Tissue distribution and metabolism of 5-acetyltryptamine in the mouse

(*Received* 9 *January* 1963; *accepted* 27 *February* 1963)

A NEW tryptamine derivative acetylated in the 5-position of tryptamine, 5-acetyltryptamine (5-AT)[1], has been found to have cardiovascular properties that differ in several aspects from its 5-hydroxy analogs.* Manometric experiments show that the increase in oxygen uptake caused by the addition of 5-AT to a rat liver homogenate could be inhibited by the monoamine oxidase (MAO) inhibitor, phenethylhydrazine, but not by the diamine oxidase inhibitor, isoniazid. Such experiments suggest that 5-AT is a substrate for MAO, but a poor one. Serotonin disappears three to four times more rapidly that 5-AT from a rat liver homogenate and as much as ten times more rapidly from a mouse brain homogenate. Serotonin metabolism has been shown to be inhibited (40 to 60%) by 5-AT at a concentration of 10^{-4} M, being approximately eight times more effective than amphetamine in this respect.† As a result, additional studies were undertaken to clarify 5-AT tissue-distribution pattern and its metabolism in the mouse.

EXPERIMENTAL

The Organic Chemistry Department of the Warner-Lambert Research Institute synthesized the following: ^{14}C 5-acetyltryptamine (^{14}C 5-AT), labeled on the acetyl group, 5-acetyltryptamine, and 5-acetyl indole-3-acetic acid (5-AI3AA). The other chemicals used were obtained from commercial sources. Female mice of the M-1 strains (Manor Farms, Staatsburg, New York), weighing 18 to 22 g, were used.

For measurement of ^{14}C 5-AT distribution, the compound, with a specific activity of 0·4 μc/mg, was intravenously injected at 10 mg/kg. At precisely timed intervals after the injection, the mice were sacrificed and the tissues removed, blotted dry (to remove all blood and clots), weighed, and immediately frozen in dry ice. Three pooled organs of one type were homogenized in 10·0 ml of 0·1 N HCl and brought to a pH of 10 with saturated sodium carbonate. To the whole homogenate 1·0 ml of borate buffer[2] (pH 10), 1 g of sodium chloride, and 15·0 ml of n-butanol were added. The mixture was shaken for 30 min and then centrifuged. The n-butanol layer was drawn off and passed through sodium sulfate to remove any water present. A sample of 10·0 ml of dried n-butanol was placed in a counting vial and evaporated to dryness by means of an air stream at room temperature. The residue was taken up in 20·0 ml of a toluene scintillation solution[3] and counted for its radioactive content on a Packard Tri-Carb liquid scintillation spectrometer. The counts per minute were corrected for instrument efficiency and sample quenching and converted to microcuries times 10^{-4} per gram of tissue.

Chromatographic measurements of ^{14}C 5-AT, 5-AT and 5-AI3AA were done in the following solvent systems on Whatman no. 1 paper employing an ascending unidimensional technique:

System 1: n-butanol:acetic acid:water (4:1:5)

System 2: toluene:n-butanol:acetic acid:water (2:2:1:1)

System 3: chloroform:acetic acid:water (10:4:5)

The chromatograms were scanned by ultraviolet light, and the radioactive chromatograms were scanned on a Nuclear-Chicago model C 100A Actigraph II. Fluorescent spectra were obtained with an Aminco Bowman spectrophotofluorometer, and the ultraviolet spectra were obtained with a recording Beckman DB spectrophotometer.

RESULTS

Tissue distribution of ^{14}*C 5-AT*

The distribution of ^{14}C 5-AT in the heart, liver, kidney, and brain tissues of mice (Table 1) shows that high concentration is found in the heart tissue immediately, but after 60 min the ^{14}C 5-AT concentration drops steadily in all the tissues studied. Radioactive chromatographic studies of tissue homogenates show that only ^{14}C 5-AT is present in the liver, heart, and brain in the unchanged form 60 min after administration, whereas in the kidney both ^{14}C 5-AT and traces of the metabolite are found 60 min after administration.

* Communication of Dr. Gale Boxill of the Pharmacology Department.

† Communication of Dr. Bernard Dubnick of the Biochemistry Department.

TABLE 1. ^{14}C5-AT TISSUE DISTRIBUTION *in vivo* IN MOUSE AFTER INJECTION OF 5-AT, 10 MG/KG I.V.

