THE METABOLISM OF EPINEPHRINE CONTAINING ISOTOPIC CARBON. II*

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In a previous publication (1), evidence was presented indicating that a major pathway of epinephrine metabolism involved cleavage of the molecule at some point between the β-carbon and the methyl carbon. The presence of ether-soluble substances in urine suggested that methylamine was split off, at least to some extent. A minimum of five urinary metabolites of epinephrine has been revealed by paper chromatography (2). The interpretation of these data, however, was complicated by the use of dl-epinephrine, employed because of the unavailability of the natural l isomer possessing adequate radioactivity. There was thus no assurance that some of these observations were not attributable to the unnatural d isomer and hence unrelated to the metabolism of physiological epinephrine. Recently we have synthesized methyl-C\(^{14}\)-dl-epinephrine of higher specific activity and prepared its l isomer as well as the l isomer of β-C\(^{14}\)-epinephrine. Using the two types of radioactive l-epinephrine we have confirmed previously reported metabolic studies and have found no observable differences in the fates of l-epinephrine and dl-epinephrine in the rat. Evidence is also presented to demonstrate that methylamine is split from epinephrine.

EXPERIMENTAL

Synthesis of Methyl-C\(^{14}\)-dl-epinephrine—Radioactive barium carbonate\(^{1}\) was converted to sodium cyanide (3) which was catalytically hydrogenated to methylamine as described by Jones and Skraba.\(^ {2}\) Adrenalone was prepared in a closed system from methylamine hydrochloride (3 parts by weight) in water (4 parts), by distilling aqueous methylamine into a flask containing chloroacetyl catechol (2 parts) and permitting the reaction to proceed 20 hours under nitrogen. Isotopic methylamine hydrochloride was recovered and the entire process repeated several times. Adrenalone

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\(^{1}\) Radioactive barium carbonate was obtained on allocation from the Isotopes Division, United States Atomic Energy Commission.

was converted to epinephrine as previously described (4). The activity of the methyl-C^{14}-dl-epinephrine was $1.30 \times 10^6$ c.p.m. per mg. measured with a flow counter. Criteria of purity of epinephrine synthesized by this method have been published (2).

Resolution of dl-Epinephrine—A portion of the methyl-C^{14}-dl-epinephrine was diluted with non-isotopic dl-epinephrine to give 145 mg. Resolution as $l$-epinephrine-$d$-bitartrate$^3$ followed the method of Flächer (5). After three recrystallizations from methanol, 32.4 mg. of the salt, m.p. 146-146.7°, were obtained; Flächer found a melting point of 149° for the pure compound. Free epinephrine, 15.3 mg., was precipitated by addition of ammonia to an aqueous solution of the salt. 5.08 mg. of epinephrine were dissolved in 0.300 ml. of 0.3 N HCl, the observed rotation in a 1 dm. micropolarimeter tube being $-0.822^\circ$ at 25°; $[\alpha]_D^{25} = -48.6^\circ$. Flächer reported $[\alpha]_D^{19.5} = -51.4^\circ$ (5); we have found for pure $l$-epinephrine $[\alpha]_D^{25} = -52.7^\circ$. Based on the value of $-52.7^\circ$, the methyl-labeled epinephrine contains 96 per cent of the $l$ isomer. The activity was $2.08 \times 10^6$ c.p.m. per mg.

For the resolution of $\beta$-C^{14}-dl-epinephrine the use of a larger quantity (183 mg.) permitted an additional recrystallization yielding 21.6 mg. of $l$-epinephrine-$d$-bitartrate, m.p. 147.9-148.5°, and 11.1 mg. of epinephrine, $[\alpha]_D^{25} = -51.0^\circ$, indicating about 98 per cent purity. The activity was $7.2 \times 10^4$ c.p.m. per mg.

Assay of Urine for Radioactivity—In order to reduce errors in assay due to uneven surfaces in plating of samples, and due to the use of large factors required in correcting to zero thickness, all urine samples were assayed at infinite thickness. After ascertaining the amount of dry residue in each urine sample, a volume sufficient to give a residue of 90 mg. (20 mg. per sq. cm. for the plates used) was plated. If the weight varied from 90 mg., a small, experimentally derived factor was applied to correct to 90 mg. thickness. The radioactive solutions administered to animals were assayed by addition of a known amount to a volume of normal rat urine containing 90 mg. of dry residue. All counts were made in a flow counter.

