Journal of Chromatography, 162 (1979) 169—176
Biomedical Applications
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CHROMBIO. 255

LONGITUDINAL URINARY TRACE AMINE EXCRETION IN A HUMAN MALE

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SUMMARY

The urinary excretion of a β-phenylethylamine (PE), *m*-tyramine (*m*TA), *p*-tyramine (*p*TA) and tryptamine (TR) in their unconjugated (free) and conjugated (except tryptamine) forms, was examined in a male human subject over a total period of 28 days. The average excretion values were (in μg per 24 h, mean ± standard error): PE, free 3.19 ± 0.21, conjugated 6.85 ± 0.52; *m*TA, free 98.0 ± 2.2, conjugated 106.1 ± 18.6; *p*TA, free 427 ± 12, conjugated 571 ± 142; and TR, free 79.4 ± 2.8.

This data, when considered along with other published information, permits the suggestion that probably *m*TA and *p*TA in the unconjugated form are exclusively formed endogenously; whether or not this also pertains to PE and TR is less clear. In all cases, the conjugated amines derive from both exogenous and endogenous sources.

INTRODUCTION

The normal urinary excretion of the trace amines, β-phenylethylamine (PE), *m*-tyramine (*m*TA), *p*-tyramine (*p*TA), and tryptamine (TR) in the human is well documented (see recent review by Boulton [1]). In the unconjugated form, the values (as μg per 24 h, mean ± standard error; $n = 16—21$) listed by Slingsby and Boulton [2] are: PE, 4.9 ± 1.0; *m*TA 83 ± 7; *p*TA, 489 ± 40; and TR, 100 ± 13, respectively. The variation in the excretion of the amine conjugates is much larger in all cases. Abnormalities in the excretion of both the free and conjugated forms of the trace amines have been claimed to occur in Parkinsonism, migraine, depression, schizophrenia, phenylketonuria, mania and hypertension [1—3].

In a recent investigation [2, 4], a significant proportion of mentally disordered individuals admitted to a general hospital psychiatric ward exhibited increases in their urinary excretion of unconjugated *p*TA, PE and TR. As a consequence of this, we have decided to investigate, in a longitudinal manner, the

excretion of PE, *m*TA, *p*TA and TR in their free and conjugated forms (except TR), in normal subjects and in selected patients suffering from certain mental disorders. This particular paper describes the excretion of the above amines in a human male, who ingested a "normal" diet and certain likely precursors labelled with deuterium, over a period of 28 days.

MATERIALS AND METHODS

All of the solvents, chemicals and materials used were commercially available with the exception of the deuterated internal standards [1,1,2,2-tetradeutero-2-(4-hydroxyphenyl)ethylamine, (*p*-tyramine-d_4); 1,1,2,2-tetradeutero-2(3-hydroxyphenyl)-ethylamine, (*m*-tyramine-d_4); 1,1,2,2-tetradeutero-2-phenylethylamine, (β-phenylethylamine-d_4), and 1,1-dideutero-2-indolylethylamine (tryptamine-d_2)], which were prepared as previously described [5, 6]. With the exception of tryptamine, all the amine internal standards were tetradeuterated. The use of d_4 internal standards possesses two distinct advantages when compared with the dideutero standards that were incorporated when the integrated ion current (IIC) method was first introduced [2, 6]. First, the correction factor (incorporated into the formula used to calculate the amounts present) is much smaller and secondly, and more importantly, the use of d_4 standards permits not only the quantitation of the endogenous (protonated) amine but also any amines synthesised from precursors labelled with a different number of deuteriums (see ref. 28 for further details).

The amino acid and amine precursors listed in Fig. 2—5, suitably labelled with deuterium, were synthesised in gram quantities for this study by Dr. Bruce Davis of the Psychiatric Research Division in Saskatoon.

