ON THE UTILIZATION OF L- AND D-ALANINE FOR THE ACETYLATION REACTION IN VIVO*

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Bloch and Rittenberg (1, 2) have reported that deuterio-DL-alanine and acetylamino acids labeled with deuterium in the acetyl group give rise to a large isotope concentration in the acetyl groups of amines containing an α-hydrogen atom, in contrast to amines not having an α-hydrogen atom. With deuterioacetic acid itself, no such difference in the acetylation of the two types of foreign amines was found, while deuteriopyruvic acid gave qualitatively similar though lower values than DL-alanine. Bloch and Rittenberg suggested that pyruvic acid, arising by deamination of alanine, could condense directly with phenylaminobutyric acid by the mechanism proposed by Knoop (3) and others (4) and that acetylation of p-aminobenzoic acid, on the other hand, took place only after conversion of the labeled pyruvic acid to acetic acid.

In a previous communication (5), differential acetylation of the two types of amines with carbon-labeled pyruvic acid was observed in a laboratory strain of rats of unknown origin.† In the Sprague-Dawley strain, on the other hand, it was found that the acetyl groups of the two types of amines had identical isotope concentrations after administration of carbon-labeled pyruvic acid (5). This has now been found to be the case also with three additional strains of rats (Table I).

The ability of carbon-labeled DL-lactic acid to serve as a precursor for the acetyl groups of the two kinds of amines has also been tested and compared to that of pyruvic and acetic acids. The resulting isotope concentrations (Table II) are identical in both types of acetylamines.

Since the data obtained with carbon-labeled pyruvic and lactic acids were in disagreement with the results of Bloch and Rittenberg with deuterio-DL-alanine, acetylation experiments with carbon- and deuterium-labeled L- and D-alanine were carried out.

* Presented in part at the meeting of the Federation of American Societies for Experimental Biology, Atlantic City, April, 1950.
† Aided by a grant from the United States Public Health Service.
† In view of the results reported here the data obtained with this strain indicate that its behavior is exceptional. In particular the use of differential acetylation for the calculation of the size of the pyruvic acid pool does not seem to be justified any longer.
EXPERIMENTAL

The synthesis of 2-C\textsuperscript{14}-pyruvic acid has been reported previously (6).

\textbf{2-C\textsuperscript{14}-DL-Lactic Acid—}This compound was prepared by hydrogenation of 2-C\textsuperscript{14}-pyruvic acid in aqueous solution with hydrogen and Adams' catalyst at atmospheric pressure and room temperature.

\begin{table}[h]
\centering
\caption{Acetylation of Foreign Amines by 2-C\textsuperscript{14}-Pyruvic Acid in Various Rat Strains}
\begin{tabular}{|l|c|c|c|c|}
\hline
Rat strain & \multicolumn{2}{|c|}{Dose (D.) of 2-C\textsuperscript{14}-pyruvic acid administered} & \multicolumn{2}{|c|}{Acetly groups of} \\
& & & Acetylphenylamino- & Acetyl-\(\text{\(p\)}\)-amino- \\
& & & butyric acid & benzoic acid \\
& & & \(\text{R. i. c.}\)\textsuperscript{*} & \(\text{R. i. c.}\) \(\text{D.}\) & \(\text{R. i. c.}\)\textsuperscript{*} & \(\text{R. i. c.}\) \(\text{D.}\) \\
\hline
Sprague-Dawley & & & & & & & \\
0.98 & & 3.2 & 2.5\textsuperscript{†} & 3.0 & 3.0 & \\
Wistar & 1.0 & 3.2 & 3.2 & 2.8 & 2.8 & \\
Sherman & & 0.74 & 3.8 & 2.8 & 3.8 & \\
Yale & & & & & & \\
\hline
\end{tabular}
\begin{tablenotes}
\item * The relative isotope concentration is calculated for 100 per cent isotope in the administered compound.
\item † Average of several experiments.
\end{tablenotes}
\end{table}

\begin{table}[h]
\centering
\caption{Acetylation of Foreign Amines by 2-C\textsuperscript{14}-DL-Lactic, 2-C\textsuperscript{14}-Pyruvic, and Deuterioacetic Acids in Same Animal}
\begin{tabular}{|l|c|c|c|c|}
\hline
Compound & \multicolumn{2}{|c|}{Dose} & \multicolumn{2}{|c|}{Acetly groups of} \\
& & & Acetylphenylamino- & Acetyl-\(\text{\(p\)}\)-amino- \\
& & & butyric acid & benzoic acid \\
& & & \(\text{R. i. c.}\)\textsuperscript{*} & \(\text{R. i. c.}\) \(\text{D.}\) & \(\text{R. i. c.}\)\textsuperscript{*} & \(\text{R. i. c.}\) \(\text{D.}\) \\
\hline
2-C\textsuperscript{14}-DL-Lactic acid & 1.02 & 1.1 & 1.1 & 1.1 & 1.1 & \\
2-C\textsuperscript{14}-Pyruvic & 1.07 & 3.3 & 3.2 & 3.8 & 3.7 & \\
Deuterioacetic & 1.02 & 3.3 & 3.2 & 3.8 & 3.7 & \\
\hline
\end{tabular}
\begin{tablenotes}
\item * See foot-note to Table I.
\end{tablenotes}
\end{table}

