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SENSITIVE DETERMINATION OF DEUTERATED AND NON-DEUTERATED TRYPTOPHAN, TRYPTAMINE AND SEROTONIN BY COMBINED CAPILLARY GAS CHROMATOGRAPHY AND NEGATIVE ION CHEMICAL IONIZATION MASS SPECTROMETRY

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SUMMARY

Sensitive methods for the determination of deuterated and non-deuterated tryptophan, tryptamine and serotonin by combined capillary gas chromatography and negative ion chemical ionization mass spectrometry were developed. [3,3-$^2H_2$]-L-Tryptophan, which was used as a tracer, was synthesized for studies of their in vivo metabolism. Tryptophan was converted into its trifluoroacetylmethyl derivative after prepurification with an AG 50W-X2 cation-exchange column. Tryptamine and serotonin were extracted with 20% butanol in diethyl ether and derivatized with trifluoroacetic anhydride. These derivatives were separated and determined by selected ion monitoring. In these determinations, [2′,3,3,4′,5′,6′,7′-$^2H_7$]-D,L-tryptophan, [$\alpha,\alpha,\beta,\beta$-$^2H_4$]tryptamine and [$\alpha,\alpha,\beta,\beta$-$^2H_4$]serotonin were used as internal standards.

INTRODUCTION

Since the proposal of the biogenic amine hypothesis of affective disorders, many investigations have been carried out. These attempts to verify the amine hypothesis have not given uniform results, suggesting either multifactorial etiologies of the disease or complexity in metabolism and transport of biogenic amines in both the central nervous and peripheral systems. Therefore, it is important to develop

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a new method to investigate in detail the in vivo metabolism of biogenic amines.

Recently, there has been an almost exponential growth rate in the application of stable isotopes to investigate the in vivo metabolism of certain compounds. Many investigators have reported that gas chromatography–mass spectrometry (GC–MS) using a compound labelled with a stable isotope as a tracer is a powerful tool for these metabolic studies. The rapidly developing technique of negative ion chemical ionization mass spectrometry (NICIMS) has been used to achieve highly sensitive analyses of compounds with high electron affinity [1]. In a previous paper [2], it was reported that trifluoroacetylation and pentafluoropropionylation are very effective for enhancing sensitivity, by producing favourable fragmentation of different kinds of indolic compounds.

In the present paper, we describe both the synthesis of [3,3-$^2H_2$]-L-tryptophan as a tracer and also sensitive GC–NICIMS methods for the determination of tryptophan, tryptamine and serotonin. Tracer experiments were also carried out.

EXPERIMENTAL

Apparatus

A Finnigan 4000 gas chromatograph–mass spectrometer equipped with a pulsed positive ion negative ion chemical ionization accessory (Finnigan, CA, U.S.A.) was used. Sample injections were performed with a solvent cut injector (Gaskuro Kogyo, Tokyo, Japan). GC separation was carried out with an OV-101 coated fused-silica capillary column (25 m×0.25 mm I.D.). The capillary column was connected directly to the mass spectrometer. Methane was used as the GC carrier gas and the chemical ionization reagent gas. The capillary column head-pressure was held at 0.6 kg/cm^2, which gave a flow-rate of methane of 1 ml/min. The ion-source pressure, the ion-source temperature, the electron energy and the emission current were maintained at 0.15 Torr, 250°C, 90 eV and 300 μA, respectively.

Reagents

[$^2H_2$]Paraformaldehyde, [$\alpha,\alpha,\beta,\beta$-$^2H_4$]serotonin creatinine sulphate and [$\alpha,\alpha,\beta,\beta$-$^2H_4$]tryptamine hydrochloride were purchased from Merck Frosst Canada. Deuterium oxide (D=99.75%) was purchased from Merck Japan. L-Tryptophan, serotonin creatinine sulphate, tryptamine hydrochloride and trifluoroacetic anhydride were purchased from Wako. Acylase "Amano 15000" was kindly supplied by Amono Seiyaku. Cation-exchange resin AG 50W-X2 (200–400 mesh), obtained from Bio-Rad Labs, was used after treatment with a 10-fold volume of 2 *M* hydrochloric acid at 80°C for 4 h and washing with a 50-fold volume of redistilled water. The other reagents and solvents were all reagent grade.

