by reserpine is metabolized by MAO, while that released by sympathomimetic amines directly into the circulation is methylated by catechol O-methyltransferase.[39] The mode of action of guanethidine differs from both that of sympathomimetic amines and reserpine.[37] The liberation of norepinephrine-$^3$H by these three classes of compounds may be influenced by a variety of inhibitors. Cocaine blocks the release of norepinephrine by sympathomimetic amines.[5,40] Ganglionic blocking agents,[14] MAO inhibitors, and phenothiazines[41] block release caused by reserpine. Ganglionic blocking agents[14] inhibit release caused by guanethidine.

The various relationships between drugs, biosynthesis, and the disposition and metabolism of norepinephrine may be further probed by the rapid assay utilized here. The method provides a simple, convenient method for studying structure–activity relationships with respect to chemorelease of norepinephrine. The method makes possible the study of interactions between drugs which release norepinephrine and drugs which inhibit the normal or drug-induced release of norepinephrine.

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**Acknowledgments.**—The authors would like to express their gratitude to the following pharmaceutical firms: Abbott Laboratories, Burroughs Wellcome and Co., Chas. Pfizer, Ciba, Hoffman-La Roche Inc., McNeil Laboratories, Merck Sharp and Dohme, Smith Kline and French, Wm. S. Merrell Co., and Wyeth Laboratories for their generosity in making various drugs available for these studies. The skillful technical assistance of Mrs. Louise Atwell is gratefully acknowledged.

# The Depletion of Norepinephrine-$^3$H from Heart by α-Methyl-*m*-tyrosine. A Novel and Convenient Method for Assaying the Inhibition of Aromatic Amino Acid Decarboxylase *in Vivo*

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*Received December 16, 1965*

Cardiac norepinephrine of mice was prelabeled with 5 μcuries of norepinephrine-$^3$H. After 1 hr 10 mg/kg of α-methyl-*m*-tyrosine was administered subcutaneously. After 3 hr the activity of cardiac norepinephrine-$^3$H was 50% of that of control animals. This release of norepinephrine which requires enzymatic decarboxylation of α-methyl-*m*-tyrosine to the active releasing agent α-methyl-*m*-tyramine is blocked by inhibitors of aromatic amino acid decarboxylase. The resulting decrease in α-methyl-*m*-tyrosine-releasing activity is a direct measure of inhibition of the enzyme *in vivo*, and provides a convenient method for determining the effectiveness of decarboxylase inhibitors in intact animals.

The chemorelease of cardiac norepinephrine in mice is conveniently assayed by prelabeling the endogenous norepinephrine with an intravenous injection of 5 μcuries of norepinephrine-7-$^3$H; after 1 hr the drug to be tested is administered and the activity of cardiac norepinephrine-$^3$H after 3 hr is compared with that in control animals.[1,2]

In screening various enzyme inhibitors for their effect on norepinephrine release,[2] we observed that of the α-methyl aromatic amino acids which cause a marked depletion of norepinephrine over a period of days, *only* α-methyl-*m*-tyrosine caused a release of norepinephrine during the assay period of 2 hr.

The depletion of norepinephrine in heart, brain, and other tissues by α-methyl aromatic amino acids, such as α-methyl-*m*-tyrosine and α-methyldopa, is caused by the amine formed *in vivo* by the enzymatic decarboxylation of the amino acid.[3,4]

An earlier report that the amino acid, α-methyl-*m*-tyrosine, had a releasing activity of its own was based on experiments in animals in which aromatic amino acid decarboxylase was only partially inhibited by a hydrazine analog of dopa, *i.e.*, α-(3,4-dihydroxybenzyl)-α-hydrazinopropionic acid.[5]

Because α-methyl aromatic amino acids are poor substrates for aromatic amino acid decarboxylase[6] and because of the unique releasing activity of α-methyl-*m*-tyrosine, the release caused by this amino acid was reinvestigated. The effect of various amino acids on the liberation of norepinephrine-$^3$H from heart is shown in Table I.