Comparison of Percentage Excretion of C^{14} in Urine after Administration of Methyl-C^{14}-l-epinephrine and $\beta$-C^{14}-l-Epinephrine—Rats were injected intravenously with 0.05 $\gamma$ of labeled $l$-epinephrine per gm. of body weight and urine was collected quantitatively for 17 hours from each rat separately. Urine was assayed as described above. The percentages of total C^{14} excreted in urine after administration of methyl-C^{14}-l-epinephrine were 67, 63, 52, 69, 67, 49, 67, 57, 70, and 50, averaging 61. The previously published figure obtained with methyl-C^{14}-dl-epinephrine in the combined

$^3 l$-Epinephrine-$d$-bitartrate was supplied by the Sterling-Winthrop Research Institute, to which the authors are greatly indebted.
urine of six rats after 19 hours was 61 per cent (1). After administration of $\beta$-$\text{C}^{14}$-$l$-epinephrine, the amounts excreted were 95, 100, and 106 per cent. The previously published figure obtained with $\beta$-$\text{C}^{14}$-$dl$-epinephrine for the combined urine of six rats after 19 hours was 100 per cent (1).

**Percentage Excretion of $\text{C}^{14}$ in Urine after Administration of Radioactive Methylamine Hydrochloride**—After intravenous injection of rats with 0.01 γ of methylamine hydrochloride per gm. of body weight, the percentages of radioactivity excreted in urine (not including radioactivity due to unchanged methylamine$^4$) were 20, 23, 17, and 19, averaging 20.

**Formation of Methylamine from Epinephrine in Vitro**—Amine oxidase was prepared essentially according to the procedure of Alles and Heegaard (6) by homogenizing rat liver in a Potter-Elvehjem homogenizer with 2 volumes of 0.1 M phosphate buffer, pH 7.4, centrifuging, dialyzing the supernatant against distilled water for 5 hours, bringing the pH to 6.0, centrifuging, and suspending the residue in 1 volume of 0.1 M phosphate buffer, pH 7.4. Oxidations were carried out in air at 30° in the presence of semicarbazide (to stabilize the aldehyde produced) and of potassium cyanide (to reduce autoxidation). Labeled epinephrine was incubated with the enzyme and the above components for 2 hours; then a solution of non-isotopic epinephrine (to retard the autoxidation of the small amount of isotopic epinephrine) was added, followed by a solution containing 97 mg. of carrier methylamine hydrochloride. Finally, the mixture was made alkaline with sodium bicarbonate solution and immediately frozen for lyophilization. Volatile constituents were evaporated from the frozen state in vacuo and caught in a trap containing hydrochloric acid and cooled in liquid air. The methylamine hydrochloride thus obtained was converted to its $p$-toluenesulfonamide and recrystallized from two different solvents to constant radioactivity. The results are shown in Table I.

$^4$ A considerable amount of intravenously injected methylamine is excreted unchanged in the urine of rats. In one experiment five rats received 0.05 γ of $\text{C}^{14}$-methylamine hydrochloride per gm. of body weight and urine was collected directly in acid for 4 hours. By assaying the urine with and without addition of ammonia, it was found that 65 per cent of the urinary radioactivity (equivalent to about 25 per cent of the injected radioactivity) was lost from the ammoniacal urine, owing presumably to loss of methylamine. Similar experiments with methyl-labeled epinephrine urine failed to demonstrate the presence of methylamine. However, under these conditions methylamine may be formed so slowly that it is completely metabolized. Rats injected subcutaneously with minute amounts of $\text{C}^{14}$-methylamine hydrochloride expired 30 per cent of the total radioactivity as carbon dioxide in 4 hours and 42 per cent in 6 hours; no free methylamine was present in expired air. As methylamine is readily oxidized to carbon dioxide, it might be expected that the urinary metabolites would include urea and a large number of trace substances which had picked up this labile carbon.
Methylation by Epinephrine and Methylamine in Vivo—To test the methylating behavior of the methyl carbon of epinephrine and methylamine, three rats were injected subcutaneously with 1.0 γ of methyl-C\(^{14}\)-\(dl\)-epinephrine per gm. of body weight and urine was collected 24 hours at 0°. To a large volume of urine were added 100 mg. of creatinine as carrier and then an excess of picric acid in alcoholic solution. After one recrystallization of the potassium creatinine picrate from water, with a large quantity of norit, the radioactivity reached a low constant figure. The percentage of total urinary radioactivity found as creatinine was 0.4 per cent. In a similar experiment two rats were injected subcutaneously with 0.50 γ of methylamine hydrochloride per gm. of body weight, urine was collected for 24 hours, and the creatinine found to contain 1.2 per cent of the total urinary radioactivity. The results of both experiments were confirmed.

