THE SYNTHESIS OF GLUTATHIONE IN ISOLATED LIVER*

BY KONRAD BLOCH

(From the Department of Biochemistry and the Institute of Radiobiology and Biophysics, University of Chicago, Chicago)

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The mechanism which is responsible for the formation of individual peptide bonds in biological systems has so far remained obscure. There has as yet been no successful demonstration of an in vitro enzymatic synthesis of a simple peptide composed of natural amino acids. Various investigators have approached this problem by employing model substances which contain a CO—NH linkage but differ from natural peptides in that either the carboxylic or amino component or both are not α-amino acids. This is true for the acyl peptides studied by Bergmann and Behrens (1) and also for acetylsulfanilamide (2), p-aminohippuric acid (3), hippuric acid (4), and glutamine (5). In all these cases the enzymatic formation of peptide bonds has been demonstrated. The incorporation of amino acids into the proteins of isolated tissues has recently been investigated with the aid of isotopic tracers (6–9), but experiments of this type do not lend themselves readily to a study of the mechanisms involved in the formation of individual peptide bonds.

We have previously reported the formation of glutathione from its constituent amino acids in rat liver slices (10). It was felt that glutathione was particularly suited to the study of peptide bond synthesis, because no other peptide is obtainable with comparable ease and purity from small amounts of animal tissues. These experiments dealing with the in vitro synthesis of glutathione were originally undertaken to test the hypothesis that N-acetylamino acids which appear to be intermediates in the normal metabolism of amino acids (11) might be concerned in peptide synthesis (12–14). Experiments with liver slices yielded inconclusive results in that glycine and N-acetylglycine were utilized equally well for glutathione formation.

In the course of attempts to differentiate between the behavior of the free amino acid and its acetyl derivative it was observed that glutathione synthesis proceeds readily in liver homogenates. In this system acetylglycine, in contrast to glycine, proved to be ineffective. This report describes some experiments which have been carried out to study the con-

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ditions associated with the formation of glutathione in these broken cell preparations.

EXPERIMENTAL

Synthesis of Isotopic Amino Acids—Glycine and L-glutamic acid labeled with N\textsuperscript{15} were prepared as described by Schoenheimer and Ratner (15). The amino acids contained 31.5 atom per cent excess N\textsuperscript{15}.

C\textsubscript{14}-Glycine—Carboxyl-labeled glycine was synthesized by the following series of reactions:

\begin{align*}
(1) & \quad \text{CH}_3\text{C}^{14}\text{O} \text{OK} \xrightarrow{\text{C}_3\text{H}_4\text{COBr}} \text{CH}_3\text{C}^{14}\text{OBr} \\
(2) & \quad \text{CH}_3\text{C}^{14}\text{OBr} + \text{Br}_2 \longrightarrow \text{BrCH}_2\text{C}^{14}\text{OBr} + \text{HBr} \\
(3) & \quad \text{BrCH}_2\text{C}^{14}\text{OBr} + \text{H}_2\text{O} \longrightarrow \text{BrCH}_2\text{C}^{14}\text{OOH} + \text{HBr} \\
(4) & \quad \text{BrCH}_2\text{C}^{14}\text{OOH} + \text{NH}_3 \longrightarrow \text{NH}_2\text{CH}_2\text{C}^{14}\text{OOH} + \text{HBr}
\end{align*}

CH\textsubscript{3}C\textsuperscript{14}OOH was prepared by the interaction of CH\textsubscript{3}MgBr with C\textsubscript{14}O\textsubscript{2}. Anhydrous potassium acetate was heated with benzoic acid in the presence of benzoic acid to yield acetyl bromide as described by Anker (16). The acetyl bromide was collected in an ice-cooled flask and 1.1 moles of bromine per mole of acetyl bromide were added slowly. The mixture was warmed and heated on a steam bath for 1.5 hours and then freed of excess bromine and of HBr by a stream of nitrogen. An excess of water was added dropwise to the ice-cooled bromoacetyl bromide. The clear aqueous solution of bromoacetic acid was then added to a volume of concentrated NH\textsubscript{3} containing 70 M excess. The solution was kept at room temperature for several hours and then evaporated in vacuo to dryness. The residue was dissolved in a small volume of water and the glycine precipitated by addition of 4 volumes of methanol. The yield after one recrystallization from water-ethanol was 55 to 60 per cent, based on the potassium acetate used. The radioactivity of the glycine was 100,000 counts per minute counted as an infinitely thick sample after conversion to BaCO\textsubscript{3}.

