other examples of pre-column reactions to fluorochromes are those with fluorescamine [10], OPA/ME [11] and OPA/ethanethiol [12]. Cation-exchange HPLC with post-column OPA/ME fluorimetry is another option [13]. Compared with acid chlorides, fluorescamine and OPA are more selective agents since they merely react with primary amino groups. They have, however, limited stability [4]. OPA/ethanethiol–PA adducts proved more stable than those prepared with the OPA/ME reaction [12]. A number of pre-column derivatisation techniques have been developed for HPLC with UV-Vis spectrophotometric detection [4]. They include reactions with benzoyl chloride [14], *p*-toluenesulphonyl chloride (tosyl chloride), 2,4-dinitrofluorobenzene, 4-fluoro-3-nitrobenzotrifluoride, quinoline-8-sulphonyl chloride and 4-dimethylaminoazobenzene-4′-sulphonyl chloride (dabsyl chloride). Wagner et al. [15] described an ion-pair reversed-phase HPLC method that enables profiling of S-adenosylmethionine, decarboxylated S-adenosylmethionine and analogues (by initial UV detection), in a single run with PA (by OPA/ME derivatisation of the UV-cell eluate and fluorimetric detection). The method is applicable for both tissues and body fluids. Maruta et al. [16] described octanesulphonic acid reversed-phase HPLC followed by on-line enzymatic reaction with immobilized soybean polyamine

oxidase. Liberated H_2O_2 was measured with electrochemical detection. The method was applied for Pu, Cad, Sd and Sp in tissue and acid-hydrolysed urine.

4. HPLC of polyamines, N-acetylpolyamines and their metabolites

Growing interest in PA retroconversion and terminal metabolism prompted development of appropriate assays for PA, NacPA and n-α-AA metabolites. HPLC analyses of tissue and urine extracts were e.g. used to demonstrate conversion of Δ^1pyrroline to pyrrolidin-2-one [17], Sd to Putr and ISOGA, and N^1acSd to an ISOGA conjugate tentatively identified as ACISOGA [18]. Early methods for NacPA in urine employed thin-layer chromatography of dansyl derivatives [19,20], combined with HPLC [19]. The subsequently developed direct HPLC method of Seiler and Knödgen [21] was based on octane sulphonic acid reversed-phase separation of PA and NacPA in perchloric acid-pretreated tissue and urine, followed by post-column derivatisation with OPA/ME and fluorimetric detection. However, DAP and Pu peaks coincided and NacPu proved contaminated. The method was subsequently improved by the use of a high-resolution column, that not only enabled additional measurement of NacPu, but also of Putr, Isoputr and histamine [22]. NacPA isolation with silica gel followed by dansylation necessitated two HPLC systems to obtain separation between urinary NacPu, N^1acSd and N^8acSd [23]. Profiling of urinary NacPA, but not the parent compounds, was achieved by a 74-min programme on a four-buffer cation-exchange HPLC system, followed by derivatisation with OPA/ME and fluorimetric detection [24]. As subsequently demonstrated, sample prepurification improved the selectivity and sensitivity of PA and NacPA profiling methods. Prepurification of cerebrospinal fluid and urine by cation-exchange chromatography, followed by dansylation, C_{18}-column derivative clean up and reversed-phase HPLC with fluorimetric detection was one of these improvements [25]. Comparable results were obtained by Löser et al. [26], who improved the method of Seiler and Knödgen [22]. They employed silica prepurification of urine, octanesulphonic acid reversed-phase HPLC, post-column derivatisation with OPA/ME and fluorimetric detection. Chromatograms of PA in acid-hydrolysed urine, and PA and NacPA in unhydrolysed urine, both from a healthy person, are depicted in Fig. 2.