Twenty-four hour urine samples starting with the first voiding each day, were collected onto 10 ml conc. HCl in polyethylene bottles; after recording the total volume, a 250-ml aliquot was removed and stored at —16° until analysed. The collection period was over 16 days in May, 1976, followed by a 12 day period in May, 1977 (28 days in total), during which time various deuterated likely metabolic precursors (see below) of the amines were ingested. The content of the diet which was "normal" for North America, was recorded. Precursors labelled with deuterium were ingested, in equally divided doses (at 8 a.m. and 6 p.m.), according to the following regimen: DL-phenylalanine-d_2 (3 gm) on day 2; DL-*p*-tyrosine-d_2 (3 gm) on day 6; DL-tryptophan-d_5 (3 gm) on day 10; β-phenylethylamine-d_2 (200 mg) on days 13 and 24.

At the time of analysis (the general procedure is summarized in Fig. 1) samples were completely thawed and mixed before aliquots were removed (10 ml in the case of PE, 1 ml for *m*TA, *p*TA and TR). Each aliquot was diluted to 15 ml, to which was added each internal standard (*p*TA-d_4, 1000 ng; *m*TA-d_4, 200 ng; PE-d_4, 200 ng; and TR-d_2, 500 ng). The aliquot was then adjusted to pH 7.0 with 2 *N* NaOH and percolated through a Bio-Rad AG 50W-X2 ion-exchange chromatography column (6 × 1 cm) prepared as described by Kakimoto and Armstrong [7]. The initial eluate and first water wash were retained for the determination of conjugated amines. The eluted amine fractions (ethanol—ammonium hydroxide—water, 65 : 25 : 10, in the case of *m*TA, *p*TA and TR, and methanol—hydrochloric acid, 86 : 14 in the case of PE) were

