ON THE OCCURRENCE OF GLYCINAMIDE RIBOTIDE AND ITS FORMYL DERIVATIVE*

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(Received for publication, October 12, 1955)

Inosinic acid is synthesized from small molecules such as glycine, formate, and CO₂ by a multienzyme system present in pigeon liver (1-3). This paper reports the occurrence of two new aliphatic ribotides synthesized by this system and describes their isolation and metabolic interrelationships. These compounds have been shown to be precursors of inosinic acid (4-6) and have been assigned the following tentative structures: N-glycyl-5-phosphoribofuranosylamine (glycinamide ribotide) (I) and N(α-N-formyl)glycyl-5-phosphoribofuranosylamine (formylglycinamide ribotide) (II).

Evidence for the structure of these compounds will be presented in a subsequent paper (7).

Materials

Most of the substrates employed have been described previously (3). Glycine-1-C¹⁴ was prepared by the method of Sakami et al. (8). For radio-

* This work has been supported by grants from Eli Lilly and Company, the Elisabeth Severance Prentiss Foundation, and the National Institutes of Health, United States Public Health Service, grant No. S-3840.
† Oglebay Fellow in Medicine and Biochemistry, 1952-55.
‡ DuPont Predoctoral Fellow, 1954-55. A portion of this work is included in the thesis submitted by Richard A. Peabody to the Graduate School of Western Reserve University in September, 1955, in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Biochemistry.
autographic studies a specific activity of 77,000 c.p.m. per μmole was employed, while for isolation of intermediates on ion exchange columns the activity was 3000 to 5000 c.p.m. per μmole.

The preparation of the fresh acetone powder extract has been described (3). The Dowex-treated and dialyzed extract was prepared by extracting 10 gm. of acetone powder (3) with 100 ml. of 0.05 M K₂HPO₄ for 30 minutes. This material was centrifuged at 5000 × g for 20 minutes, and the supernatant solution was passed through a Dowex 1 column (HCO₃⁻ form, 1.82 sq. cm. × 12 cm.) for a period of 1 to 2 hours. The column was washed with 20 ml. of water, and the combined effluents were dialyzed against running 0.05 M K₂HPO₄ solution (20 liters) for 18 to 24 hours and lyophilized (yield 3.4 gm.). All operations prior to lyophilization were performed at 0–4°. The powder was taken up in water (100 mg. per ml.) immediately before use. The protein concentration of this solution was 70 to 80 mg. per ml.

**Methods**

**Determination of C¹⁴-Formate Incorporation into Purine and Non-Purine Fractions**—The C¹⁴-formate incorporated into the 2 and 8 carbons of inosinic acid and hypoxanthine was determined by hydrolysis with sulfuric acid, oxidation of the resulting CO to CO₂, and measurement of the radioactivity as BaCO₃ (3). The results were corrected to an infinitely thin layer. The incorporation of formate into all fractions (total formate fixation) was determined by pipetting a 0.05 ml. aliquot of the trichloroacetic acid filtrate of the reaction mixture into a glass planchet (Tracerlab, Inc.). After addition of 5 drops of water and 5 drops of alcohol, the solution was dried slowly under an infra-red lamp, and the radioactivity of the residue was measured. These results were corrected to give values equivalent to those obtained when the samples were counted as BaCO₃. The non-purine fraction was calculated as the difference between the total formate fixed per vessel and the formate incorporated into the 2 and 8 carbons of purine.

**Assay for Formylglycinamide Ribotide**—The synthesis of formylglycinamide ribotide was measured by the incorporation of C¹⁴-formate into a form which was unstable to acid hydrolysis. This was calculated as the difference between the total formate fixation (described above) and the fixation into a fraction stable to acid hydrolysis. To determine the radioactive fraction not hydrolyzed by acid, 0.2 ml. of the trichloroacetic acid filtrate was heated at 100° for 15 minutes with 0.1 ml. of 3 N HCl. After dilution with water to 2.0 ml., a 0.5 ml. aliquot was pipetted into a glass planchet, dried, and counted.