2-C\textsuperscript{14}-D- and L-Alanines—2-C\textsuperscript{14}-Pyruvic acid was treated with an excess of phenylhydrazine hydrochloride. The hydrazone was obtained in 95 per cent yield and hydrogenated quantitatively to DL-alanine with Adams' catalyst and hydrogen at atmospheric pressure and room temperature in water suspension. Alkali was added, the aniline formed during hydrogenation removed by extraction with ether, and the aqueous layer treated
with benzoyl chloride. After dilution with normal and deuteriobenzoyl-\(DL\)-alanine (1),\(^2\) the optical antipodes were separated via the brucine and strychnine salts by the method of Fischer (7). The alkaloid salts were recrystallized to constant isotope concentration. \(D\)-alanine, \([\alpha]_D = -12.9^\circ\), 15,700 c.p.m. as \(\text{BaCO}_3\), 9.3 per cent \(D\); \(L\)-alanine, \([\alpha]_D = +12.5^\circ\), 20,600 c.p.m. as \(\text{BaCO}_3\), 9.5 per cent \(D\).

The feeding experiments and the isotope analyses were carried out as previously described (5).

**TABLE III**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Dose</th>
<th>Acetyl groups of</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Acetylphenylamino-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>butyric acid</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R. i. c.*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>per cent</td>
</tr>
<tr>
<td>2-(\text{C}^\text{14})-L-Alanine</td>
<td>0.93</td>
<td>1.1</td>
</tr>
<tr>
<td></td>
<td>0.96</td>
<td>1.8</td>
</tr>
<tr>
<td></td>
<td>0.41</td>
<td>0.92</td>
</tr>
<tr>
<td></td>
<td>0.48</td>
<td>0.68</td>
</tr>
<tr>
<td>Deuterio-L-alanine</td>
<td>0.48</td>
<td>0.12</td>
</tr>
<tr>
<td>2-(\text{C}^\text{14})-D-Alanine</td>
<td>0.52</td>
<td>4.8</td>
</tr>
<tr>
<td>Deuterio-D-alanine</td>
<td>0.52</td>
<td>3.0</td>
</tr>
</tbody>
</table>

* See foot-note to Table I.

**Results**

Results similar to those observed with pyruvic acid were obtained with \(L\)-alanine (Table III). The lower absolute values may be ascribed to dilution of the ingested \(L\)-alanine by \(L\)-alanine present in the tissues. \(D\)-Alanine, on the other hand, yielded a substantially higher isotope concentration in the acetyl group when phenylaminobutyric acid was administered than when \(p\)-aminobenzoic acid was given. The absolute isotope concentration in acetyl-\(p\)-aminobenzoic acid is of the same order of magnitude after \(D\)-alanine as after \(L\)-alanine administration, indicating that acetylation of the aromatic amine may well proceed by way of pyruvic and acetic acids. The difference in the ability of the optical antipodes of alanine to acetylate foreign amines containing an \(\alpha\)-hydrogen atom accounts for the results obtained by Bloch and Rittenberg, and the results obtained with labeled pyruvic acid and \(L\)-alanine make acetylation of foreign amines in vivo by the Knoop mechanism unlikely.

* The deuterio-\(DL\)-alanine was kindly supplied by K. Bloch.
An experiment was carried out to investigate the possibility that d-alanine condenses with a keto acid to form an acetylamino acid after decarboxylation. This acetylamino acid could then transfer its acetyl group to a foreign amine with an α-hydrogen atom by a mechanism involving acetyl transfer, which has been shown to yield high isotope concentrations in acetylphenylaminobutyric acid (2). Unlabeled d-alanine was fed, together with labeled pyruvic acid. If these two compounds would condense to form acetylanline, the double bond of the intermediary Schiff base would shift rapidly between the nitrogen and the two α-carbon atoms. The isotope would therefore be distributed in both the alanine and acetyl moieties and transacetylation would be expected to yield a higher isotope concentration in acetylphenylaminobutyric acid. The experiment, however, gave identical isotope concentrations in both acetyl- amines (Table IV).