Synthesis of [3,3-$^2H_2$]-L-tryptophan

N-Acetyl[3,3-$^2H_2$]-D,L-tryptophan was synthesized according to the procedure reported by Snyder and Smith [3] with a minor change: the use of [$^2H_2$]paraformaldehyde instead of formaldehyde in the preparation of gramine. N-Acetyl[3,3-$^2H_2$]-D,L-tryptophan (24.8 g) was suspended in redistilled water (490 ml) and dissolved by addition of 2 *M* sodium hydroxide; the pH of the solu-

tion was adjusted to 7.0. The solution was incubated at 35 ± 2 °C for 23 h after addition of acylase "Amano 15000" (518 mg) and cobalt chloride hexahydrate (58 mg). The pH of the solution was adjusted to 5.85 after filtration of the suspended material. Then the solution was concentrated to ca. 100 ml and allowed to stand overnight in a refrigerator. The precipitate was separated by filtration and the second fraction was obtained by adjusting the pH (5.85) and by concentrating the filtrate and allowing it to stand in a refrigerator. The two fractions of the product were combined and recrystallized from 70% aqueous ethanol and dried (yield, 6.8 g; m.p., 254–255 °C; $[\alpha]_D^{20}$, −30.4).

Synthesis of [2′,3,3,4′,5′,6′,7′-$^2H_7$]-D,L-tryptophan, [2′,4′,5′,6′,7′-$^2H_5$]-L-tryptophan, [2′,4′,5′,6′,7′-$^2H_5$]tryptamine, [2′,4′,6′,7′-$^2H_4$]-5-hydroxytryptophan and [2′,4′,6′,7′-$^2H_4$]serotonin

These deuterated compounds were prepared from [3,3-$^2H_2$]-D,L-tryptophan, L-tryptophan, tryptamine, 5-hydroxytryptophan and serotonin according to the method reported by Matthews et al. [4] with slight modifications.

Administration of [3,3-$^2H_2$]-L-tryptophan

[3,3-$^2H_2$]-L-Tryptophan (10 mg/kg) was administered orally at 11:00 am and heparinized blood was obtained from the forearm vein at certain intervals. An aliquot of the blood sample was centrifuged at 10 000 *g* for 15 min in a refrigerated centrifuge. Plasma obtained was stored at −20 °C until analysed. The remaining blood sample was diluted with an equal volume of water and stored at −20 °C until analysed. Urine was also collected at certain intervals. After measurement of the volume, an aliquot of the urine sample was stored at −20 °C until analysed.

Procedure for the determination of deuterated and non-deuterated tryptophan in plasma

Plasma samples (20 μl) were deproteinized with 0.5 ml of 80% aqueous ethanol containing 200 ng of [2′,3,3,4′,5′,6′,7′-$^2H_7$]-D,L-tryptophan and centrifuged in a microcentrifuge (Sakuma, Model M-15-3). The supernatant was evaporated to dryness on a rotary evaporator and the residue was redissolved in 1 ml of 25 m*M* pyridine–formate buffer (pH 2.5). The solution was applied to a 150 mm × 5 mm I.D. glass column containing 0.3 ml of AG 50W-X2. The column was drained, then washed with 5 ml of 25 m*M* pyridine–formate buffer (pH 3.0). Then the amino acids were eluted from the column with 6 ml of 25 m*M* pyridine–formate buffer (pH 4.5). The eluate was evaporated to dryness and the residue was redissolved in 300 μl of ethanol. The solution was transferred to a siliconized glass ampoule, and the ethanol was evaporated under a stream of nitrogen.

A 100-μl volume of a trifluoroacetic anhydride (TFAA)–acetonitrile (1:1) mixture was added to the residue. After a reaction for 5 min at room temperature, the excess TFAA and acetonitrile were evaporated under a stream of nitrogen. Then 300 μl of diazomethane solution in diethyl ether (ca. 1%) were added to the residue, and the solution allowed to stand for 10 min at room temperature. The excess diazomethane solution was removed under a stream of nitrogen. The

residue was redissolved in 50 μl of the TFAA–acetonitrile mixture, and 1 μl of the resulting solution was subjected to GC–NICIMS.