5. GC of polyamines, N-acetylpolyamines and their metabolites

GC is mostly preceded by derivatisation of polar functional groups. Derivatisation usually necessitates isolation of analytes in a water and salt free form. For this, early prepurification methods for urinary PA employed alkaline butanol extraction [1] or cation-exchange chromatography [27]. It was followed by derivatisation with trifluoroacetic anhydride and packed-column GC with flame-ionisation (FID) or electron-capture (ECD) detection [27,28]. Both prepurification methods proved applicable for mass spectrometric (MS) detection, and led to the identification of NacSd in urine [28]. Removal of pigments from acid-hydrolysed urine by Porapak-Q contributed to long term maintenance of GC column performance [29]. Nevertheless, PA analyses by GC remained time-consuming and unattractive (e.g. alkaline butanol extraction). With simultaneous development of assays with amino acid analyzers and later HPLC, most efforts were directed at liquid chromatography. Further developments in GC analyses comprised improvements in isolation procedures, derivatisation techniques, columns and detectors. Some of these will be discussed.

Makita et al. [30] derivatised PAs in acid-hydrolysed urine with pentafluorobenzoyl chloride in aqueous solution. Derivatives were isolated by organic liquid extraction and analyzed by packed column GC–ECD. Formation of isobutyloxycarbonyl derivatives in aqueous solution, followed by post-derivatisation organic solvent extraction, was successfully applied for PA analyses in urine [31] and plasma [32] by packed-

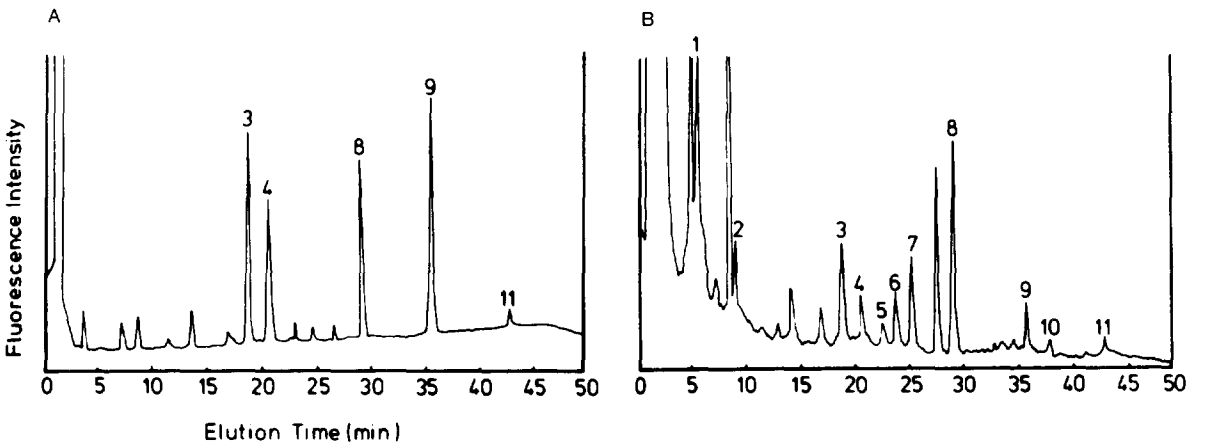

Fig. 2. High-performance liquid chromatograms of polyamines (A, B) and N-acetylpolyamines (B) in acid-hydrolysed (A) and unhydrolysed (B) urine from a healthy subject. Chromatograms were obtained by silica gel prepurification, sodium acetate/acetonitrile gradient-octanesulphonic acid reversed-phase high-performance liquid chromatography, post-column derivatisation with *o*-phthalaldehyde/2-mercaptoethanol and fluorimetric detection. Peaks: 1 = NacPu; 2 = NacCad; 3 = Pu; 4 = Cad; 5 = histamine; 6 = N^1acSd; 7 = N^8acSd; 8 = 1,7-diaminoheptane (internal standard); 9 = Sd; 10 = N^1acSp; 11 = Sp. Reprinted from Löser et al. [26], with permission.

column GC–FID [31] and GC with nitrogen detection [32]. PAs in urine [33] and erythrocytes [34] were measured by packed-column GC–ECD, following Porapak Q/cation-exchange isolation [33] or adsorption on activated Permutit [34] and conversion into pentafluoropropionyl [33] or heptafluorobutyryl [34] derivatives. Employing the alkaline butanol extraction method, Beninati et al. [35] developed a packed-column GC–FID method for underivatised PA in tissue extracts. Adsorption of the free PA bases was circumvented by addition of 1% KOH to the stationary-phase support. Yamamoto et al. [36] were the first to report a convenient packed-column GC–FID assay for urinary PAs and NacPAs. The method comprised silica gel isolation, reaction with ethyl chloroformate in aqueous solution and organic solvent extraction of derivatives. Sp did, however, not elute from the GC column and GC–MS analysis showed that Pu was not completely resolved from an unidentified compound.