α-Methyl-*m*-tyrosine was the only amino acid which caused significant release. The methyl ester of α-methyl-*m*-tyrosine was as active as the amino acid, suggestive of rapid ester hydrolysis *in vivo*. The release by α-methyl-*m*-tyrosine and by its decarboxylation product, α-methyl-*m*-tyramine, as a function of time is shown in Figure 1. Norepinephrine was released by the

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Table I

The Effect of Various Aromatic Amino Acids on the Release of Norepinephrine-$^3$H from Mice Hearts[a]

| Substance | Dose, mg/kg | mμcuries/g of tissue | % of control |
|---|---|---|---|
| Control | .. | 340 ± 20 | 100 |
| L-Tyrosine | 10 | 354 | 104 |
| L-Dopa | 10 | 286 | 84 |
| 3,4-Dihydroxyphenylserine | 10 | 334 | 98 |
| *threo-m*-Hydroxyphenylserine | 10 | 334 | 98 |
| α-Methyltyrosine | 10 | 289 | 85 |
| α-Methyl-*m*-tyrosine | 10 | 157 | 46 |
| α-Methyl-*m*-tyrosine methyl ester | 10 | 170 | 50 |
| α-Methyldopa | 10 | 340 | 100 |
| α-Methyl-2,3-dihydroxyphenylalanine | 10 | 310 | 91 |
| 5-Hydroxytryptophan | 10 | 367 | 108 |
| α-Methyl-5-hydroxytryptophan | 10 | 340 | 100 |
| Mimosine | 10 | 425 | 125 |
| 3-(γ-Tropyl)alanine | 10 | 445 | 130 |

[a] Norepinephrine-$^3$H (5 μcuries, 1 mμmole) was injected into the tail vein of 14–18-g mice. After 1 hr, amino acid was injected subcutaneously and the animals (5/assay) were sacrificed after 2 additional hr. Hearts were removed, homogenized in 0.4 *N* $HClO_4$, and centrifuged, and an aliquot was counted in scintillation equipment as previously described.[4]

Table II

Effect of Inhibitors of Aromatic Amino Acid Decarboxylase on the α-Methyl-*m*-tyrosine-Induced Release of Norepinephrine-$^3$H[a]

| Inhibitors | Dose, mg/kg | Norepinephrine-$^3$H, mμcuries/g | % of control | % inhibition of release |
|---|---|---|---|---|
| None | ... | 157 | 46 | 0 |
| α-Methyldopa | 50[b] | 176 | 57 | 20 |
| α-Methyl-2,3-dihydroxyphenylalanine | 50[b] | 164 | 54 | 15 |
| α-Methyl-5-hydroxytryptophan | 50[b] | 160 | 48 | 4 |
| Hydralazine | 25[c] | 162 | 46 | 0 |
| Pheniprazine | 25[c] | 248 | 69 | 43 |
| *m*-Hydroxy-*p*-bromobenzyloxyamine | 50[b] | 186 | 56 | 18 |
| N′-(*m*-Hydroxybenzyl)-N′-methylhydrazine | 50[b] | 265 | 83 | 69 |
| N-DL-Seryl-2,3,4-trihydroxybenzylhydrazine | 10[c] | 283 | 86 | 74 |
| N-DL-Seryl-2,3,4-trihydroxybenzylhydrazine | 50[b] | 299 | 95 | 91 |
| β-Phenyl-α-hydrazinopropionic acid | 30[b] | 275 | 87 | 80 |
| β-(*p*-Hydroxyphenyl)-α-hydrazinopropionic acid | 30[b] | 255 | 83 | 69 |
| α-(3,4-Dihydroxybenzyl)-α-hydrazinopropionic acid | 10[c] | 258 | 81 | 65 |
| α-(3,4-Dihydroxybenzyl)-α-hydrazinopropionic acid | 30[b] | 301 | 96 | 93 |

[a] Norepinephrine (5 μcuries, 1 mμmole) injected intravenously into 14–18-g mice. To induce release α-methyl-*m*-tyrosine was injected subcutaneously 60 min after norepinephrine-$^3$H. Animals (5/assay) were sacrificed 3 hr after norepinephrine. Hearts were removed, homogenized in 0.4 *N* $HClO_4$, and centrifuged, and an aliquot was counted in scintillation equipment as previously described. Controls for inhibition studies were animals that received the inhibitor but not α-methyl-*m*-tyrosine. In most cases only slight changes from the normal control value of 340 mμcuries/g of tissue were observed. [b] Inhibitor was injected subcutaneously 30 and 90 min after norepinephrine-$^3$H. Dose (mg/kg) is a total of the two injections. [c] Inhibitor was injected subcutaneously 30 min after norepinephrine-$^3$H.