Procedure for the determination of deuterated and non-deuterated tryptamine and serotonin in blood samples and urine samples

An internal standard solution was prepared by diluting (1:200) stock solution ([α,α,β,β-$^2H_4$]tryptamine, [α,α,β,β-$^2H_4$]serotonin 400 μg/ml 0.01 *M* hydrochloric acid), and 100 μl were added to 1.0 ml of diluted blood or urine sample. The amines were extracted with 5 ml of 20% *n*-butanol solution in diethyl ether after addition of 1 ml of 0.75 *M* sodium phosphate buffer (pH 10.0), which contained 260 mg of sodium chloride and 1 mg of ethylenediaminetetraacetic acid disodium salt. Then 4 ml of *n*-hexane were added to the organic layer, and the amines were extracted from the organic layer with 300 μl of 0.1 *M* hydrochloric acid by shaking for 5 min. After the aqueous phase had been frozen in the dry ice–acetone bath, the upper organic layer was removed by decantation. The aqueous phase was washed with 4 ml of *n*-hexane, and the *n*-hexane layer was removed in the same way. The aqueous layer was transferred to a glass ampoule and evaporated to dryness under a stream of nitrogen. Then 50 μl of the TFAA–acetonitrile (1:1) mixture was added to the residue. The ampoule was sealed and allowed to react at 60°C for 30 min. The reaction mixture was subjected to GC–NICIMS.

RESULTS AND DISCUSSION

It is well known that NICIMS can provide a sample ion current 100–1000 times greater than that available from positive ion methodology for particular classes of molecules, and that the electron affinity is the most important factor for the sensitivity [1]. Most biological compounds, however, do not have a strong electron affinity, and it is essential to convert them into effective derivatives as a means of enhancing the sensitivity for the ultramicro determination. Furthermore, the usefulness of NICIMS for medical and biological applications also depends on the intrinsic property of the fragmentation of the molecular ion, which requires that the negative charge is retained on the main fragment of the molecule to be analysed. In a previous paper [2], it was reported that the introduction of the trifluoroacetyl (TFA) or the pentfluoropropionyl (PFP) group onto the indole ring resulted in a large increase in the sensitivity, and that the derivative showed useful prominent negative fragment ions that contained the moiety of the original compound to be analysed.

From these data and the results of other preliminary investigations, the derivatives illustrated in Figs. 1–3 were adopted for the highly sensitive GC–NICIMS. Fig. 1 shows a methane NICI mass spectrum of the TFA-methyl derivative of tryptophan which consists of $(M-H-\quad)^-$ at *m/e* 183 and $(M-HF)^-$ at *m/e* 390. In the methane NICI mass spectrum of this derivative of [2′,3,3,4′,5′,6′,7′-$^2H_7$]tryptophan, the related fragment peaks were found at *m/e*

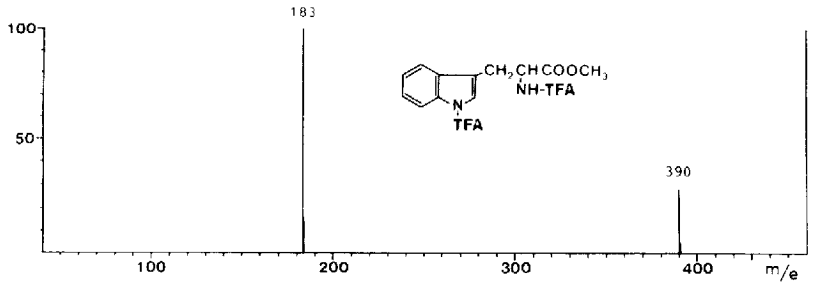

Fig. 1. Methane NICI mass spectrum of the TFA-methyl derivative of tryptophan.

183 and 397. These data suggest that a hydrogen atom at the 2-position or a hydrogen of the NH group might be eliminated as HF to give the fragment ion $(M-HF)^-$.

In Figs. 2 and 3, methane NICI mass spectra of TFA derivatives of tryptamine and serotonin are shown. The base peak, which could be assigned to $(M-HF)^-$, was found in both spectra. By comparing their mass spectra with those of the TFA derivatives of [2′,4′,5′,6′,7′-$^2H_5$]tryptamine, [α,α,β,β-$^2H_4$]tryptamine and [α,α,β,β-$^2H_4$]serotonin it was deduced that the hydrogen atom of the NH group in the side-chain might be eliminated as HF.

