Journal of Chromatography, 230 (1982) 219—230
Biomedical Applications
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROMBIO. 1260

PLASMA CONCENTRATIONS OF *p*- AND *m*-HYDROXYPHENYLACETIC ACID AND PHENYLACETIC ACID IN HUMANS

GAS CHROMATOGRAPHIC—HIGH-RESOLUTION MASS SPECTROMETRIC ANALYSIS

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(First received October 13th, 1981; revised manuscript received February 17th, 1982)

SUMMARY

Conjugated and unconjugated phenylacetic acid and *m*- and *p*-hydroxyphenylacetic acid have been determined in the plasma of normal, healthy subjects after fasting, consumption of a meal and ingestion of deuterium-labelled amine precursors, by high-resolution gas chromatography—high-resolution mass spectrometry with selected ion monitoring of their trifluoroethyl-pentafluoropropionyl derivatives.

We observed that all three conjugated acids are higher in fasting than in non-fasting subjects, and unconjugated phenylacetic acid was lower. Ingestion of deuterium-labelled amine precursors resulted in the appearance in the blood of the correspondingly labelled acids, a peak in the concentrations being reached about 1 h after consumption. Conjugated and unconjugated acids as expected increased following the consumption of a meal.

Unconjugated phenylacetic acid was significantly higher in females than in males. Most values tended to increase with age, with male unconjugated and conjugated *m*-hydroxyphenylacetic acid and female conjugated phenylacetic and *m*-hydroxyphenylacetic acids increasing significantly.

INTRODUCTION

It has been claimed that phenylacetic acid levels in blood plasma in aggressive psychopaths are elevated [1]. In cerebrospinal fluid, it has similarly been claimed to be elevated in schizophrenics [2] but reduced in depressive patients [3]. Such findings have been interpreted as indicating a role for phenylethylamine in the aetiology of these disease states.

We have previously reported that some of the trace amines, phenylethyl-

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amine and *m*- and *p*-tyramine, are abnormally excreted in Parkinsonism [4] and schizophrenia [4, 5]. In addition it has been shown that the trace amines are found in the brain and in nerve endings [6] and exhibit potent physiological effects when iontophoresed either singly or in combination with noradrenaline and dopamine [7, 8]. Some of them are differentially affected by stimulant and neuroleptic drugs [9] and are taken up and released by unique carrier systems [10—14]. In addition, their role in the brain has been proposed to be that of synaptic activators or modulators [15]. As a preliminary step in a study of the trace amine deamination products (i.e. trace acids) in the blood plasma of aggressive psychopaths, schizophrenics, depressives and agoraphobics, we have first investigated these levels in normal, healthy individuals. In particular, we were interested to investigate the effects of fasting on their plasma concentrations, to assess whether values obtained from the same individual but on different days were reproducible, and to examine the effect of ingestion of some of the trace amines suitably labelled with deuterium on plasma trace acid levels. In addition, the effects of sex and age on the acids levels were also determined.

EXPERIMENTAL

Materials

Phenylacetic acid-α,α-d_2 was synthesized from benzoic acid by reduction with $LiAl^2H_4$ to give benzyl alcohol-α,α-d_2, which was converted to benzyl chloride-α,α-d_2 with thionyl chloride, and finally by treatment of the Grignard complex of benzyl chloride with dry ice to give phenylacetic acid-α,α-d_2. Phenylacetic acid-ring-d_5 was synthesized similarly, starting, however, from toluene-d_8 which was oxidized with permanganate to give benzoic acid-d_5. $LiAlH_4$ was used in the reduction.

m- and *p*-hydroxyphenylacetic acids-α,α-d_2 were prepared starting from the hydroxybenzoic acids. The acids were esterified and the phenolic groups benzylated, reduced with $LiAl^2H_4$ to obtain the deuterated alcohols, which were converted to the chlorides with thionyl chloride and then to the nitriles using potassium cyanide (deuterated solvents required). Hydrolysis of the nitriles in 30% NaO^2H in ^2H_2O gave the O-benzyl acids. Hydrogenolysis over Pd/C yielded *m*- and *p*-hydroxyphenylacetic acids-α,α-d_2. The yield for *m*-hydroxyphenylacetic acid was high, but that for the *p*-hydroxyphenylacetic acid was lower since the nitrile appeared to undergo some kind of dimerization during the basic hydrolysis.

m-Hydroxyphenylacetic acid-d_5 (ring-d_3,α,α-d_2) and *p*-hydroxyphenylacetic acid-d_4 (ring-d_2,α,α-d_2) were synthesized as described previously by exchanges in $^2HCl/^2H_2O$ at elevated temperatures [16]. The syntheses of phenylethylamine-β,β-d_2, *m*- and *p*-tyramine-β,β-d_2 have also been previously described [12].