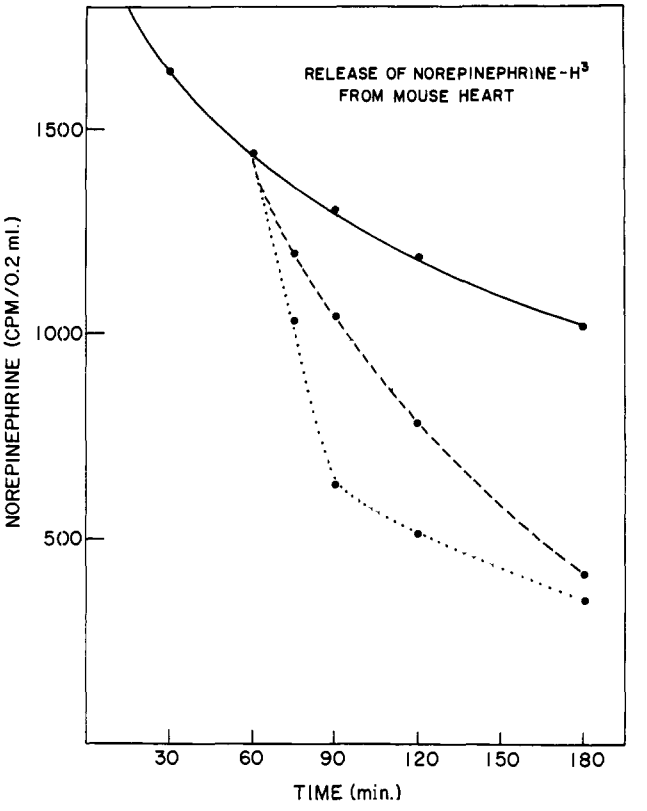


Figure 1.—Release of norepinephrine-$^3$H from mouse heart by α-methyl-*m*-tyrosine and α-methyl-*m*-tyramine as a function of time. Control, —; following α-methyl-*m*-tyrosine, - - - - ; following α-methyl-*m*-tyramine, .....

amino acid over the entire 2-hr period. By contrast, the amine, α-methyl-*m*-tyramine, released a major fraction of the norepinephrine during the first 30 min. After this initial "burst" the decrease in cardiac norepinephrine was virtually identical with the control. These observations are explicable in terms of a continuous decarboxylation of α-methyl-*m*-tyrosine to α-methyl-*m*-tyramine. Inhibitors of decarboxylase effective *in vivo* should block the decarboxylation of α-methyl-*m*-tyrosine and consequently also the release of norepinephrine. With this technique virtually complete inhibition of α-methyl-*m*-tyrosine decarboxylation is observed with decarboxylase inhibitors such as β-phenyl-α-hydrazinopropionic acid,[7] α-(3,4-dihydroxybenzyl)-α-hydrazinopropionic acid,[8] β-(*p*-hydroxyphenyl)-α-hydrazinopropionic acid,[9] DL-seryl-2,3,4-trihydroxybenzylhydrazine,[10] and N′-(*m*-hydroxybenzyl)-N′-methylhydrazine[3] (see Table II).

*m*-Hydroxy-*p*-bromobenzyloxyamine[3] caused moderate inhibition, while the α-methylamino acids[11] did not significantly interfere with the decarboxylation of α-methyl-*m*-tyrosine. The inhibitors tested did not appreciably effect release of norepinephrine-$^3$H by α-methyl-*m*-tyramine.

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Because of its simplicity the method is well suited for determining the inhibition of aromatic amino acid decarboxylase *in vivo*. It has the additional advantage of measuring the effect of decarboxylase inhibitors *in situ* rather than in homogenates prepared from a single organ. The method in its present form does not assay the inhibition of decarboxylase in the central nervous system.

# Metabolism of Metaxalone

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The metabolism of metaxalone (I) has been studied in the dog and man. The major product is formed by oxidation of one of the methyl groups to yield 5-(3-methyl-5-carboxyphenoxymethyl)-2-oxazolidinone. This also occurs in the urine as the glucuronide. The ether linkage is also cleaved to give 3,5-xylenol and 5-hydroxymethyloxazolidinone. The oxazolidinone ring appears to be stable. In order to identify these metabolites the above acid and its triacetyl β-glucuronide methyl ester were synthesized.

Metaxalone, which is 5-(3,5-dimethylphenoxymethyl)-2-oxazolidinone (I), was first synthesized by Lunsford, *et al.*[1] This drug has been shown to be an effective muscle relaxant with low toxicity.[2] In an earlier attempt to study absorption and excretion of this compound in animals and man using the colorimetric method of Titus, *et al.*,[3] only extremely low concentrations in blood and urine could be found, in spite of the fact that recoveries of added amounts to biological fluids were satisfactory. The method described by Titus is based on the extraction of the drug from alkaline solution, and it was thus apparent that metaxalone was extensively metabolized and probably to an acidic compound. The following studies were carried out to investigate the metabolism of metaxalone in man and animals.

## Experimental Section

**Synthesis of Related Compounds.**—A general review of the literature of the metabolism of structurally related compounds and the chemistry of metaxalone suggested a number of possible metabolites. The synthesis of these are described below.