In tracer experiments using stable-isotope-labelled compounds, deuterium-labelled compounds are often used as tracers and internal standards. In such cases, it is important to use deuterium-labelled compounds that do not suffer from the loss of labels. Curtius and co-workers used [2′,4′,5′,6′,7′-$^2H_5$]-L-tryptophan as a tracer for in vivo studies of the tryptophan-5-hydroxylase system [5]

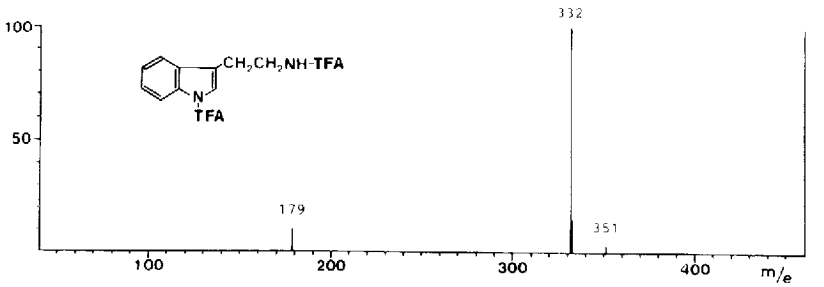

Fig. 2. Methane NICI mass spectrum of the TFA derivative of tryptamine.

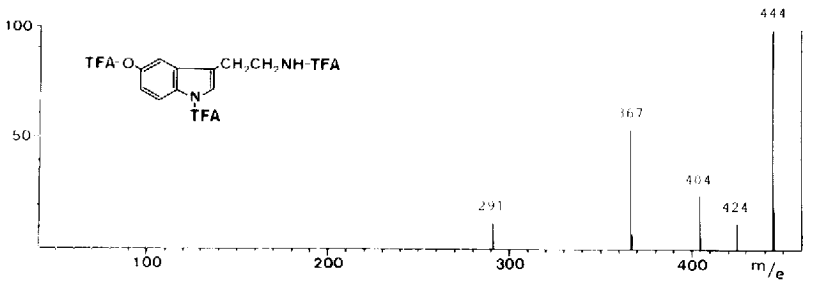

Fig. 3. Methane NICI mass spectrum of the TFA derivative of serotonin.

TABLE I

REPRODUCIBILITY OF THE PEAK-HEIGHT RATIOS AFTER TFA OR TFA-METHYL DERIVATIZATION OF MIXTURES OF DEUTERATED AND NON-DEUTERATED COMPOUNDS

Abbreviations: TA = tryptamine; TRP = tryptophan; 5-HTP = 5-hydroxytryptophan; 5-HT = serotonin.

Sample No.	$[^2H_0]/[^2H_5]$		$[^2H_0]/[^2H_4]$		
	$[^2H_5]$TA	$[^2H_5]$TRP	$[^2H_4]$-5-HTP	$[^2H_4]$-5-HT	$[\alpha,\alpha,\beta,\beta\text{-}^2H_4]$-5-HT
1	2.00	0.76	21.79	50.84	2.79
2	1.96	0.76	6.39	6.77	2.84
3	2.00	0.74	21.59	23.55	2.82
4	2.08	0.76	9.08	47.18	2.75
5	2.00	0.74	2.83	40.30	2.78

and for measurement of tryptophan pyrolase activity in vivo [6]. However, they do not report on the stability of the labels.

Table I shows the reproducibility of the peak-height ratios of non-deuterium-labelled indolic compounds to their ring-deuterium-labelled ones after TFA or TFA-methyl derivatization. The derivatization and the measurement were repeated for each mixture containing non-deuterated and deuterated compounds in a certain ratio. The mixture of ring-deuterated 5-hydroxytryptophan and serotonin, which both have a hydroxyl group at the 5′-position, gave a broad range of the ratios, whereas the mixture of tryptamine and tryptophan provided reproducible values. Similar experiments were carried out for $[\alpha,\alpha,\beta,\beta\text{-}^2H_4]$tryptamine and $[\alpha,\alpha,\beta,\beta\text{-}^2H_4]$serotonin. In these cases, reproducible results were obtained for both compounds. These results seem to recommend the use of side-chain-labelled tryptophan as a tracer for the tracer experiments. Therefore we decided to synthesize $[3,3\text{-}^2H_2]$-L-tryptophan as a tracer for the metabolic investigation of tryptophan to serotonin and tryptamine in vivo.