Radioactivity in Ether-Extractable Fractions of Urine after Administration

After incubation of methyl-C\(^{14}\)-epinephrine with homocysteine in the presence of liver or adrenal homogenates under conditions favoring transmethylation (7), isolated carrier methionine contained no radioactivity. Similarly, transmethylation could not be demonstrated with methylamine.

### Table I

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Weight of substrate</th>
<th>Per cent of radioactivity as methylamine</th>
</tr>
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<tbody>
<tr>
<td>Methyl-C(^{14})-(l)-epinephrine</td>
<td>117</td>
<td>60</td>
</tr>
<tr>
<td>&quot;</td>
<td>117</td>
<td>3</td>
</tr>
<tr>
<td>Methyl-C(^{14})-(dl)-epinephrine</td>
<td>18</td>
<td>53</td>
</tr>
<tr>
<td>&quot;</td>
<td>18</td>
<td>3</td>
</tr>
<tr>
<td>β-C(^{14})-(dl)-Epinephrine</td>
<td>100</td>
<td>&lt;0.2</td>
</tr>
</tbody>
</table>

* Boiled enzyme.

### Table II

Per Cent of Total Radioactivity in Ether-Extractable Fractions in Urine of Rats Injected Intravenously with 0.06 γ per gm. of Body Weight of β-C\(^{14}\)-\(l\)-Epinephrine or β-C\(^{14}\)-\(dl\)-Epinephrine

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Isomer</th>
<th>No. of rats</th>
<th>Ether Fraction I</th>
<th>Ether Fraction II</th>
<th>Ether Fraction III</th>
<th>Ether Fraction IV</th>
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<tr>
<td>1</td>
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<tr>
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<tr>
<td>4</td>
<td>dl</td>
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<td>0</td>
<td>1.6</td>
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<tr>
<td>5</td>
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<td>3</td>
<td>2.7</td>
<td>5.0</td>
<td>0</td>
<td>1.9</td>
</tr>
</tbody>
</table>
of \(\beta-C^{14}\)-l-Epinephrine and \(\beta-C^{14}\)-dl-Epinephrine—After intravenous injection of both types of \(\beta\)-labeled epinephrine at a level of 0.05 \(\gamma\) per gm. of body weight to groups of rats, ether-soluble fractions were assayed as previously described (1). The results are shown in Table II.

Chromatography of Urine—Paper chromatograms, developed in 4 parts of butanol, 1 part of acetic acid, and 1 part of water (8) were run on urine collected for 4 hours from a 125 gm. female rat injected subcutaneously with 2.5 \(\gamma\) of methyl-\(C^{14}\)-l-epinephrine per gm. of body weight (Fig. 1) and on the combined urines collected for 6 hours from two 105 gm. male rats injected subcutaneously with 0.50 \(\gamma\) of methyl-\(C^{14}\)-dl-epinephrine per gm. of body weight (Fig. 2). The paper strips were cut into 1 cm. segments, counted in a flow counter, and corrected for background only.

**Fig. 1.** Radioactivity on paper chromatogram of urine from rat injected subcutaneously with 2.5 \(\gamma\) of methyl-\(C^{14}\)-l-epinephrine per gm. of body weight.

**Fig. 2.** Radioactivity on paper chromatogram of urine from rats injected subcutaneously with 0.50 \(\gamma\) of methyl-\(C^{14}\)-dl-epinephrine per gm. of body weight.