**5-(3-Methyl-5-carboxyphenoxymethyl)-2-oxazolidinone.**—Three grams of metaxalone was suspended in 100 ml of water and stirred continuously at 75–80° for 3 hr while 2.14 g (0.5 equiv) of $KMnO_4$ in 25 ml of water was added. Removal of the $MnO_2$ and excess metaxalone by filtration and ether extraction of the filtrate gave a clear aqueous solution of pH 8–9. After concentrating to 25 ml, the solution was acidified; a gum (480 mg) separated. Thin layer chromatography (benzene–methanol–formic acid, 25:8:2) of this gum showed a major substance and a minor amount of a more polar acidic material, probably the phthalic acid derivative. Crystallization was induced, and, after several recrystallizations, the melting point was 157–192° (the material was poorly recrystallizable and the melting point did not improve; however, it was chromatographically pure).

*Anal.* Calcd for $C_{12}H_{13}NO_5$: C, 57.38; H, 5.22; N, 5.58. Found: C, 57.15; H, 5.16; N, 5.73.

**5-(3-Methyl-5-carboxyphenoxymethyl)-2-oxazolidinone Triacetyl β-Glucuronide Methyl Ester.**—A mixture of 1.5 g of 5-(3-methyl-5-carboxyphenoxymethyl)-2-oxazolidinone, 2.4 g of methyl bromacetylglucuronate, and 3.0 g of fresh silver oxide was stirred in 30 ml of pyridine with cooling in an ice bath for 15 min. The dark solution was filtered through Celite, and the filtrate was pumped to dryness. The residue was taken into $CHCl_3$ and the solution was extracted with 1 *N* HCl, dilute bicarbonate, and finally with water. Removal of the $CHCl_3$ gave 980 mg of dark gum, $R_f$ 0.65 (tlc, benzene–acetone, 1:1). Florisil chromatography produced 591 mg of a clear glass, $[\alpha]^{25}D$ −30.6° (*c* 3, methanol). The material was chromatographically homogeneous.

*Anal.* Calcd for $C_{25}H_{29}NO_{14}$: C, 54.44; H, 5.30; N, 2.53. Found: C, 54.17; H, 5.31; N, 2.68.

**3-Aminopropanediol** was synthesized by the procedure of Bergmann, *et al.*[4]

**Isolation of Metabolites. 5-(3-Methyl-5-carboxyphenoxymethyl)-2-oxazolidinone from Dog Urine.**—A 14.5-kg, male, mongrel dog was given a daily oral dose of 5.73 g of metaxalone (395 mg/kg) for a 7-day period. The urine was collected during this period and preserved by the addition of NaF to the daily collection beaker. A 1.8-l. aliquot of the total collection (4.8 l.) was boiled and filtered through Celite to remove a flocculent precipitate. Saturation of the urine with $(NH_4)_2SO_4$ (about 1 kg) produced a gummy precipitate which was separated by decantation of the urine and which was extracted from excess $(NH_4)_2SO_4$ by treatment with warm ethanol. Removal of the ethanol produced 4.4 g of a glassy brown gum. A 500-mg sample of this gum was induced to crystallize from hot water and after three recrystallizations had a melting point of 145–170°; two further recrystallizations produced 120 mg, mp 153–173°.

*Anal.* Calcd for $C_{12}H_{13}NO_5$: C, 57.38; H, 5.22; N, 5.58. Found: C, 57.56; H, 5.27; N, 5.62.

This material cochromatographed on thin layer chromatography with the synthesized material. Their infrared and ultraviolet spectra were identical. The $[\alpha]^{26}D$ of the metabolite was −15° (*c* 3, methanol).

In order to confirm the structure of the metabolite, it was cleaved with HI. Treatment of 400 mg of the urinary acid with 10 ml of refluxing 57% HI for 4 hr followed by ether extraction gave 182 mg of extracted material. This was taken into bicarbonate solution which was extracted twice with ether to remove neutral material. Acidification of the aqueous bicarbonate solution and ether extraction gave 65 mg of crude *m*-hydroxytoluic acid. Recrystallization from methanol–water gave a pure sample, mp 208.5–210°. The material did not depress the melting point of authentic *m*-hydroxytoluic acid and the infrared curves were identical.

*Anal.* Calcd for $C_8H_8O_3$: C, 63.15; H, 5.30. Found: C, 63.48; H, 5.36.

**5-(3-Methyl-5-carboxyphenoxymethyl)-2-oxazolidinone Triacetyl β-Glucuronide Methyl Esters.**—A 75-kg male subject (J. E. P.) ingested orally 12.8 g of metaxalone (85 mg/kg per day) over a 2-day period, and the urine (1.93 l.) was collected

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