N-Acetyl-$[3,3\text{-}^2H_2]$-D,L-tryptophan was synthesized according to the method for N-acetyltryptophan reported by Snyder and Smith [3] with some modifications. The deuterium was introduced in the gramine preparation step by using $[^2H_2]$paraformaldehyde, and $[3,3\text{-}^2H_2]$-L-tryptophan was obtained by the enzymic hydrolysis of N-acetyl-$[3,3\text{-}^2H_2]$-D,L-tryptophan. Three multiplets at ca. 7.7 ppm, 7.5 ppm and 7.2 ppm, which could be assigned to indole ring protons (5H), and a singlet at ca. 4.0 ppm, a methine proton (1H), were found on the ^1H NMR spectrum of $[3,3\text{-}^2H_2]$-L-tryptophan measured in deuterium oxide. ^1H NMR measurements indicated that two deuterium atoms were at the methylene group in the side-chain. The percentages of non-, mono- and dideuterated species in $[3,3\text{-}^2H_2]$-L-tryptophan preparation were calculated from the relative intensities of peaks at *m/e* 411–416 in a methane CI positive mass spectra measured for L-tryptophan and $[3,3\text{-}^2H_2]$-L-tryptophan, assuming that the relative peak heights of the isotopic ions $(M+1)^+$, $(M+1+1)^+$, $(M+1+2)^+$ and $(M+1+3)^+$ of each species are analogous. The isotopic purity of $[3,3\text{-}^2H_2]$-L-tryptophan was 94.1% (Table II).

TABLE II

PERCENTAGES OF NON-, MONO- AND DIDEUTERATED L-TRYPTOPHAN IN THE [3,3-$^2H_2$]-L-TRYPTOPHAN PREPARATION

Species (%)	Percentage
$^2H_0$	0.57
$^2H_1$	5.2
$^2H_2$	94.1

In this work, [2′,3,3,4′,5′,6′,7′-$^2H_7$]-D,L-tryptophan, [α,α,β,β-$^2H_4$]tryptamine and [α,α,β,β-$^2H_4$]serotonin were used as the internal standards for the determination of non-deuterated and deuterated tryptophan, tryptamine and serotonin in biological samples. [2′,3,3,4′,5′,6′,7′-$^2H_7$]-D,L-tryptophan was prepared by ^2H-^1H exchange of [3,3-$^2H_2$]-D,L-tryptophan according to the method reported by Matthews et al. [4] for the synthesis of [$^2H_5$]tryptophan. Table III shows the relative intensities of the isotopic peaks that were mesured for the derivatized internal standards.

For the analysis of these compounds in physiological fluids a prepurification step was necessary. Tryptophan in plasma was purified using an AG 50W-X2 column after deproteinization. In this step, pyridine–formate buffer was used as the eluent because it provided a salt-free sample after evaporation.

For tryptamine and serotonin in blood, plasma or urine, the solvent extraction method reported by Maruyama and Takemori [7] was used with some modifications. In each prepurification step, [2′,3,3,4′,5′,6′,7′-$^2H_7$]-D,L-tryptophan, [α,α,β,β-$^2H_4$]tryptamine or [α,α,β,β-$^2H_4$]serotonin showed no detectable loss of the labels. These data also suggest that [3,3-$^2H_2$]-L-tryptophan and its metabolites, [$^2H_2$]serotonin and [$^2H_2$]tryptamine must suffer no detectable loss of the labels, because [2′,3,3,4′,5′,6′,7′-$^2H_7$]-D,L-tryptophan, [α,α,β,β-$^2H_4$]serotonin and [α,α,β,β-$^2H_4$]tryptamine have deuterium atoms at the positions where [3,3-$^2H_2$]-L-tryptophan, [$^2H_2$]serotonin and [$^2H_2$]tryptamine have deuterium atoms.