**Assay for Glycinamide Ribotide**—The formation of the glycinamide ribotide was estimated by employing glycine-1-C¹⁴ as a precursor. The amide
linkage in glycinamide ribotide and in formylglycinamide ribotide was not affected by heating to 100° at pH 5.4. Therefore, on treatment of an aliquot of the reaction filtrate with ninhydrin, the radioactivity which remained after the residual glycine-1-C\(^{14}\) was decarboxylated represented glycinamide ribotide (or formylglycinamide ribotide). A 0.1 ml. aliquot of the trichloroacetic acid filtrate was pipetted carefully into the bottom of a small test-tube and neutralized with 1 M sodium hydroxide by using brom thymol blue. 1 ml. of 0.1 M potassium phosphate buffer, pH 5.4, 0.1 ml. of 0.1 M non-radioactive glycine, and 1.0 ml. of ninhydrin solution (30 mg. per ml.) were added. The tube was covered with a glass marble and heated at 100° for 30 minutes. The mixture was cooled, and after the addition of 1 drop of caprylic alcohol it was aerated with CO\(_2\) for 15 minutes. Water was added to 10 ml., and a 2 ml. aliquot was pipetted into a glass planchet, dried, and counted. The factor for the correction of the results to an infinitely thin layer in a glass planchet was found to be approximately 2.4. Evidence for the specificity of these assays is presented under "Results."

**Chromatography**—For all paper chromatographic studies except the large scale preparations, Whatman No. 1 paper was employed. Two-dimensional chromatograms were developed by the ascending technique. For large scale preparations a thick paper (Eaton and Diekman No. 627-030) was used. A single thickness of Whatman No. 1 was sewn on as a leader to control the rate of solvent flow from the trough to the starting line. The following solvent systems were employed (all ratios are in volume proportions): (1) propanol and water (65:34) in an atmosphere of ammonia (0.5 ml. of 1 M ammonium hydroxide per liter of jar volume), (2) pyridine and water (65:35), (3) n-butanol-17.6 \(N\) acetic acid-water (2:1:1), (4) isopropanol-water (70:30) with 0.35 ml. of concentrated ammonium hydroxide per liter of jar volume, (5) propanol-water (60:40), (6) 95 per cent ethanol-water (77:23), and (7) methanol-formic acid (88 per cent by weight)-water (80:15:5). With Solvent 6 the paper was dipped in 1.2 per cent sodium citrate buffer, pH 5.4, and dried prior to application of compounds.

Radioactive compounds were located with a monitor and were eluted with water from the papers by descending chromatography. For radioautograms, precursors with a specific activity of 60,000 to 120,000 c.p.m. per pmole were employed. 0.1 ml. of the trichloroacetic acid reaction filtrate was applied to a spot 1 cm. in diameter and was chromatographed in two dimensions. The dried chromatogram was exposed to x-ray film for 4 to 7 days. A permanent photographic record of a film occasionally was made on Kodagraph Contact Standard paper.

For anion exchange chromatography Dowex 1 (4 per cent cross-linked, 250 to 400 mesh, formate form) was employed. Reaction mixtures were
adjusted to pH 9.0 with ammonium hydroxide solution and applied to the column.

Results

Incorporation of C\textsubscript{14}-Formate and Glycine-1-C\textsubscript{14} into Unidentified Fractions

—Fig. 1 shows the incorporation of C\textsubscript{14}-formate into the purine and non-purine fractions at different time intervals. The total C\textsubscript{14}-formate in the non-purine fraction increased to a maximum at 40 minutes, while the total activity in the purine fraction increased during the 120 minute period. This suggested a precursor-product relationship.

![Figure 1](http://www.jbc.org/)

**Fig. 1.** The rate of incorporation of C\textsubscript{14}-formate into purine and non-purine fractions. The reaction system was similar to that described under Table I.