Positive- and negative-ion packed-column GC–MS of PAs [37–41] and NacPAs [40] were developed for both identification purposes and stable-isotope dilution (SID) GC–MS [37,38]. Ionisation was performed in both electron-impact and chemical-ionisation modes. Derivatisation occurred by trifluoroacetylation [37,38,40], trimethylsilylation [37,40], pentafluoropropionylation [39] or N-ethyloxycarbonylation [36]. With these methods Ohki et al. [39] identified the alcoholic counterpart of Isoputr in urine, whereas Yamamoto et al. [41] identified symmetrical homospermidine and symmetrical norspermine in mosses and ferns.

Developments in capillary GC, notably column availability, injection systems and automated injection, allowed its introduction for routine laboratory usage. Employing silica gel purification of acid-hydrolysed urines we developed a capillary GC–FID method with split injection for the determination of heptafluorobutyryl PAs [42]. A urine sample of a patient with non-African non-Hodgkin's Burkitt-type lymphoma showed several large peaks of unknown identity. With GC–MS they were identified as (methyl)heptafluorobutyryl DAP, ISOGA and Isoputr. The method was further developed to capillary GC–nitrogen–phosphorus (NPD) profiling of urinary total DAP, Pu, Cad, Sd, Sp, Isoputr, Putr and spermic acid-1 as their (methyl)heptafluorobutyryl derivatives [43]. Experiments with deuterium labelled PAs in rats