In the metabolic studies, the tracer compound and its metabolites were stable for ^2H-^1H exchange under in vivo conditions. To check the loss or scrambling of the labels, an investigation of ^2H-^1H exchange of [3,3-$^2H_2$]-L-tryptophan,

TABLE III

RELATIVE INTENSITIES OF THE ISOTOPIC PEAKS OF INTERNAL STANDARDS

Abbreviations: TRP = tryptophan; 5-HT = serotonin; TA = tryptamine.

Sample	Relative peak heights		
[$^2H_7$]TRP	*m/e* 390	*m/e* 392	*m/e* 397
	0.1	1.1	100.0
[$^2H_4$]-5-HT	*m/e* 444	*m/e* 446	*m/e* 448
	0.1	0.4	100.0
[$^2H_4$]TA	*m/e* 332	*m/e* 334	*m/e* 336
	0.2	0.4	100.0

TABLE IV

PEAK-HEIGHT RATIOS OF [3,3-$^2H_2$]-L-TRYPTOPHAN, [$\alpha,\alpha,\beta,\beta$-$^2H_4$]SEROTONIN AND [$\alpha,\alpha,\beta,\beta$-$^2H_4$]TRYPTAMINE TO THE NON-DEUTERATED COMPOUNDS FOR THE SAMPLE CONTAINING THE DEUTERATED AND NON-DEUTERATED COMPOUNDS BEFORE AND AFTER INCUBATION

Incubation conditions: 4 h at 37°C. Abbreviations: TRP = tryptophan; 5-HT = serotonin; TA = tryptamine.

Medium	Peak-height ratio		
	[$^2H_2$]TRP/TRP	[$^2H_4$]-5-HT/5-HT	[$^2H_4$]TA/TA
-	0.420	0.933	0.961
0.01 *M* Hydrochloric acid	0.419	0.912	0.958
0.01 *M* Phosphate buffer (pH 7.0)	0.420	0.928	0.961
0.01 *M* Phosphate buffer (pH 9.0)	0.420	0.925	0.946

[$\alpha,\alpha,\beta,\beta$-$^2H_4$]serotonin and [$\alpha,\alpha,\beta,\beta$-$^2H_4$]tryptamine in 0.01 *M* hydrochloric acid and 0.01 *M* phosphate buffers (pH 7.0, 9.0) was carried out. Table IV shows the peak-height ratios of [3,3-$^2H_2$]-L-tryptophan, [$\alpha,\alpha,\beta,\beta$-$^2H_4$]serotonin and [$\alpha,\alpha,\beta,\beta$-$^2H_4$]tryptamine to the non-deuterated compounds for the sample containing the deuterated and non-deuterated compounds before and after incubation (4 h).

Calibration curves for [3,3-$^2H_2$]-L-tryptophan and non-deuterated tryptophan, serotonin, and tryptamine were prepared by plotting the corresponding peak-height ratios against that of the internal standard after correction for background due to the isotopic peak on the y axis, and the amount (nmol) of each compound on the x axis. The curves were linear and passed through the origin in the concentration range from at least 1 pmol to 5 nmol of [3,3-$^2H_2$]-L-tryptophan and tryptophan, from at least 0.5 pmol to 2 nmol of serotonin and from at least 0.1 pmol to 2 pmol of tryptamine.

The curve for [$^2H_2$]serotonin was prepared in the following manner. When the calibration curve for non-deuterated serotonin was expressed as $y = ax$, that for [$^2H_2$]serotonin was expressed as $y = (K_2/K_0)ax$, where K_2 and K_0 are the ratios of the ion abundances at m/e 446 or m/e 444 to total abundance of the isotopic ions corresponding to $(M-HF)^-$ for the derivatives of [$^2H_2$]serotonin and serotonin samples, respectively. The value of K_2 was calculated from the relative isotopic intensities for serotonin and the percentage of deuterated species of [3,3-$^2H_2$]-L-tryptophan (Table II), assuming that the isotopic peak-height ratios corresponding to $(M-HF)^-$ of each species are analogous and that each species was equally metabolized to serotonin in vivo. The curve for [$^2H_2$]tryptamine was prepared in a similar manner to that for [$^2H_2$]serotonin.