In Table I the effect of bicarbonate and boiled extract on the incorporation of C\textsubscript{14}-formate into the purine and non-purine fractions is presented. When bicarbonate was omitted, the non-purine fraction decreased from 0.22 to 0.14 μmole, while the purine fraction decreased from 0.14 to 0.03 μmole. This resulted in a shift of the non-purine to purine ratio from a value of 1.6 to 4.7. While in this experiment a fall in the non-purine fraction occurred when bicarbonate was omitted, in others an increase was observed. Omission of the boiled extract appeared to decrease purine synthesis and to have no significant effect on the non-purine fraction. It should be pointed out that the extract used in these experiments had not been treated with Dowex and had not been dialyzed.

The rate of fixation of glycine-1-C\textsubscript{14} was found to be proportional to time (Table II) for 90 minutes. After 30 minutes the total C\textsubscript{14}-glycine fixation exceeded purine synthesis by a factor of 5.6. This indicated that C\textsubscript{14}-glycine as well as C\textsubscript{14}-formate (Table I) was incorporated into compounds.
other than inosinic acid and hypoxanthine. In Table II\textsuperscript{1} purine synthesis was measured by the incorporation of C\textsuperscript{14}O\textsubscript{2} into carbon 6 (3). C\textsuperscript{14}O\textsubscript{2} was not significantly diluted by endogenous carbon in such experiments.\textsuperscript{2}

**Table I**

*C\textsuperscript{14}-Formate Incorporated into Carbons 2 and 8 of Total Hypoxanthine Fractions and into Non-Purine Fraction*

<table>
<thead>
<tr>
<th>Omissions</th>
<th>Purine (C2 + C8)</th>
<th>Non-purine</th>
<th>(b)</th>
<th>(k)/(a)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(\mu\text{mole})</td>
<td>(\mu\text{mole})</td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>0.14</td>
<td>0.22</td>
<td>1.6</td>
<td></td>
</tr>
<tr>
<td>KHCO\textsubscript{3}</td>
<td>0.03</td>
<td>0.14</td>
<td>4.7</td>
<td></td>
</tr>
<tr>
<td>Boiled extract</td>
<td>0.09</td>
<td>0.20</td>
<td>2.2</td>
<td></td>
</tr>
</tbody>
</table>

The reaction mixture contained 0.4 ml. of an extract of pigeon liver acetone powder, 1.0 \(\mu\text{mole}\) of IMP, 5 \(\mu\text{moles}\) of ATP, 15 \(\mu\text{moles}\) of PGA, 7 \(\mu\text{moles}\) of KHCO\textsubscript{3}, 8 \(\mu\text{moles}\) of MgCl\textsubscript{2}, 16 \(\mu\text{moles}\) of DL-homocysteine, 0.2 ml. of boiled extract of pigeon liver (3), and 5 \(\mu\text{moles}\) of C\textsuperscript{14}-formate in a final volume of 0.65 ml. The mixture was incubated 40 minutes at 38° in air.

**Table II**

*Total Glycine "Fixation" and De Novo Synthesis of Purine*

<table>
<thead>
<tr>
<th>Time (min.)</th>
<th>Glycine-1-C\textsuperscript{14} incorporation</th>
<th>C\textsuperscript{14}O\textsubscript{2} incorporation into purine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(\mu\text{mole})</td>
<td>(\mu\text{mole})</td>
</tr>
<tr>
<td>15</td>
<td>0.22</td>
<td>0.075*</td>
</tr>
<tr>
<td>30</td>
<td>0.42</td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>1.10</td>
<td></td>
</tr>
<tr>
<td>90</td>
<td>1.60</td>
<td></td>
</tr>
</tbody>
</table>

The reaction mixture contained 0.5 ml. of acetone powder extract, 5 \(\mu\text{moles}\) of glycine-1-C\textsuperscript{14}, 5 \(\mu\text{moles}\) of sodium formate, 10 \(\mu\text{moles}\) of glutamine, 50 \(\mu\text{moles}\) of KHCO\textsubscript{3}, 1.2 \(\mu\text{moles}\) of ATP, 14 \(\mu\text{moles}\) of PGA, 5 \(\mu\text{moles}\) of MgCl\textsubscript{2}, 5 \(\mu\text{moles}\) of DL-homocysteine, and 5.5 \(\mu\text{moles}\) of KH\textsubscript{2}PO\textsubscript{4} in a final volume of 1.3 ml. The mixture was incubated at 38° in air.