HPLC grade ethyl acetate, hexane, benzene and triethylamine (Caledon, Georgetown, Canada) were used. All other reagents were obtained at the highest available purity. All glassware was soaked before use in chromic acid, washed in Contrad detergent and finally rinsed several times with distilled water.

Methods

Venous blood samples (20 ml) were drawn into tubes containing sodium citrate in the morning between 8 a.m. and 9 a.m. before breakfast for the fasting group and between 9 a.m. and 10 a.m. after breakfast for the non-fasting group. Blood samples from a few individuals were taken twice, on different days, under otherwise identical conditions to assess the constancy of the concentration in the same person. No control was exercised over the diet of the non-fasting group. The plasma was separated and stored at −70°C until analysis.

For the kinetic study, a fasting blood sample was taken at 11:45 a.m. Lunch was eaten from 12:00 to 12:30 p.m. (beef stroganoff, vegetable soup, salad and tea) and immediately after the meal at 12:30 p.m.) a gelatin capsule containing 25 mg of each of the hydrochloride salts of phenylethylamine-β,β-d_2, *m*- and *p*-tyramine-β,β-d_2 was swallowed. Blood samples were then taken 0.5, 1, 2 and 4 h after swallowing the capsule.

In order to determine unconjugated acid levels, a plasma sample (0.5 ml), to which was added an aqueous solution (100 µl) containing 160 ng of phenylacetic acid-α,α-d_2 (PAA-d_2), 157 ng of phenylacetic acid-ring-d_5 (PAA-d_5), 174 ng of *p*-hydroxyphenylacetic acid (PHPA-d_2), and 49 ng of *m*-hydroxyphenylacetic acid-α,α-d_2 (MHPA-d_2), was diluted to 1 ml with distilled water. For the measurement of labelled acids arising from the ingested labelled trace amines, PAA-d_5 (174 ng), MHPA-d_5 (29 ng) and PHPA-d_4 (48 ng) were used as internal standards. Protein was precipitated from this mixture by adding 0.2 *M* zinc sulfate (1 ml) and 0.2 *M* barium hydroxide (1 ml) followed by centrifugation at 6500 *g* for 20 min. The supernatant was decanted from the precipitate, acidified with 1 *N* hydrochloric acid (200 µl) and the clear solution saturated with about 0.5 g sodium chloride. This mixture was then extracted with ethyl acetate (3 × 2 ml) using a vortex mixer and a small centrifuge for breaking the emulsions. The extracts were combined in a conical test tube and concentrated to about 200 µl by evaporation in a stream of nitrogen while being warmed (about 60°C) in a water bath. After transfer of the concentrated extract and washings to 0.3-ml Reacti-vials (Pierce, Rockford, IL, U.S.A.), triethylamine (40 µl) was added to prevent loss of PAA and the solution evaporated to dryness in a stream of nitrogen. To ensure that the residue was free of moisture, 100 µl of benzene were added followed by re-evaporation. 2,2,2-Trifluoroethanol (150 µl) and pentafluoropropionic anhydride (40 µl) were then added to the residues and heated at 80°C for 1 h. After concentration of the reagents to 30—40 µl in a stream of nitrogen, hexane (150 µl) and pentafluoropropionic anhydride (40 µl) were then added and the mixture again heated for 1 h at 80°C. After cooling, 100 µl of phosphate buffer (1 *M*, pH 6.0) was added and the mixture shaken for 30 sec. The hexane (upper) layer was withdrawn, concentrated to about 20 µl under a stream of nitrogen, and submitted to high-resolution gas chromatographic—high-resolution mass spectrometric analysis using selected ion monitoring (SIM).

For these analyses, 0.3—0.5 µl of the hexane solution was injected without splitting onto a 50-m OV-101 SCOT capillary column, which was mounted in a Hewlett-Packard 5710A gas chromatograph with a splitless injector, and coupled directly via SGE 0.4 mm I.D. glass-lined tubing to the ion source of a