This method for the preparation of carbon-labeled glycine has been found to be more convenient and to give higher yields than that described in the literature (17). Little handling of the radioactive intermediates is necessary and after the distillation of acetyl bromide all further operations can be carried out in the same flask.

Acetylglycine was prepared either from C\textsubscript{14}-glycine or N\textsuperscript{15}-glycine as previously described (18).

Incubation Experiments—In the experiments with intact tissue (Table I, Experiments 1 to 3) 1.5 gm. of liver slices were suspended in a medium of the following composition: labeled glycine or acetylglycine 0.01 M, Krebs'
phosphate buffer of pH 7.4, and 25 mg. of carrier glutathione. The total volume per flask was 20 ml.

The liver homogenates were prepared by dispersing pigeon liver in a Waring blender in a medium of the following composition: phosphate buffer of pH 7.4 0.05 M, KCl 0.03 M, MgSO₄ 0.0024 M, glutamic acid 0.01 M, cysteine 0.003 M, C¹⁴-glycine 0.016 M, and 25 mg. of carrier glutathione. The homogenates contained 1.6 gm. of pigeon liver in a total volume of 20 ml. The final pH was 7.4, and the time of incubation 1 hour.

The medium in Experiment 1, Table IV, contained N¹⁵-glutamic acid instead of non-isotopic glutamic acid. The molar ratio of C¹⁴-glycine to N¹⁵-glutamic acid was 1:1. In Experiment 2, Table IV, there were added C¹⁴-glycine 0.016 M, N¹⁵-NH₄Cl 0.008 M, and non-isotopic glutamic acid 0.016 M.

Isolation of Glutathione—25 mg. of non-isotopic glutathione per flask were added to the incubation medium in order to facilitate the isolation of isotopic glutathione in quantities sufficient for purification and isotope analysis. No differences in the isotope concentration of glutathione were observed whether the carrier was added before or after incubation. After incubation the reaction mixture was deproteinized with trichloroacetic acid and glutathione was precipitated first as the cadmium salt and then as the cuprous mercaptide as described by Waelsch and Rittenberg (19). The purity of the glutathione samples which were obtained from the experiments with amino acids labeled by N¹⁵ was checked by determination of Kjeldahl nitrogen. The majority of the glutathione samples which contained C¹⁴ were redissolved by the addition of an excess of cuprous oxide and reprecipitated by aeration (20). This treatment did not change the isotope concentration in the mercaptides, indicating that the product obtained in the first precipitation with cuprous oxide was pure. Reprecipitation of glutathione containing C¹⁴ in the presence of non-isotopic glycine likewise failed to depress the isotope concentration.

Glutathione formed in homogenates in the presence of C¹⁴-glycine contained isotopic carbon only in the glycine moiety, as shown by the following experiment. 100 mg. of glutathione, with a C¹⁴ content of 151 counts per minute as an infinitely thick sample after combustion to barium carbonate, were hydrolyzed by refluxing with 20 per cent HCl for 8 hours. Glutamic acid was isolated from the hydrolysate as the hydrochloride, cysteine as the cuprous mercaptide, and glycine in the form of its toluenesulfonyl derivative. No radioactivity was detectable in the glutamic acid and in cysteine. The C¹⁴ content of the toluenesulfonylglycine was 171 counts per minute as compared to 167 counts calculated from the C¹⁴ content of glutathione. The radioactivity of glutathione therefore resided exclusively in the glycine moiety of the peptide.

The quantity of glutathione (in mg.) which is newly synthesized (x)
can be calculated from the specific activity \( C_0 \) of the \(^{14}C\)-glycine added, the specific activity \( C \) of glycine carbon in the isolated glutathione, and the sum of glutathione (in mg.) originally present in the tissue \( G_1 \) and carrier glutathione added \( G_2 \).

\[
x = \frac{C \times (G_1 + G_2)}{C_0}
\]

(1)

This involves the assumption that the added isotopic glycine is not significantly diluted by glycine from the tissues. That this is the case is shown by the observation that doubling the molarity of isotopic glycine in the incubation medium did not raise the isotope concentration of glutathione. Since the amount of carrier glutathione was always much larger (10 to 15 times) than the tissue glutathione, fluctuation in the original glutathione content of the tissues will introduce only a small error in the calculation of \( x \). Glutathione determined iodometrically \((21)\) in aliquot samples of the tissue used for incubation was found to vary from 1.3 to 1.7 mg. per gm. of wet tissue. An average value of 1.5 mg. for \( G_1 \) was used for the calculation of the quantities of newly synthesized glutathione according to equation (1).