* In this vessel 50 \(\mu\text{moles}\) of KHC\textsuperscript{14}O\textsubscript{3} and 10 \(\mu\text{moles}\) of non-radioactive glycine were used. The C\textsuperscript{14} activity of carbon 6 of the purine fraction was determined (3).

**Radioautographic Observations on Accumulation of Glycinamide Ribotides**

- Fig. 2 is a radioautogram of a typical two-dimensional chromatogram. It demonstrates the occurrence of a number of compounds which are la-

\textsuperscript{1} The abbreviations used are: ATP, adenosine triphosphate; IMP, inosine-5’-phosphate; AMP, adenosine-5’-phosphate; PGA, 3-phosphoglycerate; R5P, ribose-5-phosphate; CF, calcium leucovorin.

\textsuperscript{2} Goldthwait, D. A., unpublished data.
beled by glycine-1-C\textsuperscript{14}. The area \textit{A} denotes the location of glycinamide ribotide and formylglycinamide ribotide, \textit{B} the position of glycine, and \textit{C} the position of hypoxanthine. The identities of the less dense spots are not known. In a comparable experiment, C\textsuperscript{14}-glycine was added to one vessel and C\textsuperscript{14}-formate to another. Fig. 3 shows radioautograms of chromatograms of the two reaction filtrates. Some of the compounds labeled by C\textsuperscript{14}-glycine and some labeled by C\textsuperscript{14}-formate have similar chromato-

![Fig. 2. Incorporation of glycine-1-C\textsuperscript{14} into various compounds. Area \textit{A} represents nucleotides, \textit{B} glycine-1-C\textsuperscript{14}, and \textit{C} hypoxanthine. The reaction mixture contained 0.5 ml. of acetone powder extract, 5 \textmu moles of glycine-1-C\textsuperscript{14}, 10 \textmu moles of sodium formate, 50 \textmu moles of KHCO\textsubscript{3}, 10 \textmu moles of glutamine, 5.0 \textmu moles of ATP, 10 \textmu moles of PGA, 8 \textmu moles of MgCl\textsubscript{2}, and 0.2 ml. of boiled extract of liver in a final volume of 1.1 ml. The mixture was incubated for 2 hours at 38° under N\textsubscript{2}. Then 0.25 ml. of 20 per cent trichloroacetic acid was added, and 0.1 ml. of the filtrate was placed on the lower left corner of the paper and chromatographed in the vertical direction with solvent System 1 and in the horizontal direction with solvent System 2.](http://www.jbc.org/)

graphic behavior; compare the spots marked \textit{A}. Fig. 4 provides evidence that the radioactive compounds in \textit{A} of Figs. 2 and 3 were not compounds known to incorporate C\textsuperscript{14}-formate or glycine-1-C\textsuperscript{14}. Fig. 4 is a reproduction of a chromatogram of a reaction filtrate to which methionine, cystathionine, glycine, serine, hypoxanthine, inosine, and inosinic acid were added as marker substances. When HC\textsuperscript{14}O\textsubscript{3}\textsuperscript{-} was used as a tracer under the same conditions and the reaction filtrate was chromatographed, no radioactivity was detected in the spot comparable to Fig. 2, \textit{A}.

A preliminary experiment was carried out to determine whether these compounds were purine precursors. A portion of the compounds in \textit{A} labeled with C\textsuperscript{14}-glycine was found to be converted to inosinic acid in the
presence of a large pool of unlabeled glycine. More detailed experiments on the incorporation of glycaminamide ribotide and formylglycinamide ribotide have been published (6).