VG 70 70F high-resolution double-focussing mass spectrometer which was equipped with a digital selected ion monitor and a VG M82 multispec data system. The column was operated isothermally at 170°C with a helium flow-rate of 29 cm/sec. Usually after every fourth injection the temperature was increased to 220°C to remove residual compounds. The mass spectrometer was set up for selected ion monitoring at 5000 resolution such that the following ions could be analyzed: molecular ion of PAA [as trifluoroethyl (TFE) derivative] at *m/z* 218.0555; molecular ion of PAA-d_2-TFE at *m/z* 220.0680; molecular ion of PAA-d_5-TFE at *m/z* 223.0868; fragment ion resulting from loss of $COOCH_2CF_3$ for MHPA and PHPA [as pentafluoropropionyl (TFE) derivative], at *m/z* 253.0288; and MHPA-d_2 and PHPA-d_2 at *m/z* 255.0413. Since all three acids are well separated by the capillary column, only three or four masses needed to be recorded at any one time, the mass reference ion, the proteo ion and the deuterated internal standard ions. The retention times of the acid derivatives of PAA, MHPA and PHPA were 4.1 min, 5.8 min and 6.3 min, respectively. The labelled and unlabelled compounds were almost isographic for each of the three acids with the labelled acids eluting a few seconds before the unlabelled acids [16] (see Fig. 1).

Calibration curves for each acid were prepared by carrying through the entire procedure known concentrations of the labelled and unlabelled acids (see Fig. 2). This permitted the results obtained for the blood samples to be corrected for chemical and isotopic impurities in the internal standards and also for differences in the mass spectrometric sensitivity between the labelled and unlabelled compounds. The absolute amount of any particular acid in plasma is given by the equation:

$$A_{\text{plasma}} = \frac{A_{\text{int. std.}} \times H_{\text{plasma}}}{H_{\text{int. std.}}} \times F \times 2 \text{ ng/ml}$$

where A_{plasma} is the amount in ng of acid in the plasma, $A_{\text{int. std.}}$ is the amount in ng of deuterated internal standard added, H_{plasma} and $H_{\text{int. std.}}$ are the heights of the mass spectrometric SIM signals for the plasma (unlabelled acid) and internal standard (labelled acid) respectively, and F is the correction factor obtained from the calibration curves (always less than 1). The whole is multiplied by 2 to give the answer in ng/ml. The peak heights were used instead of peak areas since the widths were constant due to very reproducible chromatography.

With each batch of eight plasma samples, two blanks and two standards were run simultaneously through the entire procedure. In the case of blanks this meant starting with 0.5 ml distilled water and for the standards 0.5 ml of an aqueous solution containing a known amount of the three unlabelled acids.

Total acids (that is, unconjugated plus conjugated) were determined using 0.25 ml plasma. The internal standards were added and the protein precipitated as described for the unconjugated acids, 2 ml concentrated hydrochloric acid were then added and the solutions heated for 2 h in a heating block at 100°C. After cooling, each solution was partially neutralized with 9 pellets of sodium hydroxide and then extracted and processed as described above for the unconjugated acids. The only difference in procedure was that triethylamine