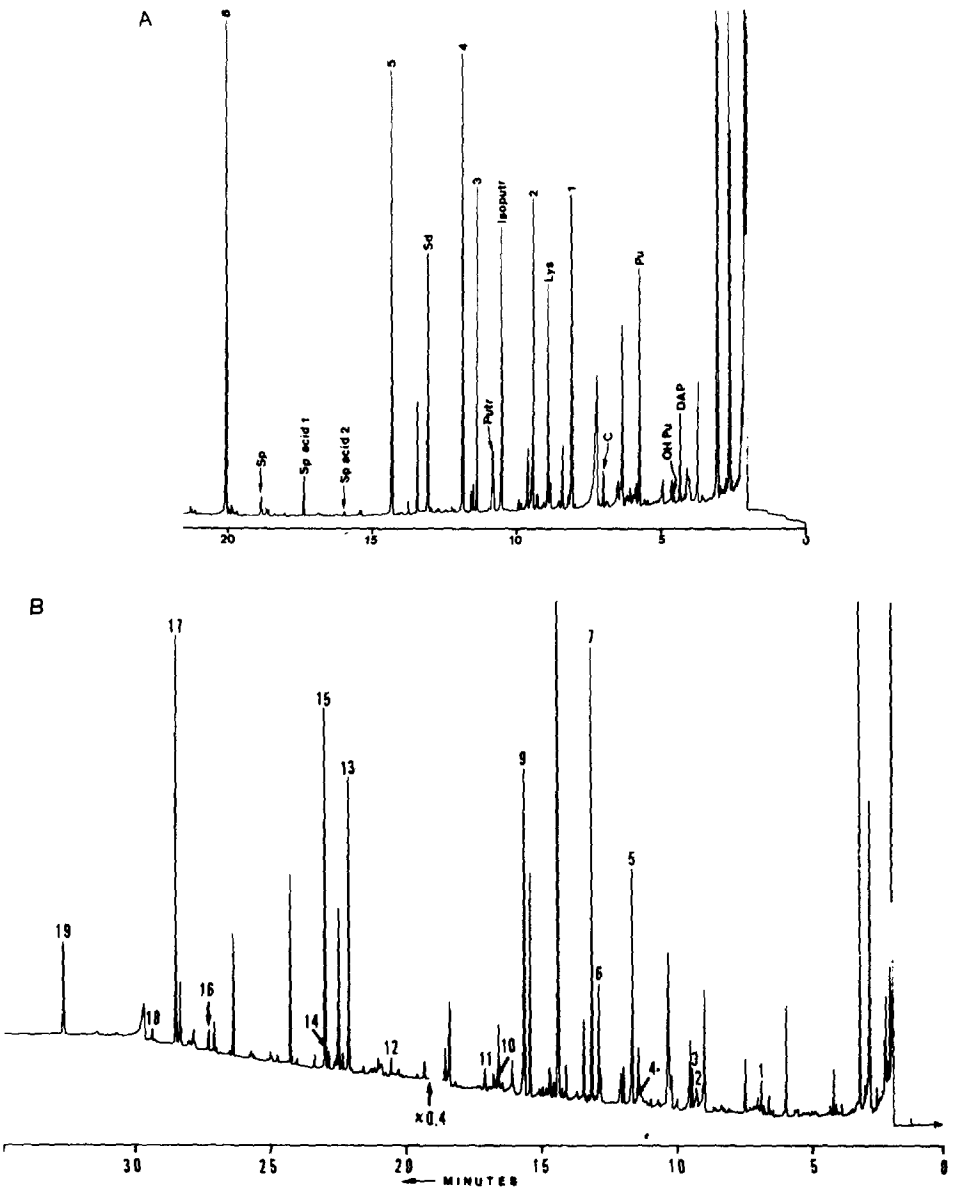

Fig. 3. Gas chromatograms of polyamines and non-α-amino acid metabolites in acid-hydrolysed urine of a healthy adult (A) and polyamines, N-acetylpolyamines, and their non-α-amino acid metabolites in unhydrolysed urine from a patient with non-Hodgkin's lymphoma (B). Chromatograms were obtained by silica gel prepurification, conversion into (methyl)heptafluorobutyryl derivatives, derivative isolation by organic solvent extraction, and capillary gas chromatography with nitrogen–phosphorus detection. Peak identification chromatogram A: OH Pu = 2-hydroxyputrescine; C = Cad; 1 = 1,6-diaminohexane (internal standard); Lys = lysine; 2 = 1,7-diaminoheptane (internal standard); 3 = N^1-methylisoputreanine (internal standard); 4 = bis(3-aminopropyl)amine (internal standard); 5 = N-(3-aminopropyl)-1,5-diaminopentane (internal standard); Sp acid 2 = spermic acid-2; Sp acid 1 = spermic acid-1; 6 = N,N′-bis(3-aminopropyl)-1,5-diaminopentane (internal standard). Peak identification chromatogram B: 1 = DAP; 2 = NacDAP; 3 = Pu; 4 = Cad; 5 = NacPu; 6 = ISOGA; 7 = 1,6-diaminohexane (internal standard); 8 = NacCad; 9 = N-acetyl-1,6-diaminohexane (internal standard); 10 = Isoputr; 11 = Putr; 12 = Sd; 13 = N-(3-aminopropyl)-1,5-diaminopentane (internal standard); 14 = N^8acSd; 15 = N^1acSd; 16 = Sp; 17 = N,N′-bis(3-aminopropyl)-1,5-diaminopentane (internal standard); 18 = N^1acSp; 19 = N^1,N^{12}diacSp. Reprinted from (A) Muskiet et al. [43] and (B) Van den Berg et al. [46], with permission.

and monitoring of specific urinary PA fragments by GC–MS confirmed and extended the knowledge on terminal catabolic routes [44]. δ-Val, spermidic acid-2, spermic acid-1 and spermic acid-2 were subsequently identified and quantified in urines of healthy persons and cancer patients [45]. Modification of the method enabled measurements of: PAs, NacPAs and n-α-AAs in urine by GC–NPD [46]; PAs in erythrocytes by GC–NPD [47]; PAs and n-α-AAs in cerebrospinal fluid by SID-GC–MS [48]; pyrrolidin-2-one in plasma by SID-GC–MS [49]; and ACISOGA in urine by both SID-GC–MS [50] and GC–NPD [51]. N^1,N^{12}-DiacSp was identified in urine by GC–MS [46]. Chromatograms of PAs and n-α-AAs in acid-hydrolysed urine of a healthy adult, and PAs, NacPAs and n-α-AAs in unhydrolysed urine of a patient with non-Hodgkin's lymphoma are depicted in Fig. 3.