Time after dose (min)	Tissue ($\mu c \times 10^{-4}$/g)			
	Heart	Brain	Kidney	Liver
0·5	275·1	19·2	191·6	61·5
60	16·0	2·9	623·0	43·3
120	1·5	0·3	31·8	19·3
180	1·2	0·2	20·6	10·5
300	1·3	0·3	20·6	1·8
1,500	0·6	0·6	7·9	8·0

Urine studies

A 24-hr pooled urine sample from mice injected with ^{14}C 5-AT, 10 mg/kg, was chromatographed in System 1 and compared to normal mouse urine with ^{14}C 5-AT added. There was found in the chromatogram of urine containing the metabolized compound a radioactive spot that possessed an Rf of 0·86, differing from that found in the chromatogram of urine plus ^{14}C 5-AT (Rf 0·60). Since the ^{14}C label was on the 5-acetyl group and the chromatographic spot from the urine containing the metabolized compound still possessed the radioactive label, this indicated that 5-AT is not deacetylated to form this metabolite.

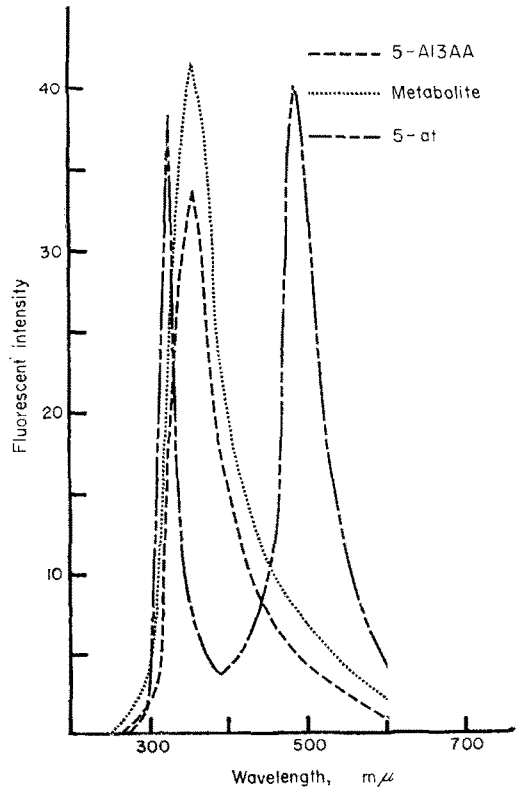

FIG. 1. Fluorescent spectra of 5-AI3AA (Aλ 290 ± 5 mμ, Eλ 355 ± 5 mμ), urinary metabolite (Aλ 290 ± 5 mμ, Eλ 355 ± 5 mμ), and 5-AT (Aλ 325 ± 5 mμ, Eλ 485 ± 5 mμ) in absolute ethanol were obtained by holding the activation wave length (Aλ) constant and scanning the emission wave lengths. All wave lengths are uncorrected.

Identification of the urinary metabolite

A fluorescent spectrum of the eluate of the chromatographic spot from the metabolized urine was determined in absolute ethanol and compared to that obtained for 5-AT (Fig. 1).

Comparative fluorescent and ultraviolet spectra were run on 5-AI3AA, which had been doubly chromatographed in System 1 and eluted with absolute ethanol, and the urinary metabolite, also doubly chromatographed and eluted with ethanol (Fig. 1, 2). The comparisons indicate that the metabolite and 5-AI3AA have the same ultraviolet and fluorescent spectra.

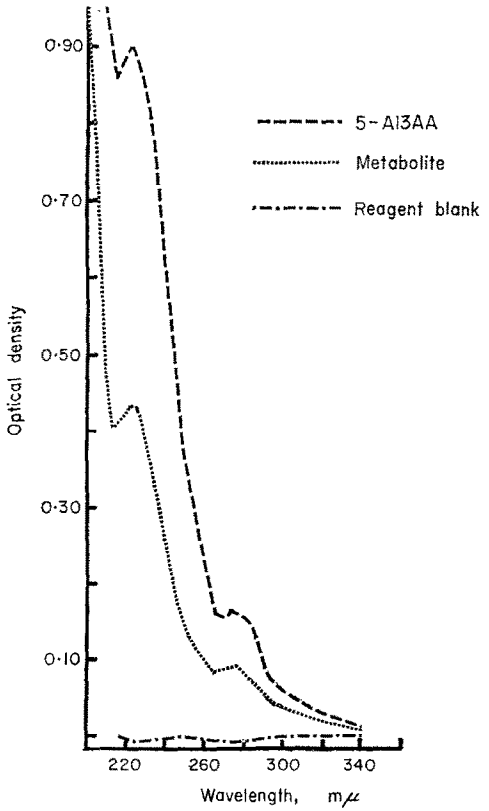

FIG. 2. Ultraviolet spectra of 5-AI3AA and urinary metabolite in absolute ethanol.