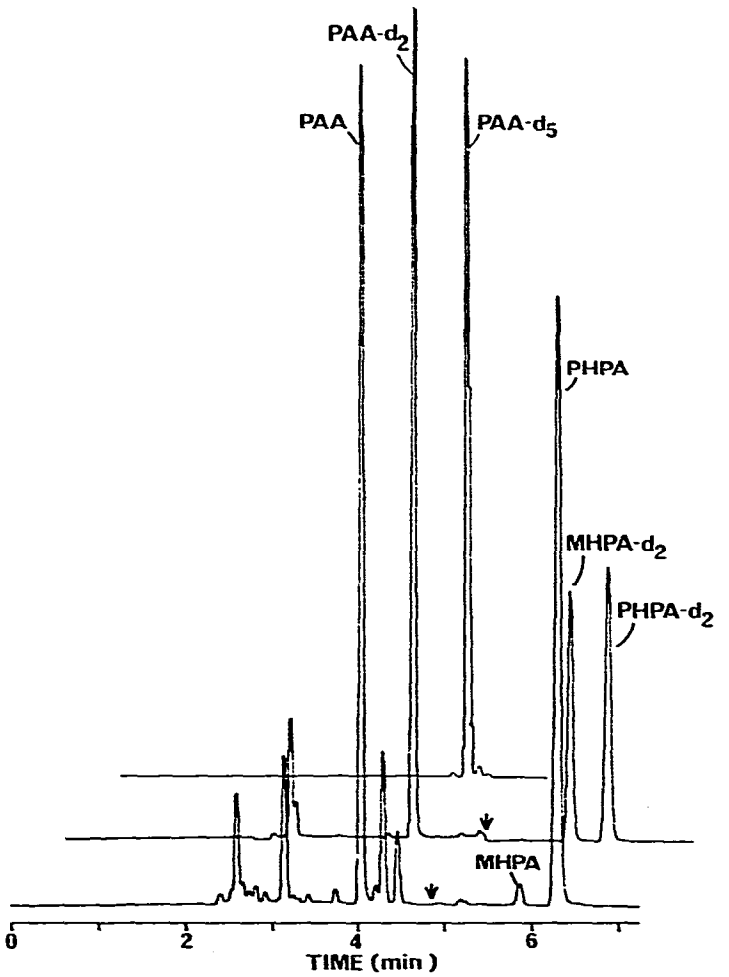

Fig. 1. Mass chromatogram of a human plasma sample showing elution of phenylacetic acid (PAA) at *m/z* 218·0555 and its internal standards PAA-d_2 at *m/z* 220·0680 and PAA-d_5 at *m/z* 223·0868, *m*-hydroxyphenylacetic acid (MHPA) and MHPA-d_2, and *p*-hydroxyphenylacetic acid (PHPA) and MHPA-d_2 at *m/z* 253·0288 and *m/z* 255·0413, respectively. The masses monitored by the SIM unit were changed at the time indicated by the arrows.

was not added after the ethyl acetate had been carefully evaporated just to dryness.

Experiments were undertaken to determine the extent, if any, of exchange of the deuterated standards during the procedure both for the unconjugated and for the total acids. In both cases, known amounts of the labelled acids (PAA-d_5, PAA-d_2, MHPA-d_2, PHPA-d_2) were separately carried through the respective procedures (in duplicate). For these analyses, the mass spectrometer was adjusted so as to measure d_2, d_1 and d_0 for the PAA-d_2, MHPA-d_2, PHPA-d_2, and d_5, d_4 and d_3 for the PAA-d_5.

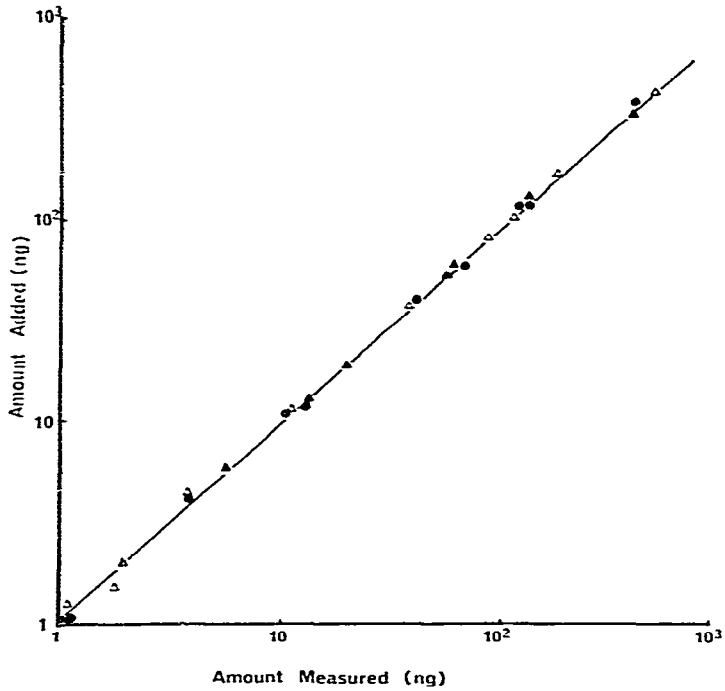

Fig. 2. Calibration curves for PAA (△); MHPA (▲); PHPA (●), using constant amounts of internal standards: 128 ng PAA-d_2, 58 ng MHPA-d_2, 172 ng PHPA-d_2.

RESULTS AND DISCUSSION

The calibration curves for the three trace acids were linear at least from 1—400 ng (Fig. 2), with slopes of 0.94, 1.00 and 0.95 for PAA, MHPA and PHPA, respectively. The three curves were so closely coincident that only one is depicted in the figure. The corresponding correlation coefficients (r) were 0.9998, 0.9985 and 0.9998, respectively. Minimum detectable quantities were 2.0 ng for PAA, 0.8 ng for MHPA and 1.5 ng for PHPA; these are the quantities in 0.5 ml plasma which give rise to signals twice those of the blanks when 40 ng of internal standard are used.

The coefficients of variation for replicate standard samples done on the same day were 4.9% for PAA, 5.7% for MHPA and 1.6% for PHPA, and for replicate samples done on different days were 5.5, 3.5 and 2.3%, respectively. For replicate plasma samples analyzed on different days the coefficients of variation for the unconjugated acids were 4.1% for PAA, 8.9% for MHPA and 4.3% for PHPA, and for total acids they were 7.4, 16.0 and 12.1%, respectively.