Fig. 4. shows the time course of the plasma levels of tryptophan and [$^2H_2$]tryptophan after the oral administration of [3,3-$^2H_2$]-L-tryptophan (10 mg/kg) to a healthy subject. The plasma level of [$^2H_2$]tryptophan increased rapidly and reached maximum ca. 40 min after administration. It then gradually

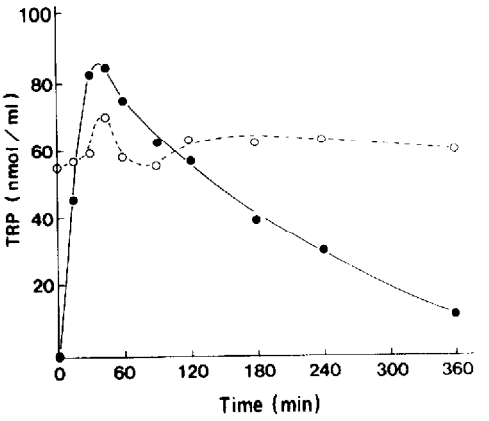

Fig. 4. Time-course of plasma levels of deuterated and non-deuterated tryptophan (TRP) after the oral administration of [3,3-$^2H_2$]-L-tryptophan (10 mg/kg) to a healthy subject. ●—●, [$^2H_2$]tryptophan; O- - -O, non-deuterated tryptophan.

decreased. Compared with daily changes in these levels, the changes caused by the administration of [3,3-$^2H_2$]-L-tryptophan (10 mg/kg) are not particularly large.

Fig. 5 shows the results of determination of serotonin and [$^2H_2$]serotonin excreted into urine after the oral administration of [3,3-$^2H_2$]-L-tryptophan. The results for tryptamine are shown in Fig. 6. It was found that amounts of [$^2H_2$]serotonin and [$^2H_2$]tryptamine comparable with endogenous levels were excreted into urine for several hours after administration. However, measurable amounts were not found in blood during the same period. Fig. 7 shows mass frag-

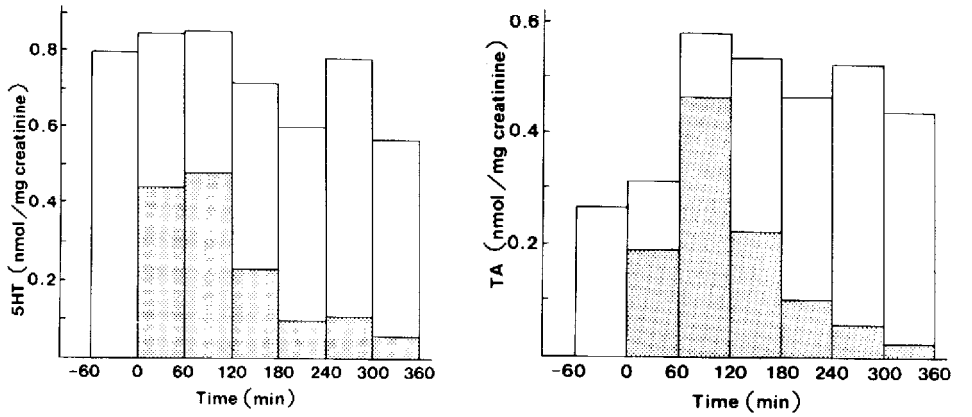

Fig. 5. Time-course of urinary levels of deuterated and non-deuterated serotonin (5HT) after the oral administration of [3,3-$^2H_2$]-L-tryptophan (10 mg/kg) to a healthy subject. Dotted areas, [$^2H_2$]serotonin; open areas, non-deuterated serotonin.

Fig. 6. Time-course of urinary levels of deuterated and non-deuterated tryptamine (TA) after the oral administration of [3,3-$^2H_2$]-L-tryptophan (10 mg/kg) to a healthy subject. Dotted areas, [$^2H_2$]tryptamine; open areas, non-deuterated tryptamine.