No attempt has been made to determine in the present experiments whether the total quantity of glutathione increases. It is conceivable that concurrently with its formation glutathione is also being removed and in this case the total quantity of glutathione may remain unchanged during the experimental period. The possibility that there is a considerable net decrease of glutathione during incubation of the homogenates under our conditions has been ruled out by the finding that the isotope concentrations in glutathione are the same whether the carrier glutathione is added before or after incubation\(^1\) and by glutathione determinations in controls before and after incubation.

Isotope Analyses—The amino acids and glutathione samples which contained \(^{15}N\) were digested by the Kjeldahl procedure and the ammonia converted to nitrogen for mass spectrometric analysis as described by Rittenberg et al. \((22)\). For \(^{14}C\) analysis the compounds were burned in a micro combustion apparatus and carbon dioxide precipitated as barium carbonate. The \(^{14}C\) content of the barium carbonate samples was measured with a thin window Geiger-Müller counter by the procedure of Reid \((23)\). Samples were counted for a sufficient length of time to insure less than 5 per cent probable error. The \(^{14}C\) values are given, unless stated otherwise, as counts of \(^{14}C\) per minute of BaCO\(_3\) samples corrected for infinite thickness.

\(^1\)Unpublished results by R. B. Johnston;
DISCUSSION

It has been shown previously that in rat liver slices isotopic nitrogen from labeled glycine and N-acetylglycine is incorporated into glutathione at a similar rate (10). On the basis of these results it could not be decided whether acetylation of the amino acid was a preliminary step in the formation of glutathione. Equivocal results are to be expected from experiments with liver slices, since both the acetylation of amino acids and the hydrolysis of the acetyl derivatives take place under these conditions (11). That acetylation does not precede the incorporation of glycine into pep-

**Table I**

**Aerobic Formation of Glutathione from Labeled Amino Acids in Isolated Liver**

The composition of the incubation medium is described in the experimental part.

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Tissue</th>
<th>Time</th>
<th>Relative isotope concentration in glutathione*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Rat liver slices</td>
<td>1/2</td>
<td>Labeled glycine added</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Labeled acetylglycine added</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>1</td>
<td>0.64</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.22</td>
</tr>
<tr>
<td>3</td>
<td>Pigeon liver slices</td>
<td>1</td>
<td>1.04</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.90</td>
</tr>
<tr>
<td>4</td>
<td>&quot;</td>
<td>1</td>
<td>1.76</td>
</tr>
<tr>
<td></td>
<td>&quot;</td>
<td></td>
<td>1.2</td>
</tr>
<tr>
<td>5</td>
<td>&quot;</td>
<td>1</td>
<td>0.52</td>
</tr>
<tr>
<td></td>
<td>&quot;</td>
<td></td>
<td>1.4</td>
</tr>
<tr>
<td></td>
<td>&quot;</td>
<td>1</td>
<td>1.89</td>
</tr>
<tr>
<td></td>
<td>&quot;</td>
<td></td>
<td>0.23</td>
</tr>
<tr>
<td></td>
<td>&quot;</td>
<td>1</td>
<td>1.89</td>
</tr>
<tr>
<td></td>
<td>&quot;</td>
<td></td>
<td>0.15</td>
</tr>
<tr>
<td></td>
<td>&quot;</td>
<td>1</td>
<td>1.7</td>
</tr>
<tr>
<td></td>
<td>&quot;</td>
<td></td>
<td>0.06</td>
</tr>
</tbody>
</table>

* Atom per cent excess N\(^{15}\) or specific activity (C\(^{14}\)) in glycine moiety calculated for 100 atom per cent excess N\(^{15}\) and a specific activity of 100 respectively in labeled amino acid added. In Experiments 1 to 4 the amino acids were labeled by N\(^{15}\), in Experiment 5 by C\(^{14}\).

Glycine linkages is suggested by experiments of shorter duration in which glycine gave rise to significantly higher isotope concentrations in glutathione than did acetylglycine (Table I). In these cases acetylglycine is evidently not used as such but only after conversion to the free amino acid. The rate of splitting of the acetyl derivative therefore appears to be slightly slower than the rate of glycine entrance into glutathione. This difference in reactivity of glycine and acetylglycine is accentuated when