6. Conclusions

Advances in PA analyses provided an arsenal of methods that allow profiling of parent PAs, NacPAs and their n-α-AA metabolites by both HPLC and GC, combined with various detectors. HPLC and GC of PAs are not competitive techniques, but rather supplementary. Their outcomes do not seem to differ much with respect to precision, recovery and reference values [5]. HPLC measurements of PAs in tissues and cells was and remains the method of choice because of minimal sample pretreatment. GC has become neglected probably because of initially laborious and unattractive prepurification. Trends in HPLC profiling methods for urinary PAs and NacPAs witness the need for more extensive prepurification, whereas GC prepurification has become more efficient. The choice between HPLC and GC, therefore, depends on desired analytes, equipment availability, costs, experience and personal taste. At present capillary GC seems more suitable for profiling of PAs, NacPAs and their n-α-AAs, because of its higher number of theoretical plates and its easy convertibility to GC–MS.

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References

[1] D.H. Russell, *Nature*, 233 (1971) 144.
[2] U. Bachrach, *Progress in Drug Research, Vol. 39*, Birkhäuser Verlag, Basel, 1992, p. 9.
[3] J. Jänne, L. Alhonen and P. Leinonen, *Ann. Med.*, 23 (1991) 241.
[4] N. Seiler, *J. Chromatogr.*, 379 (1986) 157.
[5] K. Fujita, T. Nagatsu and K. Shimpo, *Techniques in the Behavioral and Neural Sciences, Vol. 11, Methods in Neurotransmitter and Neuropeptide Research, Part 2*, Elsevier, Amsterdam, 1993, p 189.
[6] N. Seiler, F.N. Bolkenius and O.M. Rennert, *Med. Biol.*, 59 (1981) 334.
[7] L.J. Marton, O. Heby, C.B. Wilson and P.L.Y. Lee, *FEBS Lett.*, 41 (1974) 99.
[8] L.J. Marton and P.L.Y. Lee, *Clin. Chem.*, 21 (1975) 1721.
[9] N. Seiler, B. Knödgen and F. Eisenbeiss, *J. Chromatogr.*, 145 (1978) 29.
[10] M. Kai, T. Ogata, K. Haraguchi and Y. Ohkura, *J. Chromatogr.*, 163 (1979) 151.
[11] R.L. Heideman, K.B. Fickling and L.J. Walker, *Clin. Chem.*, 30 (1984) 1243.
[12] T. Skaaden and T. Greibrokk, *J. Chromatogr.*, 247 (1982) 111.
[13] J.R. Shipe and J. Savory, *Ann. Clin. Lab. Sci.*, 10 (1980) 128.
[14] C.F. Verkoelen, J.C. Romijn, F.H. Schroeder, W.P. van Schalkwijk and T.A.W. Splinter, *J. Chromatogr.*, 426 (1988) 41.
[15] J. Wagner, C. Danzin, S. Huot, N. Claverie and M.G. Palfreyman, *J. Chromatogr.*, 290 (1984) 247.
[16] K. Maruta, R. Teradaira, N. Watanabe, T. Nagatsu, M. Asano, K. Yamamoto, T. Matsumoto, Y. Shionoya and K. Fujita, *Clin. Chem.*, 35 (1989) 1694.
[17] D.W. Lundgren, H. Tevesz and E.A. Krill, *Anal. Biochem.*, 148 (1985) 461.
[18] N. Seiler, B. Knödgen and K. Haegele, *Biochem. J.*, 208 (1982) 189.
[19] M.M. Abdel-Monem and K. Ohno, *J. Pharm. Sci.*, 67 (1978) 1671.
[20] N. Seiler and B. Knödgen, *J. Chromatogr.*, 164 (1979) 155.
[21] N. Seiler and B. Knödgen, *J. Chromatogr.*, 221 (1980) 227.
[22] N. Seiler and B. Knödgen, *J. Chromatogr.*, 339 (1985) 45.
[23] M.M. Abdel-Monem and J.L. Merdink, *J. Chromatogr.*, 222 (1981) 363.