Isolation of Glycaminamide Ribotide and Formylglycinamide Ribotide—Inorganic phosphate was separated from the ribotide fraction by chromatography of the reaction filtrates on thick paper (solvent System 3). An alternative procedure involved the removal of the trichloroacetic acid by extraction with ether and the precipitation at pH 8.0 of inorganic phosphate and some phosphate esters by addition of barium hydroxide solution. The glycine ribotides remained in the supernatant solution. In preliminary large scale preparations of the glycaminamide ribotides, glycine-1-$\text{C}^{14}$ was incubated with undialyzed extract of acetone powder and with additions as described under Fig. 5. The entire trichloroacetic acid filtrate was chromatographed on thick paper, and the radioactive compounds which migrated with AMP and IMP were eluted, made alkaline with ammonium hydroxide, and placed on a Dowex 1 formate column. Fig. 5 rep-

![Fig. 3. The incorporation of glycine-1-$\text{C}^{14}$ and $\text{C}^{14}$-formate into comparable compounds. Area $A$ represents glycaminamide ribotides, and area $B$, serine and glycine. The reaction mixture contained 0.5 ml. of extract of acetone powder, 5 $\mu$moles of glycine or glycine-1-$\text{C}^{14}$, 7.5 $\mu$moles of sodium formate or $\text{C}^{14}$-formate, 37 $\mu$moles of KHCO$_3$, 10 $\mu$moles of glutamine, 2 $\mu$moles of ATP, 7 $\mu$moles of PGA, 8 $\mu$moles of MgCl$_2$, 5 $\mu$moles of dl-homocysteine, and 0.2 ml. of boiled extract in a final volume of 1.2 ml. The mixture was incubated at 38° for 2 hours. 0.3 ml. of 20 per cent trichloroacetic acid was added and 0.1 ml. of the reaction filtrate was placed on the lower left corner of the paper and chromatographed. Solvent System 2 (see “Methods”) was used for the vertical development and solvent System 3 for the horizontal.](http://www.jbc.org/)

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GOLDTHWAIT, PEABODY, AND GREENBERG 561
represents a typical elution pattern. With the large column employed in this experiment (2.0 sq. cm. X 32 cm.), glycinamide ribotide (I) was eluted with 0.05 M ammonium formate, pH 5.2. Under these conditions, two radioactive bands appeared. However, with a small column (0.64 sq. cm. X 8 cm.) the compound was eluted with buffer of the same molarity at pH 6.5 and, in this case, did not separate into the two components. With the large column formylglycinamide ribotide (II) was eluted at pH 5.0 and was separated into two components.

Fig. 4. Comparison of the paper chromatographic behavior of the aliphatic ribotides with other compounds. U represents unidentified compounds which reacted with ninhydrin.

The quantity of glycinamide ribotide varied in different experiments from 6 to 42 per cent of the total aliphatic ribotides. The factors responsible for these variations in the crude extracts are not yet clear. The first component of glycinamide ribotide eluted from the column was consistently larger than the second, while the first component of the formylglycinamide ribotide accounted for 29 to 49 per cent of its total. Elution of the Dowex 1 column at pH 9 (Fig. 5) yielded glycine and a compound which was readily decarboxylated with ninhydrin and which was not a precursor of the purine ring.

Isomeric Nature of Two Components of Each Ribotide—The evidence that these components (Fig. 5) were isomers was as follows: By exposure to acid, it was demonstrated that each of the components of formylglycinamide ribotide could be converted to a mixture of the two. Thus, when
the first component was exposed to 5 per cent trichloroacetic acid at 2°C for 24 hours and then rechromatographed, two components appeared. The elution pattern was similar to that in Fig. 5 except that the material in the first peak accounted for 68 per cent of the total. Under comparable acid conditions the second component yielded two peaks with 46 per cent of the total material in the first. As a control the second component was allowed to stand at pH 7.0 at 2°C for 48 hours. When this was passed through the column, only a single component appeared. This chromato-

FIG. 5. Elution of glycinamide ribotide (I) and formylglycinamide ribotide (II) from a Dowex 1 column. The reaction mixture contained 12 gm. of a lyophilized extract of acetone powder, 1.0 mmole of glycine-1-C14 (3 X 10⁶ c.p.m.), 2.0 mmoles of sodium formate, 0.4 mmole of IMP, 2.0 mmoles of glutamine, 0.5 mmole of ATP, 2.8 mmoles of PGA, 1.6 mmoles of MgCl₂, 1.0 mmole of dl-homocysteine, and 47 ml. of boiled extract in a final volume of 240 ml. The reaction mixture was incubated at 38°C for 1.5 hours under N₂. The graphic behavior following exposure to acid as well as the analytical data on the two components of formylglycinamide ribotide (5, 6) suggested that isomers existed. Analyses of the two purified components of glycinamide ribotide (7) and of its formyl derivative (5, 6) lend further support to the suggestion that both ribotides can exist as isomers. There is no evidence that these isomers exist in nature.