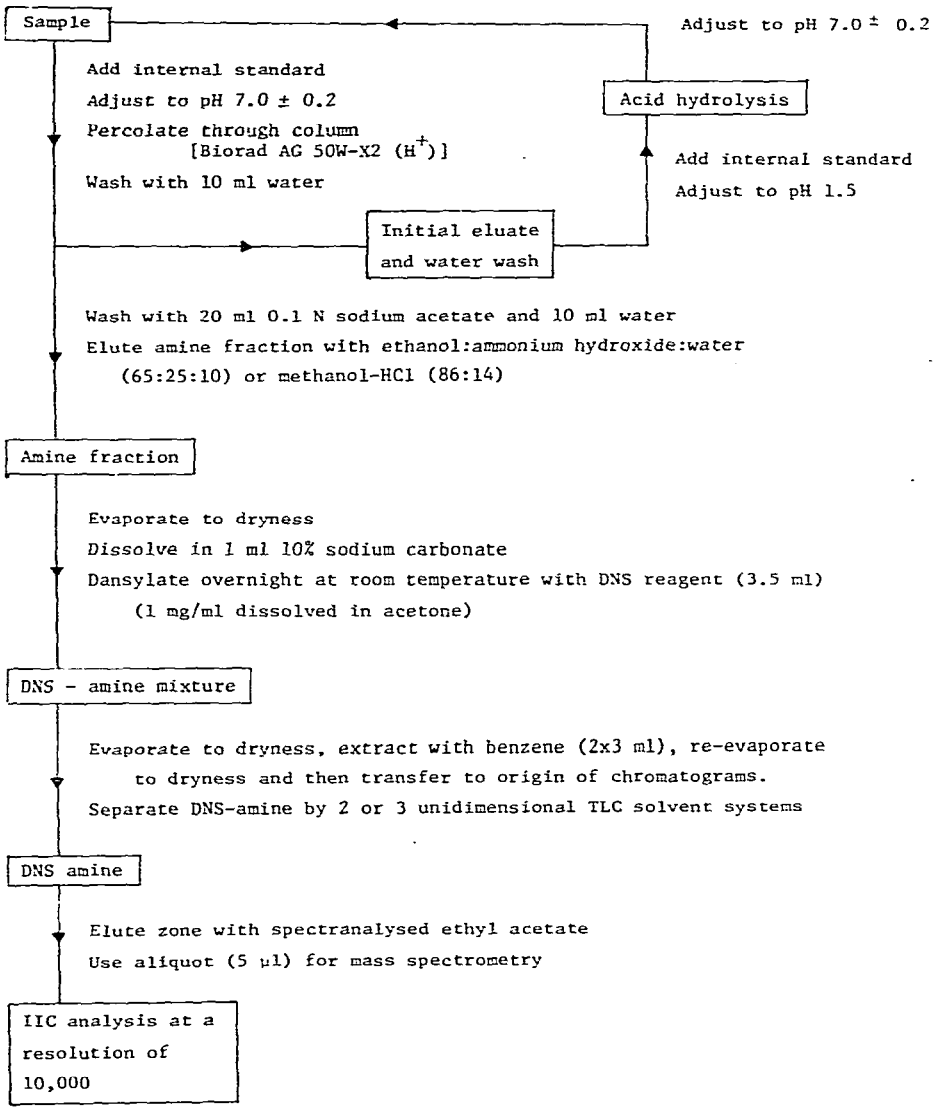

Fig. 1. Isolation, hydrolysis, derivatisation, chromatographic separation and mass spectral analysis of urinary trace amines.

dried under reduced pressure at 45°, dissolved in sodium carbonate (1 ml), dansylated, and after dansylation transferred to the origin of a silica-gel thin-layer chromatographic plate. PE was isolated by three successive unidimensional separations in solvent systems 1, 2 and 3 (see Table I), TR in systems 1 and 4 and the tyramines in systems 1 and 5. Complete details concerning the transfer and isolation of the zones, their comparison with standards run in parallel and their mass spectrometric analysis will be found in ref. 2 and the reviews by Philips [8] and Durden [9].

Conjugated amines were analysed in an identical manner in the same sample following hydrolysis of the column effluent and wash (30 min at 100° at pH 1.5).

TABLE I

CHROMATOGRAPHIC PROPERTIES OF THE DNS DERIVATIVES OF SOME TRACE AMINES

All separations on commercially prepared silica gel plates (Brinkman Instruments, Rexdale, Ontario, Canada).

Solvent system	R_F values for DNS derivatives of:			
	PE	*m*TA	*p*TA	TR
(1) Choroform—*n*-butyl acetate, 5 : 2	0.79	0.55	0.55	0.49
(2) Benzene—triethylamine, 8 : 1	0.60	—	—	—
(3) Carbon tetrachloride—triethylamine, 4 : 1	0.25	—	—	—
(4) Benzene—triethylamine, 4 : 1	—	—	—	0.11
(5) Benzene—triethylamine, 12 : 1	—	0.46	0.42	—

RESULTS

The urinary excretion profiles for free and conjugated PE, *m*TA, *p*TA and TR (free only) are shown in Figs. 2—5 and Table II. It can be seen that the daily variation in the excretion of the unconjugated tyramines is extremely small (*m*TA, 98 ± 2.2; *p*TA, 427 ± 12; in μg per day, respectively). A somewhat greater variation, although still relatively small, was seen in the case of PE