In Tables I, II and III are presented the plasma concentrations of total unconjugated and conjugated (calculated by difference) PAA, MHPA and PHPA for 42 different individuals (28 fasting, 14 non-fasting). Previously reported values by Sandler et al. [1] for PAA and Karoum et al. [17] for MHPA and PHPA are also included for comparative purposes for each acid.

It appears that food ingestion affects plasma levels of these acids and that there are, in some cases, significant differences between the levels of the fasting and non-fasting group (see Tables I, II and III). Thus fasting unconjugated PAA

TABLE I

TOTAL, UNCONJUGATED AND CONJUGATED PHENYLACETIC ACID LEVELS IN HUMAN PLASMA (ng/ml)

Number of individuals in each group are indicated in parentheses. Results are expressed as mean ± S.D.

Controls	Total	Unconjugated	Conjugated
Fasting (28)	416 ± 195	107 ± 61*	309 ± 165**
Non-fasting (14)	383 ± 182	155 ± 83.3*	228 ± 132**
Sandler et al. [1] (10)	493 ± 187	107 ± 34	386 ± 160

*These values are significantly different at $p = 0.05$ level.
**These values are significantly different at $p = 0.02$ level.

TABLE II

TOTAL, UNCONJUGATED AND CONJUGATED *m*-HYDROXYPHENYLACETIC ACID LEVELS IN HUMAN PLASMA (ng/ml)

Number of individuals in each group are indicated in parentheses. Results are expressed as mean ± S.D. n.m. = Not measured.

Controls	Total	Unconjugated	Conjugated
Fasting (28)	26.7 ± 23.8	21.5 ± 20.1	5.4 ± 5.6*
Non-fasting (14)	16.9 ± 4.2	17.9 ± 5.2	−1 ± 2.6*,**
Karoum et al. [17] (10)	n.m.	1.0 ± 0.3 (S.E.)	n.m.

*These values are significantly different at $p = 0.001$ level.
**This value is not significantly different from zero.

TABLE III

TOTAL, UNCONJUGATED AND CONJUGATED *p*-HYDROXYPHENYLACETIC ACID LEVELS IN HUMAN PLASMA (ng/ml)

Number of individuals in each group are indicated in parentheses. Results are expressed as mean ± S.D. n.m. = Not measured.

Controls	Total	Unconjugated	Conjugated
Fasting (28)	64.7 ± 40.8	54.1 ± 34.1*	10.6 ± 17.2*
Non-fasting (14)	70.8 ± 28.2	69.0 ± 26.7*	1.8 ± 16.3*
Karoum et al. [17] (10)	n.m.	11.3 ± 0.9 (S.E.)	n.m.

*These results are significantly different, fasting compared to non-fasting, at $p = 0.20$.

was found to be significantly lower than non-fasting unconjugated PAA at $p < 0.05$. Conversely, fasting conjugated PAA appeared to be higher than non-fasting conjugated PAA, but the difference was significant only at $p < 0.20$ with the number of individuals tested. Fasting MHPA levels appear to be higher than non-fasting levels, but only the conjugated acid was significantly higher ($p < 0.001$, Table II). As was the case with PAA, unconjugated PHPA was higher in non-fasting individuals whereas conjugated PHPA levels were higher in the fasting group. Fasting values were obtained on a separate occasion for ten individuals in the non-fasting group. The relationships of the various acid levels were the same as shown by comparison of the two larger groups, but statistical significance could not be obtained due to the small number of samples.

As can be seen in Table I, agreement with the data of Sandler et al. [1] for PAA is good; in the case of MHPA and PHPA (Tables II and III), however, agreement with those of Karoum et al. [17] is poor. In this latter case because Karoum et al. used different preparative procedures (protein was not precipitated for example) we undertook some additional experiments in which the plasma sample was prepared for analysis according to Karoum's method. The results obtained for each of the three acids, by the two different procedures were in agreement and so we conclude that this, at least, was not the cause of the discrepancies.

Because the fasting and non-fasting plasmas were obtained about 60 days apart, we tested for the effect of this by obtaining two fasting blood samples from three different individuals on different days separated by about four weeks. The results for the unconjugated acids with the possible exception of PHPA exhibited relatively little variation (see Table IV).