Some Chromatographic Properties of Glycinamide Ribotides—The behavior of these compounds on Dowex 1 (formate form) is illustrated in Figs. 5 and 6. Formylglycinamide ribotide was not retained on a Dowex 50 (H⁺ form) column at pH 1.0. It was immediately eluted from a charcoal column with 5 per cent aqueous ethanol. In most of the solvent systems both of the aliphatic ribotides migrated on paper chromatograms with the
mononucleotides. \( R_P \) values with several solvents are presented in Table III.

**Cofactor Requirement for Formylation of Glycinamide Ribotide**—It was considered that the formylation of glycinamide ribotide required a folic acid derivative, as had been demonstrated for the conversion of 5-amino-4-imidazolecarboxamide-5'-phosphoriboside to inosinic acid (9, 10).

Fig. 6 illustrates the effect of the cofactor upon the synthesis of the aliphatic ribotides. The natural formylation cofactor was removed by treatment of the enzyme with Dowex 1, followed by dialysis. A reduced folic acid derivative then was necessary for the incorporation of \( ^{14}C \)-formate into an acid-hydrolyzable form. The reaction mixture in each of the experiments contained ribose-5-phosphate, ATP and an ATP-regenerating system, glutamine, \( \text{MgCl}_2 \), and the Dowex-treated and dialyzed enzyme. In

<table>
<thead>
<tr>
<th>Table III</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>( R_P ) Values for Glycinamide Ribotide and Formylglycinamide Ribotide</strong></td>
</tr>
<tr>
<td>Compound</td>
</tr>
<tr>
<td>Glycinamide ribotide</td>
</tr>
<tr>
<td>Formylglycinamide ribotide</td>
</tr>
<tr>
<td>IMP</td>
</tr>
<tr>
<td>Glycine</td>
</tr>
</tbody>
</table>

* Solvent systems (see “Methods”).

Experiment 1 glycine-1-\( ^{14}C \) was employed as a tracer. Experiment 2 differed from Experiment 1 only by the addition of calcium leucovorin (ni. mixture of CF) and of unlabeled sodium formate. In Experiment 3, \( ^{14}C \)-formate was employed as the tracer, and unlabeled glycine and CF were added. At the end of the incubation the mixtures were treated as described in the legend to Fig. 6 and passed through Dowex formate columns.

Each of the columns was eluted with ammonium formate buffers as in Fig. 5. In Experiment 1 the glycinamide ribotide fraction accounted for all the fixed glycine. In Experiment 2, 8 per cent of the total glycine fixed appeared in a component eluted at pH 6.5. The remainder represented formylglycinamide ribotide. In Experiment 3 formylglycinamide ribotide accounted for 98 per cent of the acid-hydrolyzable \( ^{14}C \)-formyl compounds. In order to compare the migration of each of the major components, they were rechromatographed on new Dowex 1 columns (formate form, 0.64 sq. cm. \( \times \) 8.0 cm.). Fig. 6 shows the elution patterns and demonstrates clearly that, in the absence of the folic acid derivative and formate, glycin-
Fig. 6. Effect of citrovorum factor and formate on the synthesis of glycaminamide ribotide and formylglycinamide ribotide. The reaction mixtures were prepared by the addition of 200 mg. of lyophilized Dowex-treated and dialyzed extract, 30 μmoles of ATP, 64 μmoles of MgCl₂, 50 μmoles of sodium phosphocreatine, 0.5 ml. of a dialyzed 1:2 water extract of rabbit muscle (creatine kinase), 50 μmoles of glutamine, 25 μmoles of R5P, 25 μmoles of C¹⁴-formate (47,300 c.p.m. per μmole) or unlabeled formate, 50 μmoles of glycine-1-C¹⁴ (19,000 c.p.m. per μmole) or unlabeled glycine, and 2 mg. of calcium leucovorin in a final volume of 6.7 ml. Three vessels were prepared identically except in the additions of glycine, formate, and calcium leucovorin indicated. The vessels were incubated 40 minutes at 38° in air. The radioactive products in the trichloroacetic acid filtrates were separated on three identical Dowex 1 columns (formate form 0.64 sq. cm. × 24 cm.) as described in the text. The major component from each column was rechromatographed on a second Dowex 1 column (formate form, 0.64 sq. cm. × 8.0 cm.) as shown in Fig. 6.
amide ribotide accumulated, while in its presence formylglycinamide ribotide, labeled either with C$^{14}$-glycine or C$^{14}$-formate, was formed.