**DISCUSSION**

The data presented in this study do not reveal any significant difference between l- and dl-epinephrines with respect to urinary excretion of \(C^{14}\), formation of methylamine in vitro, ether-soluble fractions, and chromatographic patterns. These findings afford the possibility of cautious translation of results from studies of the terminal metabolic products of dl-epinephrine to l-epinephrine. They do not provide information regarding the rates at which the two isomers are metabolized.

It has now been shown that with l-epinephrine, as well as with dl-epinephrine, a major metabolic pathway involves cleavage of the molecule at
some point between the β-carbon and the methyl carbon. Methylamine is probably one, and perhaps the only, fragment formed.

The finding of low concentrations of radioactivity in urinary creatinine after administration of methyl-labeled epinephrine and C\(^{14}\)-methylamine is compatible with other indications that methylamine is the fragment cleaved from the epinephrine molecule. The data afford no evidence for transmethylation by epinephrine; however, they do not eliminate the possibility that the methyl group may be lost to some extent either by transmethylation or by oxidative demethylation. Although the position of the radioactive carbon in the creatinine molecule was not established, it may have entered the methyl position directly from the methyl group of methylamine or from formate, which may be an intermediate in the oxidation of methylamine to carbon dioxide and which is known to be a source of labile methyl (9).

Paper chromatograms of methyl C\(^{14}\)-epinephrine urines show the presence of three radioactive peaks. Free epinephrine, assayed by isotope dilution, was present to the extent of 15 per cent in the \(d\)-epinephrine urine (equivalent to about 9 per cent of administered radioactivity) and 29 per cent in the \(l\)-epinephrine urine, the high percentages excreted being related to the large doses administered.\(^6\) As the \(R_F\) of epinephrine under the chromatographic conditions employed is about 0.50, it is probable that the middle peak in both chromatograms is largely due to free epinephrine.

A chromatogram of methylamine urine showed a large but poorly defined peak at about \(R_F 0.20\) and a sharp but small peak at \(R_F 0.57\). From inspection of this chromatogram it seems unlikely that methylamine metabolites could account fully for either of the two remaining peaks of the methyl-labeled epinephrine urine chromatograms. Therefore, it appears probable that, under the conditions of these experiments, epinephrine forms at least two metabolites which retain the methyl group.

The differences in ether-soluble fractions between \(l\)- and \(d\)-epinephrine urines do not appear to be significant. It is evident, however, that at low levels of epinephrine administration these ether-soluble substances occur to a smaller extent than was previously found (1) when larger doses of epinephrine were used.

It now becomes possible to propose a tentative summary of the fate of epinephrine in the rat, although many of the data were obtained with concentrations of epinephrine greater than physiological. Based on the data from methyl-labeled epinephrine, about 50 per cent is inactivated by loss

\(^6\) It has been shown earlier (1) that at low levels of epinephrine administration the percentage of free epinephrine in urine is very small. Thus no significant percentage of the radioactivity in the urine of rats given 0.05 \(\gamma\) of epinephrine per gm. of body weight can be attributed to unchanged epinephrine.
of methylamine, presumably through the action of amine oxidase. The methylamine is partially oxidized to carbon dioxide, but about 20 per cent of its C\textsuperscript{14} (equivalent to about 10 per cent of the originally administered C\textsuperscript{14}) is excreted in urine as metabolites. The remaining 50 per cent of the epinephrine is excreted in urine as at least two metabolites still retaining the methyl group. Based on the data from \(\beta\)-C\textsuperscript{14}-epinephrine, at least three additional epinephrine metabolites are formed from the remainder of the molecule after demethylation. Two of these are found in the ether-soluble fractions and under normal conditions are minor in amount. Since the sum of the ether-soluble substances and the metabolites bearing the methyl group accounts for about 60 per cent of the total radioactivity, it is necessary to postulate the existence of at least one additional major metabolite from which methylamine has been lost.

**SUMMARY**

1. Methyl-C\textsuperscript{14}-dl-epinephrine has been synthesized and its \(l\) isomer as well as the \(l\) isomer of \(\beta\)-labeled epinephrine prepared.

2. No observable differences in the fates of \(l\)-epinephrine and dl-epinephrine could be detected in several types of experiment.

3. Formation of methylamine from epinephrine has been demonstrated in vitro.

4. A discussion of epinephrine metabolism is presented.

**BIBLIOGRAPHY**

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