TABLE IV

COMPARISON OF UNCONJUGATED ACID LEVELS IN TWO PLASMAS TAKEN ONE MONTH APART FROM THREE INDIVIDUALS (ng/ml)

Sample	PAA		MHPA		PHPA	
	Plasma 1	Plasma 2	Plasma 1	Plasma 2	Plasma 1	Plasma 2
1	140.1	119.0	17.0	10.5	28.2	42.1
2	68.6	58.0	0.0	0.8	83.2	44.6
3	317.0	371.2	1.6	2.2	711.8	559.2

Ingestion of the deuterium-labelled amines, phenylethylamine-β,β-d_2, *m*- and *p*-tyramine-β,β-d_2, resulted in the appearance, in the blood, of their deuterated metabolites, PAA-d_2, MHPA-d_2 and PHPA-d_2. In a recent paper, it has been shown that α,α-deuterium-labelled phenylethylamine and *m*- and *p*-tyramine are considerably poorer substrates for monoamine oxidase than the non-deuterated amines, whereas the β,β-dideutero- (and ring-deuterated) amines are either deaminated at the usual rate or at a slightly enhanced rate [18]. As can be seen from Figs. 3, 4 and 5 all three deuterated acids could be detected at all the indicated times of analysis. A peak level was observed in all cases 1 h following the ingestion except in the case of conjugated MHPA and PHPA

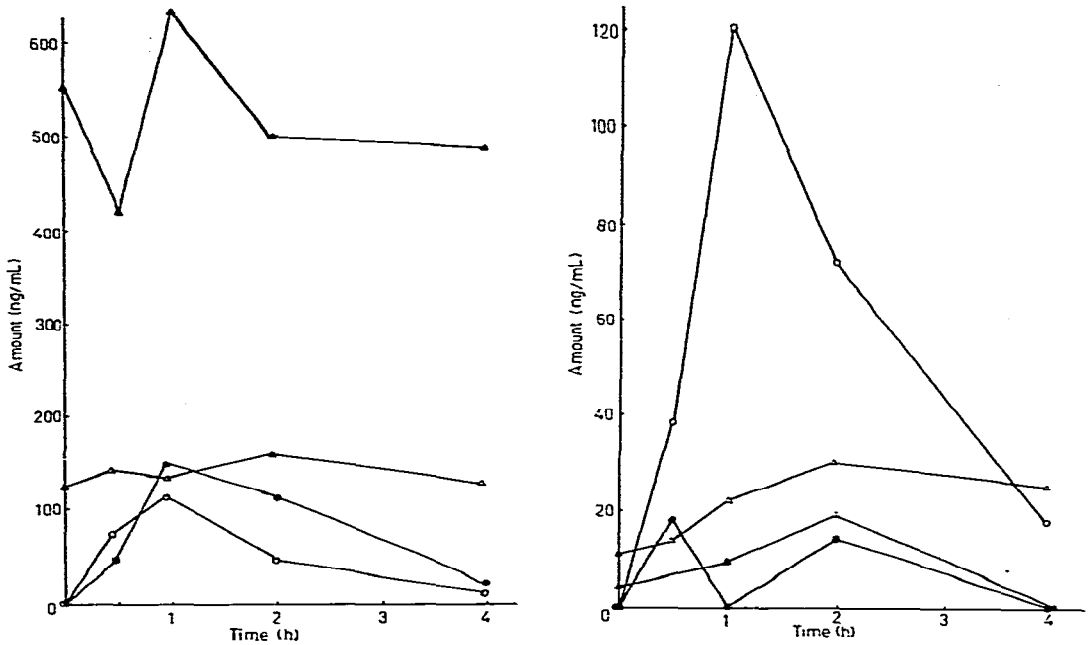

Fig. 3. Plasma levels for phenylacetic acid and phenylacetic acid-α,α-d_2 after consumption of a meal and deuterium-labelled amine precursors. (△) Unconjugated PAA; (▲) conjugated PAA; (○) unconjugated PAA-d_2 ; (●) conjugated PAA-d_2.

Fig. 4. Plasma levels for *m*-hydroxyphenylacetic acid and *m*-hydroxyphenylacetic acid-α,α-d_2 after consumption of a meal and deuterium-labelled precursors. (△) Unconjugated MHPA; (▲) conjugated MHPA; (○) unconjugated MHPA-d_2; (●) conjugated MHPA-d_2.