**DISCUSSION**

The results obtained with labeled pyruvic and lactic acids can be accounted for by assuming that most strains of rats are able to convert these acids rapidly to acetic acid, since, on an equimolar basis, acetic acid is 2 to 5 times as efficient in the acetylation reaction as are the 3-carbon compounds.

It appears that the unnatural form of alanine acetylates amines with an α-hydrogen atom more efficiently than amines which do not have an α-hydrogen atom. So far, no reaction in mammalian tissue is known which specifically utilizes an unnatural amino acid. This result, however, can be explained by the following mechanism.

It is postulated that acetic acid or an active derivative of unknown

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**Table IV**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Dose max per 100 gm, per day</th>
<th>Acetyl groups of Acetylphenylaminobutyric acid</th>
<th>Acetyl-α-aminobenzoic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>R. i. c.* per cent</td>
<td>R. i. c. per cent</td>
</tr>
<tr>
<td>2-C14-Pyruvic acid</td>
<td>0.56</td>
<td>0.9</td>
<td>1.6</td>
</tr>
<tr>
<td>+ d-alanine</td>
<td>2.5</td>
<td>1.2</td>
<td>2.1</td>
</tr>
</tbody>
</table>

* See foot-note to Table I.
constitution is the only immediate acetylating agent and that the sites of the acetylation reaction are different for the two kinds of foreign amines. For example, there is reason to assume that \( p \)-aminobenzoic acid is acetylated in the liver only. It has actually been observed, in experiments with tissue slices in \textit{vitro}, that liver is the only organ which acetylates sulfanilamide (8) which is also an aromatic amine. Any labeled compound other than acetic acid then will provide acetyl groups for the acetylation of this amine only to the extent to which it is converted to acetic acid in the liver. The labeled acetic acid formed is diluted by the large hepatic pool. This assumption is in agreement with all of the experimental data, since acetic acid itself is the most efficient precursor for the acetyl group of \( p \)-aminobenzoic acid.

On the other hand, it may be assumed that phenylaminobutyric acid can be acetylated not only in the liver but also in the kidney. Experiments in \textit{vitro} (9) support this hypothesis. Therefore, any labeled compound which is not completely metabolized in the liver and which, after transport to the kidney, can be converted in this organ to acetic acid will supply acetyl groups with high isotope concentrations for this amine, provided the dilution of the labeled metabolites in the kidney is smaller than in the liver. The experimental results with \( \delta \)-alanine can be explained adequately by this hypothesis, since the kidney has been shown by experiments in \textit{vitro} to contain high concentrations of \( \delta \)-amino acid oxidase, which converts \( \delta \)-alanine to pyruvic acid (10), and an enzyme system which is capable of converting pyruvic acid to acetic acid (11). This hypothesis can also account for the fact that the average of the values of relative isotope concentration to dose for acetylphenylamino- butyric acid after administration of labeled L-alanine is about 1.5 times greater than the average values obtained for acetyl-\( p \)-aminobenzoic acid (Table III), if it is assumed that some of the administered L-alanine reaches the kidney and is utilized there in the acetylation reaction after deamination and conversion to acetic acid. This is in contrast to the results obtained with labeled pyruvic acid where the values for the two foreign amines are always found to be identical, indicating that any pyruvic acid reaching the kidney was diluted in the liver.

The results reported here therefore appear to indicate that \( N \)-acetyl- amines are synthesized \textit{in vivo} by only one mechanism and that the differences observed with labeled precursors are due to different sites of acetylation of the two types of amines. If this is the case, acetylation of phenylaminobutyric acid in the kidney can also account for the results obtained by Bloch and Rittenberg (2) with various acetylamino acids as sources for acetyl groups of foreign amines. The part of the acetylamino acids not hydrolyzed in the liver provides acetyl groups with high isotope
concentrations in the kidney. An enzyme which hydrolyzes acetyl-L-amino acids (12) has been demonstrated to be present in high concentration in this organ. This alternative interpretation makes unnecessary the assumption of a special mechanism which transfers N-acetyl groups directly from one α-amino acid to another.

SUMMARY

Labeled pyruvic acid affords identical isotope concentrations in the acetyl groups of excreted acetylamines both with and without an α-hydrogen atom in four strains of rats in vivo. Labeled d,l-lactic acid gave the same results as did pyruvic acid.

Labeled L-alanine is utilized as a precursor for acetyl groups in a similar manner as pyruvic acid, while labeled d-alanine gives rise to exceptionally high isotope concentrations in acetylphenylaminobutyric but not in acetyl-p-aminobenzoic acid. A possible mechanism to account for these results is discussed.

The author is indebted to Dr. K. Bloch for many helpful discussions in the preparation of this manuscript and is pleased to acknowledge the technical assistance of Mr. James Hayashi.

BIBLIOGRAPHY

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