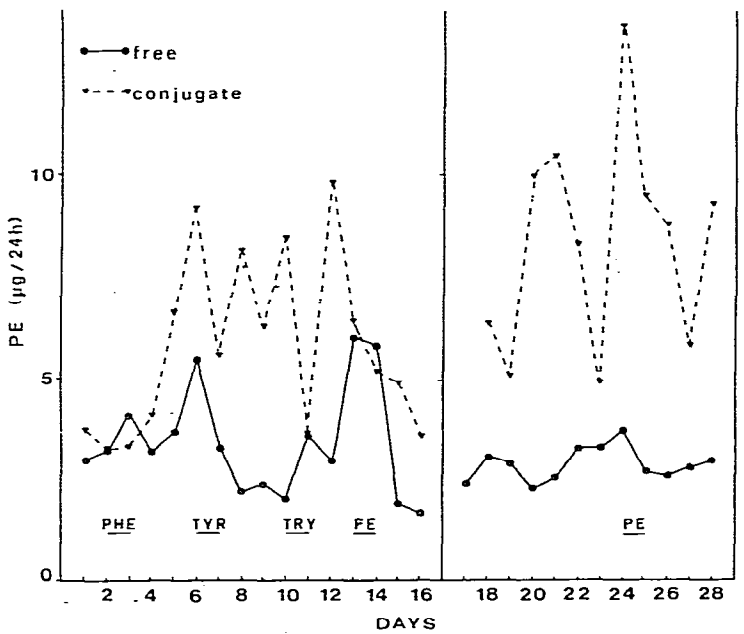

Fig. 2. Urinary excretion of conjugated and unconjugated β-phenylethylamine in a human male. The deuterated amino acids, phenylalanine, (PHE), tyrosine (TYR) and tryptophan (TRY) and deuterated phenylethylamine (PE) were ingested on days 2, 6, 10, 13 and 24 as described in the text.

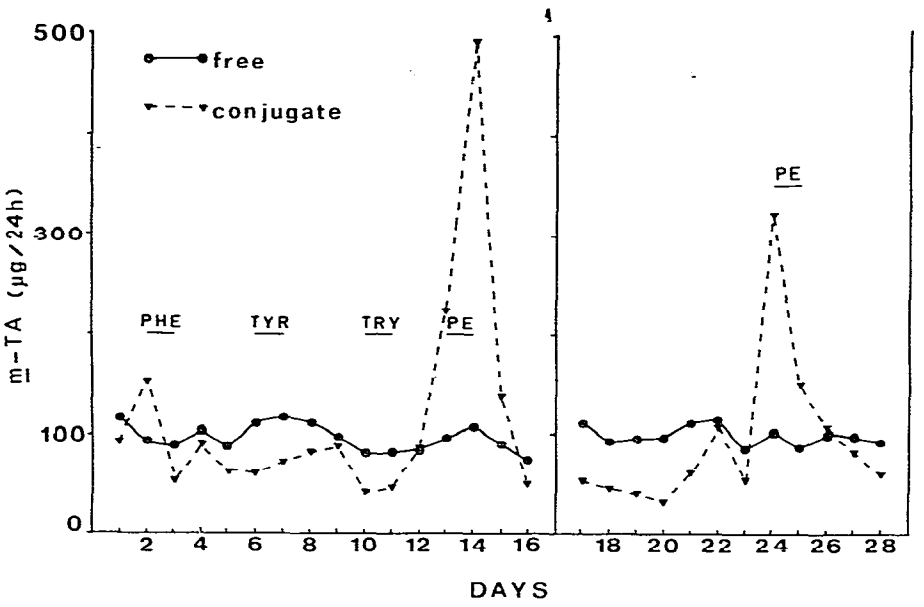

Fig. 3. Urinary excretion of conjugated and unconjugated *meta*-tyramine in a human male (see Fig. 2 and text for further details).