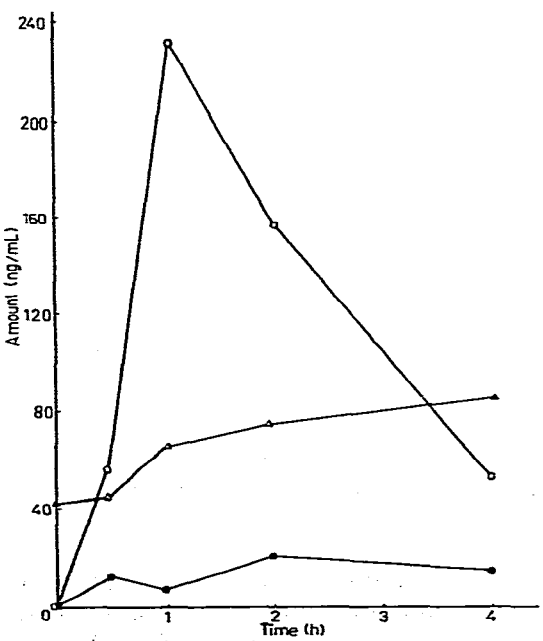

Fig. 5. Plasma levels for *p*-hydroxyphenylacetic acid and *p*-hydroxyphenylacetic acid-α,α-d_2 after consumption of a meal and deuterium-labelled precursors. (△) Unconjugated PHPA; (○) unconjugated PHPA-d_2; (●) conjugated PHPA-d_2. No conjugated PHPA was detected.

which were present only in relatively tiny quantities. A small contribution to MHPA-d_2 and PHPA-d_2 will arise by hydroxylation of the ingested phenylethylamine-d_2 as has recently been shown by Davis and Boulton [19].

The levels of unlabelled acids in the plasma of one individual were measured following the consumption of a meal (see Figs. 3, 4 and 5). Unconjugated PAA rose modestly and MHPA peaked at about 2 h whereas unconjugated PHPA levels continued unexpectedly to rise up to 4 h (samples were not obtained after 4 h). Conjugated PAA increased only slightly and reached a maximum at 1 h, whereas conjugated MHPA reached a maximum at 2 h. Conjugated PHPA could not be detected.

The results obtained in these studies indicate that in order to obtain consistency in trace acid measurements in blood, samples should be taken prior to eating the first meal of the day or at least 3 h after a meal.

The results from the fasting samples were analyzed for differences due to age and sex (Table V). Females were found to have significantly higher unconjugated PAA than males ($p < 0.01$), which is in agreement with the observations of Sandler et al. [1] that unconjugated PAA is higher in cerebrospinal fluid of control (not significant) [2, 3], depressive ($p < 0.005$) [3] and schizophrenic ($p < 0.01$) [2] females than for males.

TABLE V

CORRELATIONS OF TRACE ACID LEVELS WITH SEX AND AGE

	Mean ± S.D. (ng/ml)	Correlation coefficient (r)	p (correlation with age)
Males			
Unconjugated PAA	79.5 ± 47.6*	−0.13	n.s.**
Conjugated PAA	296.4 ± 148.3	−0.06	n.s.
Unconjugated MHPA	16.6 ± 12.9	+0.51	<0.05
Conjugated MHPA	10.0 ± 14.8	+0.49	<0.05
Unconjugated PHPA	66.6 ± 76.1	+0.14	n.s.
Conjugated PHPA	17.7 ± 14.1	−0.30	n.s.
Females			
Unconjugated PAA	119.5 ± 50.5	+0.24	n.s.
Conjugated PAA	365.6 ± 156.9	+0.51	<0.05
Unconjugated MHPA	22.6 ± 21.9	+0.22	n.s.
Conjugated MHPA	5.5 ± 4.7	+0.48	<0.05
Unconjugated PHPA	59.6 ± 39.0	+0.42	<0.07
Conjugated PHPA	13.0 ± 15.7	+0.37	n.s.

*This value is significantly different at $p < 0.01$ from female unconjugated PAA.
**n.s. = not significant.

When combined male and female values were subjected to linear regression analysis no trends according to age were observed. When the values were divided into male and female groups, however, trends were observed (Table V). Most values tended to increase with age, with male unconjugated and conjugated MHPA and female conjugated PAA and conjugated MHPA having

correlation coefficients of about 0.5, all of which proved to be significant at the $p < 0.05$ level. The mean ages were 35.7 years for males (range 24—52) and 30.2 years for females (range 21—50).