*In one experiment with pigeon liver slices considerably more isotope was incorporated into glutathione from acetylglycine than from glycine (Experiment 3 Table I). Because of the results obtained subsequently with homogenates, this observation was not further investigated.
the same process is studied in pigeon liver homogenates. In this system
the utilization of acetylglycine for glutathione synthesis is very small
(Table I, Experiments 4 and 5) while isotopic glycine is incorporated at a
rate comparable to that occurring in intact slices. It is evident therefore
that in the formation of the cysteinylglycine moiety of glutathione N-
acetylglycine is not an intermediate and that in this case at least the N-
acetylamino acid does not take part in the formation of the peptide bond.
Results by Simmonds, Tatum, and Fruton (24) on the utilization of acetyl-
amino acids by Neurospora mutants and those by Cohen and McGilvery
(3) on the formation of p-aminohippuric acid have led these authors to the
same conclusion. It is worthy of note that the enzyme system which
converts the N-acetyl derivative to the free amino acid appears to be
almost completely inactivated by homogenization of the liver tissue.

As is shown by the data in Table I the rate of aerobic glutathione syn-
thesis in pigeon liver homogenates, measured by the incorporation of iso-
topic glycine, is of the same order of magnitude as that in intact slices.
While the variations in duplicate experiments with aliquots of the same
liver homogenate never exceeded 10 per cent, the rate of glutathione syn-
thesis was found to fluctuate considerably with tissue from different
animals. A total of fourteen experiments was carried out to determine
the formation of glutathione under aerobic conditions. In nine of these
the newly synthesized glutathione, calculated from the incorporation of
glycine carbon, amounted to 0.2 to 0.4 mg. per gm. of liver per hour.
The rate of synthesis was materially lower in three experiments (0.04,
0.08, and 0.09 mg.) and greater (0.75 and 1.5 mg.) in two cases. Since
changes in the quantities of glutathione during incubation were not de-
determined, it is not possible to state whether the incorporation of isotopic
carbon results in an increase in the total quantity of the tripeptide.

The stimulating effect of adenosine triphosphate on the rate of glu-
tathione formation is shown in Tables II and III and Fig. 1. The accelera-
tion by adenosine triphosphate is optimal at a molarity of $5 \times 10^{-4}$ and
is reversed at higher concentrations. The data indicate a participation of
adenosine triphosphate in the formation of the peptide linkages in gluta-
thione, though it is not clear in what manner adenosine triphosphate
enters into the synthetic process. 2,4-Dinitrophenol has been reported
to block phosphorylations without affecting respiration (25-27). In
accord with these findings, dinitrophenol was found to interfere markedly
with glutathione formation. It should be noted, however, that the in-
hibitor was effective only in relatively high concentrations ($4 \times 10^{-4}$ m).
The results obtained on addition of succinate, fumarate, or malonate
(Table III) confirm the impression that the entrance of glycine into
peptide linkage is associated with energy-yielding reactions.
TABLE II
Effect of Adenosine Triphosphate on Incorporation of C\textsuperscript{14}-Glycine into Glutathione in Pigeon Liver Homogenates

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Gas phase</th>
<th>ATP added</th>
<th>C\textsuperscript{14} in glutathione</th>
<th>Glutathione synthesized per hr.</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>O\textsubscript{2}</td>
<td>(1 \times 10^{-3})</td>
<td>46, 47</td>
<td>62, 64</td>
</tr>
<tr>
<td></td>
<td>N\textsubscript{2}</td>
<td>(1 \times 10^{-5})</td>
<td>11</td>
<td>15</td>
</tr>
<tr>
<td>II</td>
<td>O\textsubscript{2}</td>
<td>(1 \times 10^{-4})</td>
<td>38, 33</td>
<td>51, 46</td>
</tr>
<tr>
<td></td>
<td>N\textsubscript{2}</td>
<td>(1 \times 10^{-6})</td>
<td>127</td>
<td>180</td>
</tr>
<tr>
<td></td>
<td>“</td>
<td>6.5, 3.7</td>
<td>9, 5</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>N\textsubscript{2}</td>
<td>(1 \times 10^{-3})</td>
<td>33, 30</td>
<td>46, 41</td>
</tr>
<tr>
<td>IV</td>
<td>O\textsubscript{2}</td>
<td>24</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td></td>
<td>N\textsubscript{2}</td>
<td>(1 \times 10^{-3})</td>
<td>64</td>
<td>86</td>
</tr>
<tr>
<td></td>
<td>“</td>
<td>33, 30</td>
<td>46, 41</td>
<td></td>
</tr>
<tr>
<td>V</td>
<td>O\textsubscript{2}</td>
<td>(5 \times 10^{-4})</td>
<td>110</td>
<td>179</td>
</tr>
<tr>
<td></td>
<td>N\textsubscript{2}</td>
<td>(5 \times 10^{-4})</td>
<td>235</td>
<td>381</td>
</tr>
<tr>
<td></td>
<td>“</td>
<td>782</td>
<td>1267</td>
<td></td>
</tr>
<tr>
<td>VI</td>
<td>O\textsubscript{2}</td>
<td>(5 \times 10^{-4})</td>
<td>140</td>
<td>227</td>
</tr>
<tr>
<td></td>
<td>N\textsubscript{2}</td>
<td>(7 \times 10^{-4})</td>
<td>132</td>
<td>214</td>
</tr>
<tr>
<td></td>
<td>“</td>
<td>888</td>
<td>1390</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1656</td>
<td>2833</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>230</td>
<td>372</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>297</td>
<td>481</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Each flask contained 1.6 gm. of liver, amino acids, and buffer as described in the experimental part; total volume per flask, 20 ml.; incubated for 1 hour at 37\textdegree.