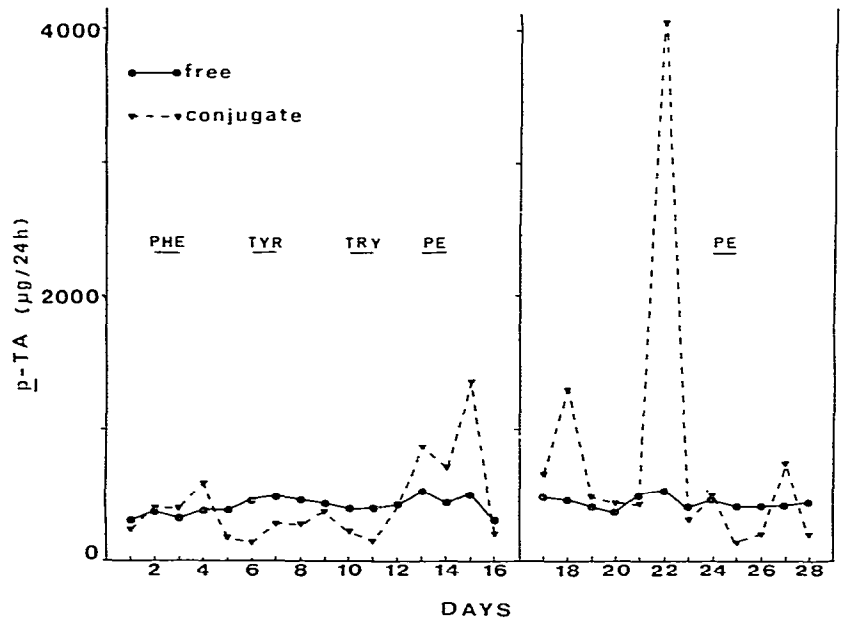

Fig. 4. Urinary excretion of conjugated and unconjugated *para*-tyramine in a human male (see Fig. 2 and the text for further details).

(3.2 ± 0.2) and TR (79.4 ± 2.8) (in µg per day).

The ingestion of the precursor substances exhibited little, if any, effect on the excretion of the unconjugated tyramines.

The excretion of all the amines in their conjugated forms was much more

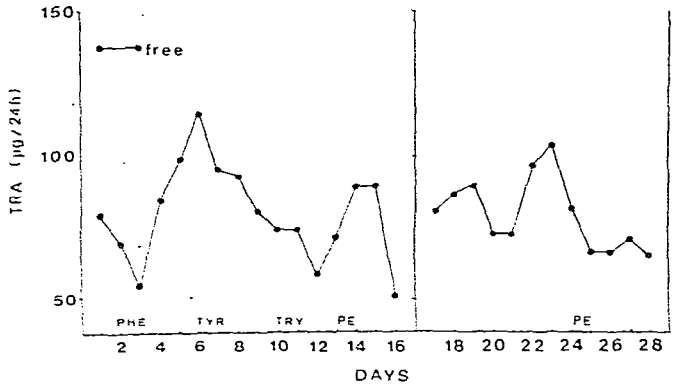

Fig. 5. Urinary excretion of unconjugated tryptamine in a human male (see Fig. 2 and the text for further details).

TABLE II

URINARY EXCRETION OF SOME TRACE AMINES IN A HUMAN MALE

All values as Mean ± S.E. are in µg per 24 h.

Amine	Free		Conjugated	
	Level	Range	Level	Range
β-Phenylethylamine	3.19 ± 0.21	1.67— 6.06	6.85 ± 0.52	3.27— 13.62
m-Tyramine	98.0 ± 2.2	74.7 —117.39	106.1 ± 18.6	34.4 — 494.0
p-Tyramine	427 ± 12	298 —549	571 ± 142	134 —4065
Tryptamine	79.4 ± 2.8	50.8 —115.3	—	—

variable, with large increases sometimes following ingestion of some of the precursors (see Figs. 2—5).

The measurement of conjugated TR after either alkaline or enzymatic hydrolysis was not possible.

DISCUSSION

The question of the origin of urinary trace amines, particularly the tyramines, has been debated for some considerable time; the principal suggestions have been that they arise from: (1) exogenous sources (diet and gut bacteria); (2) endogenous sources; or (3) a combination of endogenous and exogenous sources [1—3, 7, 10]. We believe the available evidence now permits a more definitive conclusion to be advanced concerning the origins of *m*TA and *p*TA; namely, that unconjugated tyramines (*meta* and *para*; *ortho* is not considered here) arise from endogenous sources while the conjugated tyramines are made up of a relatively constant endogenous component along with a variable exogenous component. The argument that the unconjugated tyramines may arise from the gut flora (aerobic or anaerobic) with any variations being a consequence of change in the intestinal flora composition [10], could equally well be interpreted as the availability of, and changes in, the absorption of the

precursor substances which in vivo are then converted to the tyramines. As can be seen from Figs. 3 and 4, Table II and ref. 5, however, the excretion of the unconjugated tyramines occurs with remarkable consistency and little, if any, variation.