[24] C.E. Prussak and D.H. Russell, *J. Chromatogr.*, 229 (1982) 47.
[25] P.M. Kabra, H.K. Lee, W.P. Lubich and L.J. Marton, *J. Chromatogr.*, 380 (1986) 19.
[26] C. Löser, U. Wunderlich and U. Fölsch, *J. Chromatogr.*, 430 (1988) 249.
[27] C.W. Gehrke, K.C. Kuo, R.W. Zumwalt and T.P. Waalkes, *Polyamines in Normal and Neoplastic Growth*, Raven Press, New York, NY, 1973, p. 343.
[28] T. Walle, *Polyamines in Normal and Neoplastic Growth*, Raven Press, New York, NY, 1973, p. 355.
[29] R.F. McGregor, M.S. Sharon, M. Atkinson and D.E. Johnson, *Prep. Biochem.*, 6 (1976) 403.
[30] M. Makita, S. Yamamoto and M. Kono, *Clin. Chim. Acta*, 61 (1975) 403.
[31] M. Makita, S. Yamamoto, M. Miyake and K. Masamoto, *J. Chromatogr.*, 156 (1978) 340.
[32] M.T. Bakowski, P.A. Toseland, J.F.C. Wicks and J.R. Trounce, *Clin. Chim. Acta*, 110 (1981) 273.
[33] J.M. Rattenbury, P.M. Lax, K. Blau and M. Sandler, *Clin. Chim. Acta*, 95 (1979) 61.
[34] S. Fujihara, T. Nakashima and Y. Kurogochi, *J. Chromatogr.*, 277 (1983) 53.
[35] S. Beninati, C. Sartori and M.P. Argento-Ceru, *Anal. Biochem.*, 80 (1977) 101.
[36] S. Yamamoto, M. Yokogawa, K. Wakamatsu, H. Kataoka and M. Makita, *J. Chromatogr.*, 233 (1982) 29.
[37] R.G. Smith and G.D. Daves, *Biomed. Mass Spectrom.*, 4 (1977) 146.
[38] J.R. Shipe, D.F. Hunt and J. Savory, *Clin. Chem.*, 25 (1979) 1564.
[39] T. Ohki, A. Saito, N. Yamanaka, K. Ohta, J. Sakakibara, T. Niwa and K. Maeda, *J.Chromatogr.*, 228 (1982) 51.
[40] G.D. Daves, R.G. Smith and C.A. Valkenburg, *Methods Enzymol.*, 94 (1983) 48.
[41] S. Yamamoto, A. Iwado, Y. Hashimoto, Y. Aoyama and M. Makita, *J. Chromatogr.*, 303 (1984) 99.
[42] F.A.J. Muskiet, C.M. Stratingh and D.C. Fremouw-Ottevangers, *J. Chromatogr.*, 230 (1982) 142.
[43] F.A.J. Muskiet, G.A. van den Berg, A.W. Kingma, D.C. Fremouw-Ottevangers and M.R. Halie, *Clin. Chem.*, 30 (1984) 687.
[44] G.A. Van den Berg, H. Elzinga, G.T. Nagel, A.W. Kingma and F.A.J. Muskiet, *Biochim. Biophys. Acta*, 802 (1984) 175.
[45] G.A. van den Berg, G.T. Nagel and F.A.J. Muskiet, *J. Chromatogr.*, 339 (1985) 223.
[46] G.A. van den Berg, F.A.J. Muskiet, A.W. Kingma, W. Van der Slik and M.R. Halie, *Clin. Chem.*, 32 (1986) 1930.
[47] G.A. van den Berg, A.W. Kingma and F.A.J. Muskiet, *J. Chromatogr.*, 415 (1987) 27.
[48] G.A. van den Berg, J.M. Schaaf, G.T. Nagel, A.W. Teelken and F.A.J. Muskiet, *Clin. Chim. Acta*, 165 (1987) 147.
[49] G.A. van den Berg, B.G. Wolthers, G.T. Nagel, F.A.J. Muskiet, S. Banghman and R.A. de Zeeuw, *J. Chromatogr.*, 550 (1991) 239.
[50] G.A. van den Berg, A.W. Kingma, H. Elzinga and F.A.J. Muskiet, *J. Chromatogr.*, 383 (1986) 251.
[51] J. Hessels, A.W. Kingma, M.C.J.M. Sturkenboom, H. Elzinga, G.A. van den Berg and F.A.J. Muskiet, *J. Chromatogr.*, 563 (1991) 1.