**DISCUSSION**

Studies of the synthesis of glycinamide and formylglycinamide ribotide have been facilitated by relatively rapid analytical methods. These have employed the incorporation of glycine-1-C$^{14}$ into forms which did not lose their radioactivity by reaction with ninhydrin and the incorporation of C$^{14}$-formate into an acid-hydrolyzable derivative. While it is evident that these methods lack specificity, their validity in these experiments has been tested by a comparison with the reaction products isolated by ion exchange chromatography. In the absence of a folic acid derivative, all of the radioactivity derived from glycine-1-C$^{14}$ and not lost by reaction with ninhydrin was found in the glycinamide ribotide fraction. In the presence of the folic acid derivative, 92 per cent of the fixed glycine-1-C$^{14}$ was present as formylglycinamide ribotide, while the remaining 8 per cent appeared to be in glycinamide ribotide. Under these conditions, there was no evidence for the synthesis of a peptide such as glutathione which would contain glycine in a form not affected by ninhydrin. The formylglycinamide ribotide isolated by column chromatography accounted for 98 per cent of the acid-hydrolyzable radioactivity derived from C$^{14}$-formate. With the Dowex-treated and dialyzed extract, the total formate fixation was occasionally as much as 15 per cent higher than formate fixation into acid-hydrolyzable forms. This was found mainly in inosinic acid. Incorporation into serine was negligible in most instances. An exception to this appears in Fig. 3, in which area B coincides with the migration of serine. However, in these experiments untreated extract was used. No methionine synthesis was detected in the incubation mixtures under the described conditions, and the C$^{14}$-formate incorporated into folic acid derivatives was negligible with the catalytic amounts employed.

The behavior of these ribotides on an anion exchange column is a reflection of their net charge. Glycinamide ribotide and formylglycinamide ribotide were eluted from the Dowex 1 column with 0.05 M ammonium formate at pH 6.5 and 5.0, respectively. Unlike nucleotides, these compounds can be eluted while they still possess 1 negative charge. This behavior may be due to their aliphatic nature. Ribose-5-phosphate was eluted at pH 5.0 with the formylglycinamide ribotide.

The natural folic acid derivative involved in the formylation of glycinamide ribotide has not been identified. In these experiments the reduced folie acid compound employed represents a model system. The mechanism of formylation is most likely comparable to that involved in the intro-
duction of formate into 5-amino-4-imidazolecarboxamide ribotide to form inosinic acid (10).

Recently Hartman, Levenberg, and Buchanan (11) have reported the accumulation of glycinamide ribotide and the formyl derivative in pigeon liver extracts in the presence of azaserine.

**SUMMARY**

Glycinamide ribotide and formylglycinamide ribotide, which are precursors of inosinic acid, were formed by an extract of pigeon liver. The preliminary isolation of these ribotides by paper and ion exchange chromatography and the methods of determining these compounds in reaction filtrates have been described. The specificity of the analytical methods was verified by chromatographic isolation of the ribotides. In the presence of a folic acid derivative and formate, formylglycinamide ribotide was shown to accumulate, while in its absence glycinamide ribotide was synthesized.

**BIBLIOGRAPHY**

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