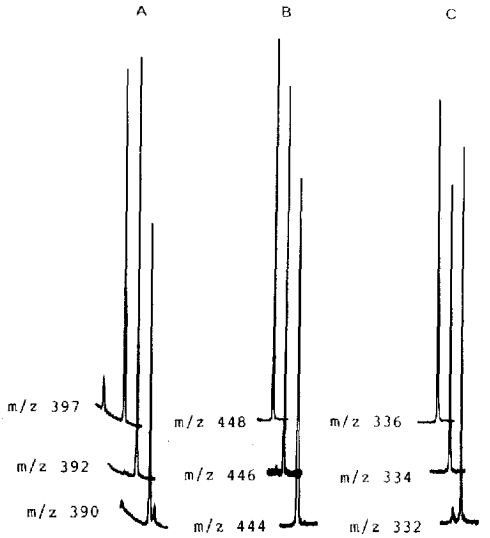

Fig. 7. Mass fragmentograms of (A) plasma tryptophan, (B) urinary serotonin and (C) urinary tryptamine.

mentograms of trytophan (plasma), serotonin (urine) and tryptamine (urine) obtained from samples after administration.

Next, an investigation was carried out to determine if the excretion of [$^2H_2$]serotonin and [$^2H_2$]tryptamine was due to biological isotope effects. A mixture of [3,3-$^2H_2$]-L-tryptophan and L-tryptophan (1:5, 30 mg/kg) was administered to the same subject, and the excretion of deuterated and non-deuterated serotonin and tryptamine was followed. As shown in Figs. 8 and 9, the

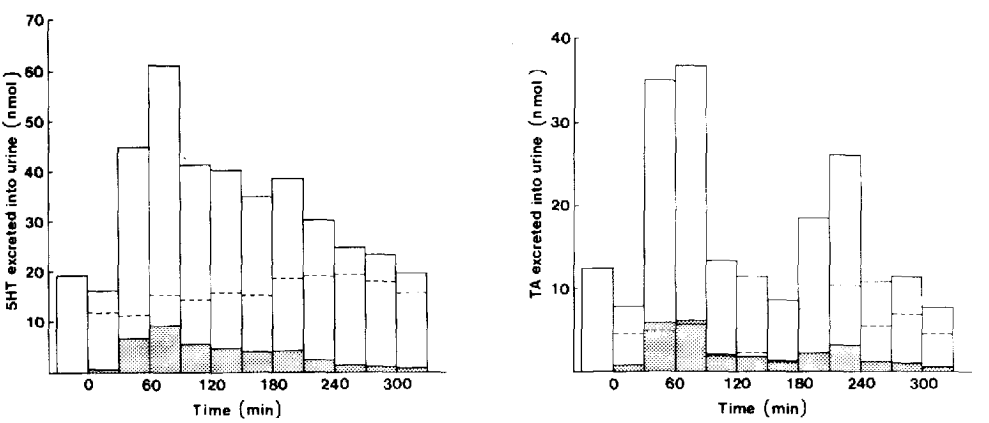

Fig. 8. Time-course of urinary levels of deuterated and non-deuterated serotonin (5HT) after the oral administration of [3,3-$^2H_2$]-L-tryptophan (5 mg/kg) and non-deuterated L-tryptophan (25 mg/kg). Dotted areas, [$^2H_2$]serotonin; open areas, non-deuterated serotonin.

Fig. 9. Time-course of urinary levels of deuterated and non-deuterated tryptamine (TA) after the oral administration of [3,3-$^2H_2$]-L-tryptophan (5 mg/kg) and non-deuterated L-tryptophan (25 mg/kg). Dotted areas, [$^2H_2$]tryptamine; open areas, non-deuterated tryptamine.

maximum excretion of [$^2H_2$]serotonin or [$^2H_2$]tryptamine was observed between 60 min and 90 min after administration, and was identical with that of serotonin or tryptamine. The levels of endogenous serotonin and tryptamine (shown as dashed lines) were estimated on the assumption that [3,3-$^2H_2$]-L-tryptophan was metabolized in vivo and excreted into urine without biological isotope effects. The estimated levels of endogenous serotonin and tryptamine matched expectations.

These data indicate that biological isotope effects might be negligible in the in vivo metabolism of [3,3-$^2H_2$]-L-tryptophan and that the turnover rate of serotonin or tryptamine in certain organs where they are rapidly synthesized and released may reflect the excretion of serotonin and tryptamine. Detailed data about these in vivo metabolisms will be discussed in a further paper.

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