For any substance found in urine to be considered to be of endogenous origin it would have to be excreted in a stable, consistent and reproducible manner over time in any particular individual, be excreted with relatively little variation among individuals in any particular species, and be normally present in tissues with enzymes for its synthesis and degradation also being present in those tissues. It is now clear that unconjugated *m*TA and *p*TA meet these criteria.

The average daily excretion of unconjugated *m*TA and *p*TA in a control human population is about 83 and 489 μg (83 ± 7 and 489 ± 40 respectively, mean ± standard error of the mean, $n = 21$) [2]. Such figures agree well with those published by Boulton and co-workers [5, 6, 11, 12, 14], and others [4, 7—10, 13] at other times and in different laboratories; as can be seen, any variation is surprisingly small. In a longitudinal study in a single individual as reported in this publication, even when there was an interruption of a year in the middle of the urine collection period, and when various potential precursors were ingested, the excretion of unconjugated *m*TA and *p*TA remained remarkably stable and reproducible (98 ± 2.2 and 427 ± 12, μg per day respectively). Further data supporting an essentially exclusive endogenous origin for the unconjugated tyramines are that virtually no free *p*TA was excreted following ingestion of *p*TA (up to 100 mg as free base) although between 2 and 6% was excreted in the free form when *p*TA was injected intravenously [11, 15] and that no *p*TA was excreted in the free form following the ingestion of foods known to be rich in *p*TA. In this latter experiment [12] there was an almost stoichiometric relationship between the *p*TA ingested and the conjugated *p*TA and free and conjugated *p*-hydroxyphenylacetic acid excreted. The unambiguous identification and quantitation of *m*TA and *p*TA in peripheral tissues, in the brain and in subcellular fractions prepared from brain, have recently been established [16—19]. In addition, it has recently been shown [20] (see also 1, 21) that decarboxylation of tyrosine in mammals is unlikely to be the primary synthetic origin of *p*TA. *m*TA arising from *meta*-tyrosine by simple decarboxylation is unlikely to occur since *meta*-tyrosine is not a normal constituent of foods or proteins. Instead, it is now known that *p*TA and *m*TA arise by hydroxylation of PE and dehydroxylation (perhaps associated in some situations with a subsequent decarboxylation) of catecholic precursors; hydroxylation probably represents the major route of synthesis for *p*TA and dehydroxylation the major route for *m*TA [18, 21—28]. The above data, we believe, make it very likely that unconjugated *m*TA and *p*TA are predominantly, if not exclusively, of endogenous origin.

It is clear from Figs. 2—5 and Table II, however, that the conjugated forms of these amines are excreted with considerable daily variation and are affected in some cases by the ingestion of possible precursors. We conclude that they arise from both exogenous and endogenous sources.

The situation with respect to PE and TR is not as clear. These amines are formed predominantly by decarboxylation in mammalian tissues as well as in

bacteria and even in the unconjugated form, are excreted with relatively large day-to-day fluctuations. At this time it is not possible to be sure of their origin, although it must be noted that they are normal constituents of peripheral and cerebral tissues [1, 16—19, 21, 29—33] and they are oxidised and lost after injection at remarkably fast rates [34, 35].

ACKNOWLEDGEMENTS

We thank Dr. B.A. Davis for the generous provision of deuterated standards and precursors and Dr. D.A. Durden for the mass spectrometric analyses. We also thank the Department of Health, Province of Saskatchewan and the Medical Research Council of Canada for continuing financial support.

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