Preliminary chromatography of 5-AI3AA and the metabolite indicated that in System 1 they reacted chromatographically alike (Table 2). In comparing chromatograms of 5-AT, 5-AI3AA, and the urinary metabolite in three different chromatographic solvent systems, it was found that 5-I3AA and the urinary metabolite possessed identical chromatographic properties in all three systems (Table 2). When the urinary metabolite was mixed with 5-AI3AA and chromatographed in these three solvent systems only one chromatographic spot was obtained with an Rf identical with the authentic 5-AI3AA and the urinary metabolite.

CONCLUSIONS

It has been reported that 5-hydroxytryptamine,[4, 5] tryptamine[5] and 5-methoxytryptamine[6] are metabolized to their indolacetic acid derivatives. The data presented above indicated that 5-AT, being an analog of these compounds, is metabolized in a similar manner to give its indoleacetic acid derivative.

The fluorescent and ultraviolet spectra and chromatographic data indicate that the metabolite and 5-AI3AA are identical.

O

TABLE 2. CHROMATOGRAPHY OF 5-AI3AA, 5-AT AND URINARY METABOLITE

Material	Rf		
	System 1	System 2	System 3
Normal mouse urine	no spot	no spot	no spot
5-AI3AA	0·86	0·86	0·71
Urine containing the metabolite	0·80		
5-AI3AA in normal urine	0·80		
Urinary metabolite doubly chromatographed from urine containing the metabolite	0·86	0·86	0·71
5-AI3AA doubly chromatographed from 5-AI3AA in normal urine	0·86	0·86	0·71
5-AI3AA and urinary metabolite doubly chromatographed	0·86*	0·86*	0·71*
5-AT in normal mouse urine	0·60		
5-AT	0·60	0·29	0·36

* Only one spot obtained.

Acknowledgements—The authors express appreciation to George A. Feri for his technical assistance and to Drs. Max von Strandtmann and John Shavel, Jr. of the Organic Chemistry Department for their synthesis of ^{14}C 5-AT, 5-AT, and 5-AI3AA.

Warner-Lambert Research Institute,
Morris Plains, N. J., U.S.A.

JAMES M. HARTIGAN, JR.
GEORGE E. PHILLIPS

REFERENCES

1. J. SHAVEL, JR., M. VON STRANDTMANN and M. P. COHEN, *J. Amer. chem. Soc.* **84**, 881 (1962).
2. G. GOMORI, *Methods in Enzymology* **1**, 146 (1955).
3. VON G. ERDTMANN and G. HERRMANN, *Z. Electrochem.* **64**, 1092 (1960).
4. W. M. MCISAAC and I. H. PAGE, *J. biol. Chem.* **234**, 858 (1959).
5. H. WEISSBACK, W. LOVENBERG, B. G. REDFIELD and S. UDENFRIEND, *J. Pharmacol. exp. Ther.* **131**, 26 (1961).
6. V. ERSPAMER, *J. Physiol.* **127**, 118 (1955).

Elimination and catabolism of ^{35}S-heparin by neoplastic mast cells in culture

(*Received* 5 *February* 1963; *accepted* 27 *February* 1963)

NEOPLASTIC mast cells, both in culture and in the mouse, synthesize histamine, 5-hydroxytryptamine,[1, 2] and heparin.[3] The turnover rate of ^{35}S-sulfate and ^{14}C-glucosamine in heparin was shown to be the same;[4] the results suggest that the turnover of ^{35}S-sulfate in heparin is a measure of the turnover of heparin, but no attempt was made to learn the fate in the cells of heparin. It has been shown that both histamine and 5-hydroxytryptamine are eliminated unchanged by these cells.[5]

X-1 cells, derived from the Dunn-Potter P-815 mastocytoma, were grown in a medium containing 2 mc of ^{35}S-sulfate.[4] The cells and all methods were essentially identical with those used to study the turnover of heparin.[4] The cells were collected by centrifugation and divided into two portions. One aliquot was washed three times with ice-cold 0·9% NaCl and extracted for ^{35}S-heparin;[4] the other was placed in isotope-free medium and incubated. After 24 hr both the medium and cells were collected; the cells were divided into two portions, one of which was washed and extracted for ^{35}S-heparin and the other incubated in isotope-free medium. At the end of the 24-hr period of incubation the heparin of the cells was extracted; in each of the fractions of medium the ^{35}S-heparin also was determined.