The Relative Metabolic Rates of Norepinephrine-7-H^3 and Epinephrine-1-C^{14}

The availability of DL-norepinephrine-7-H^3 and DL-epinephrine-1-C^{14} makes it possible to study simultaneously the metabolic pathways of these hormones *in vivo*. The extent to which epinephrine and norepinephrine are deaminated, O-methylated, N-methylated or N-demethylated after simultaneous injection has been evaluated in the present study.

A group of rats was injected with 0·5 ml of $1{\cdot}9 \times 10^{-4}$ *M* norepinephrine-7-H^3 and epinephrine-1-C^{14} with a respective activity ratio of 5:1. The urine was collected over a period of 24 h, and hydrolyzed at pH 2 by refluxing at 100°C for 20 min. The hydrolyzed urine extract was cooled to room temperature and extracted 4 times with ethyl acetate. The ethyl acetate fraction contained the acidic and neutral metabolites. The water layer was passed through an alumina column at pH 8 and the absorbed catechol amines were eluted with 0·2 *N* acetic acid. The effluent of the alumina column contained the methoxycatechol amines, and the eluate of the alumina column contained the catechol amines. The catechol amine and the methoxycatechol amine fractions were acetylated as described previously[1]. Aliquots of all fractions were counted separately in a Packard Tri-Carb scintillation counter by the discriminator ratio method. In each experiment, duplicate samples were counted and averaged, and the ratio of H^3:C^{14} was calculated (Table I). These results show an increase in ratio of H^3:C^{14} in the catechol amine fraction, a decrease of H^3:C^{14} in the methoxycatechol amine fraction and in the ethyl acetate fraction, as compared to the ratio in the injected solution. The nature of these changes in ratios was further studied. By chromatography in the Bush 'C' solvent system[2], two radioactive peaks with the same mobility as acetylated norepinephrine and epinephrine were obtained from the catechol amine fraction, and two radioactive peaks with the same mobility as acetylated 3-methoxynorepinephrine and 3-methoxyepinephrine were obtained from the methoxycatechol amine fraction.

The ethyl acetate fraction was chromatographed in chloroform acetic acid-water (2:1:1), and in isopropyl alcohol-aqueous ammonia-water (8:1:1) solvent system. Two radioactive peaks were obtained. The peak with the slower mobility was identical with 3-methoxy-4-hydroxymandelic acid and the second peak represents an unknown metabolite. This metabolite was demonstrated to be neutral since it was extractable into ethyl acetate but not into sodium bicarbonate. It is readily acetylated and from its mobility in the Bush 'C' solvent system, it appears to be an alcohol. As Table II shows the H^3:C^{14} ratio was found to be the same for 3-methoxy-4-hydroxymandelic acid and the alcohol. This shows that the alcohol is formed from norepinephrine and epinephrine in the same proportion as 3-methoxy-4-hydroxymandelic acid.

The presence of tritium in the epinephrine and 3-methoxynorepinephrine zones (Table II), indicates that norepinephrine is converted into epinephrine *in vivo*. The absence of C^{14} in the norepinephrine and 3-methoxynorepinephrine zones shows that epinephrine is not demethylated to norepinephrine, and that *N*-methylation of norepinephrine to epinephrine is an irreversible process *in vivo*.

It is evident from the H^3:C^{14} ratio in the epinephrine and 3-methoxyepinephrine zones (Table II), that epinephrine-H^3 which was formed from norepinephrine-H^3 is not O-methylated to the same extent as originally injected epinephrine-C^{14}. The failure of epinephrine-H^3 which was formed *in vivo* to undergo as extensive O-methylation as injected epinephrine-C^{14} may result from the fact that competition for the methyl donor at the metabolic site exists. This finding may also result from the fact that norepinephrine-H^3 and epinephrine-C^{14} were injected simultaneously and therefore epinephrine-H^3 had to be metabolically formed in order to compete with the O-methylation of the injected epinephrine-C^{14}.

Table I: Tritium and C^{14} ratios in catechol amine, methoxycatechol amine, and ethyl acetate fractions [a]

Experiment No.	Catechol Amine H^3/C^{14}	Methoxycatechol Amine H^3/C^{14}	Ethyl Acetate Fraction (Acidic and Neutral Metabolites) H^3/C^{14}
1	7·2/1	4·5/1	3/1 (4·0/1) [c]
2	6·5/1	4·0/1	3·5/1 (4·1/1)
3 [b]	7·0/1	4·6/1	3/1 (4·0/1)

[a] Activity ratio of injected norepinephrine-7-H^3 to epinephrine-C^{14} was 5/1.
[b] The radio chemical purity was also established by extraction of the methoxycatechol amine fraction with ethylene dichloride prior to acetylation[3].
[c] The ratios, in parentheses, were obtained after enzymatic hydrolysis of the urine.

Table II: Tritium and C^{14} total activity of epinephrine, norepinephrine, and their metabolites isolated from rats urine after separation by paper chromatography

	Total activity c. p. m. × 10^4		Ratio of H^3/C^{14}
	H^3	C^{14}	
norepinephrine	37·70	0	
3-methoxynorepinephrine	97·60	0	
epinephrine	16·40	8·40	1·9/1
3-methoxyepinephrine	18·00	29·20	0·61/1
3-methoxy-4-hydroxymandelic acid	8·10	2·00	4·0/1
neutral metabolite [a]	5·1	1·2	4·2/1

[a] A recent publication by AXELROD *et al.* [4], describes a similar compound identified as 3-methoxy-4-hydroxyphenylglycol

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Zusammenfassung

Ratten wurden gleichzeitig mit Noradrenalin-H^3 und Adrenalin-C^{14} belastet und die Ausscheidung im Harn verfolgt. Die Analyse der Metabolite hat unter anderem ergeben, dass Noradrenalin-H^3 zu Adrenalin-H^3 verwandelt wird, und dass diese Reaktion irreversibel ist. Das *in vivo* synthetisierte Adrenalin-H^3 wird in geringerem Masse inaktiviert als das injizierte Adrenalin-C^{14}. Ein neutrales Stoffwechselprodukt (wahrscheinlich ein Alkohol) wurde aus dem Harn isoliert.

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[3] J. AXELROD, S. SENOH, B. WITKOP, J. biol. Chem. *233*, 697 (1958).
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