In order to determine total acid levels (or conjugated levels which are obtained by subtracting unconjugated values from the total value) plasma must be hydrolyzed by heating with hydrochloric acid. The customary practice of adding the internal standards to the plasma before processing can lead to errors, since back-exchange of some of the label is possible, depending on the location of the deuterium atoms. Deuterium atoms on the side-chain undergo exchange during acid hydrolysis. Under the conditions used here (approximately 5 *N* hydrochloric acid at 100°C for 2 h) 23% of the PAA-d_2 was converted to PAA-d_1 (about 90%) and PAA (about 10%). Similar amounts of exchange were observed for MHPA-d_2 and PHPA-d_2. This problem can be circumvented in a number of ways. The internal standards can be added before hydrolysis and the results corrected for the exchange; alternatively the internal standards can be added after hydrolysis. We have analysed each of the three acids using both methods and have found that the results compare very well. A third option would be to use standards whose label cannot undergo exchange. This is not feasible in the case of MHPA and PHPA (where most of the ring protons or deuterons are exchangeable), but it is feasible for PAA. Another alternative would be to use ^{13}C- or ^{18}O-labelled acids; however, the high cost of the very highly enriched isotopically-labelled compounds which would be required makes them impractical for routine use in most laboratories. We have opted for, and therefore recommend, the addition of both PAA-d_2 (side-chain) and PAA-d_5 (ring) as well as MHPA-d_2 and PHPA-d_2 (both side-chain) prior to hydrolysis. A comparison of the results obtained using PAA-d_5 as internal standard with those using PAA-d_2 permits the determination of the correction factor for every sample (this turns out to be consistently in the range 0.76—0.78 for PAA-d_2). This correction factor for PAA-d_2 has been found also to apply to MHPA-d_2 and PHPA-d_2. It should be noted that the extent of exchange is highly temperature dependent. At 110°C, nearly 35% of the $^2H_2$-acid is lost, whereas at 95°C, about 13% is lost.

No exchange was found to occur during the determination of unconjugated acids, where no heating under strong acid conditions is required.

The method described here is sensitive and specific as a consequence of both the high resolution of the capillary column in the gas chromatograph and of the high-resolution double-focussing mass spectrometer. The method is presently being applied to an analysis of PAA, MHPA and PHPA in plasma obtained from patients suffering with schizophrenia, manic depression, agoraphobia and violent criminal behavior.

ACKNOWLEDGEMENTS

We wish to thank Ms. S. Smith for the mass spectrometric analyses, Ms. T. Osis for the sample preparations and Saskatchewan Health and the Canadian Medical Research Council for continuing financial support.

REFERENCES

1 M. Sandler, C.R.J. Ruthven, B.L. Goodwin, H. Field and R. Matthews, Lancet, ii (1978) 1269.
2 M. Sandler, C.R.J. Ruthven, B.L. Goodwin, G.S. King, B.P. Pettit, G.P. Reynolds, S.P. Tyrer, M.P. Weller and S.R. Hirsch, Commun. Psychopharmacol., 2 (1978) 199.
3 M. Sandler, C.R.J. Ruthven, B.L. Goodwin and A. Coppen, Clin. Chim. Acta, 93 (1979) 169.
4 A.A. Boulton, R.J. Pollitt and J.R. Majer, Nature (London), 215 (1967) 132.
5 A.A. Boulton, G.L. Marjerrison and J.R. Majer, J. Med. Acad. Sci. (USSR), 5 (1971) 68.
6 A.A. Boulton and G. Baker, J. Neurochem., 25 (1975) 477.
7 R.S.G. Jones and A.A. Boulton, Can. J. Physiol. Pharmacol., 58 (1980) 222.
8 R.S.G. Jones and A.A. Boulton, Life Sci., 27 (1980) 1849.
9 A.V. Juorio, Brit. J. Pharmacol., 66 (1979) 377.
10 E.H. Petrali, A.A. Boulton and L.E. Dyck, Neurochem. Res., 4 (1979) 633.
11 L.E. Dyck, A.A. Boulton and R.S.G. Jones, Eur. J. Pharmacol., 68 (1980) 33.
12 L.E. Dyck and A.A. Boulton, Res. Commun. Psychol. Psychiatry Behav., 5 (1980) 61.
13 A.A. Boulton and L.E. Dyck, Res. Commun. Psychol. Psychiatry Behav., 5 (1980) 79.
14 L.E. Dyck, Eur. J. Pharmacol., 69 (1981) 371.
15 A.A. Boulton, in E. Usdin and M. Sandler (Editors), Trace Amines and the Brain, Marcel Dekker, New York, 1976, p. 21.
16 D.A. Durden and A.A. Boulton, J. Neurochem., 36 (1981) 129.
17 F. Karoum, J. Moyer-Schwing, S.G. Potkin and R.J. Wyatt, Commun. Psychopharmacol., 1 (1977) 343.
18 P.H. Yu, S. Barclay, B. Davis and A.A. Boulton, Biochem. Pharmacol., 30 (1981) 3089.
19 B.A. Davis and A.A. Boulton, Eur. J. Mass Spectrom., 1 (1980) 149.