Fig. 1. Effect of adenosine triphosphate on the incorporation of C\textsuperscript{14}-glycine into glutathione in pigeon liver homogenates. Each flask contained 1.6 gm. of liver, amino acids, and buffer as described in the experimental part; total volume per flask, 20 ml.; incubated for 1 hour in oxygen at 37\textdegree.
The incorporation of glycine into glutathione is reduced to much lower, though still significant, levels under anaerobic conditions. In eight experiments in which glutathione formation in the presence of oxygen and in nitrogen was compared, the average value for anaerobic synthesis was 17 per cent of that found under the aerobic conditions. In a number of experiments the level of anaerobic synthesis was substantially raised by the addition of adenosine triphosphate. However, it was not possible to reproduce this effect with regularity. Under anaerobic conditions adenosine triphosphate was without effect in two experiments out of six (Table I) and was sometimes inhibitory at higher concentrations.

**Table III**

<table>
<thead>
<tr>
<th>Effect of Metabolites and Inhibitors on Incorporation of C(^14)-Glycine into Glutathione in Pigeon Liver Homogenates</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.6 gm. of rat liver per flask. Total volume, 20 ml.; composition of medium as described in the experimental part; incubated at 37° in oxygen for 1 hour.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Additions</th>
<th>Molarity</th>
<th>C(^14) in glutathione*</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td></td>
<td>100</td>
</tr>
<tr>
<td>Adenosine triphosphate</td>
<td>3 × 10(^{-4})</td>
<td>313</td>
</tr>
<tr>
<td>Succinate</td>
<td>1 × 10(^{-4})</td>
<td>264</td>
</tr>
<tr>
<td>Fumarate</td>
<td>1 × 10(^{-3})</td>
<td>250</td>
</tr>
<tr>
<td>Ammonium chloride</td>
<td>1 × 10(^{-3})</td>
<td>77</td>
</tr>
<tr>
<td>Glutamic acid omitted</td>
<td>1 × 10(^{-3})</td>
<td>25</td>
</tr>
<tr>
<td>Cysteine omitted</td>
<td>1 × 10(^{-3})</td>
<td>33</td>
</tr>
<tr>
<td>Malonate</td>
<td>1 × 10(^{-2})</td>
<td>39</td>
</tr>
<tr>
<td>2,4-Dinitrophenol</td>
<td>1 × 10(^{-4})</td>
<td>105</td>
</tr>
<tr>
<td>&quot;</td>
<td>4 × 10(^{-4})</td>
<td>10</td>
</tr>
</tbody>
</table>

*Since the activities of different homogenates varied considerably, the data, which are taken from different series, were recalculated for a relative activity of 100 counts of C\(^14\) in the control experiment.

The reason for the occasional failure of adenosine triphosphate to stimulate glutathione synthesis anaerobically has so far remained obscure. The synthesis of glutathione from the constituent amino acids must involve at least two steps and it is conceivable that only one of these is dependent on the supply of phosphate bond energy. The effect of adenosine triphosphate may therefore become evident only when the supply of other endogenous factors is adequate for the synthesis of the entire molecule.

The majority of the experiments which are reported here have been concerned with the incorporation of glycine into glutathione, but evidence has also been obtained to indicate that the processes under investigation include a replacement of glutamic acid residues in the tripeptide. The data in Table IV show the incorporation into glutathione of C\(^14\) and N\(^15\)
from a medium in which C\textsuperscript{14}-glycine and N\textsuperscript{15}-DL-glutamic acid were present in equimolar quantities. Under these conditions the uptake of glycine carbon into glutathione is more than twice that of nitrogen from glutamic acid. Since the isotopic glutamic acid used was in the racemic form, and since it can be assumed that only the L isomer is directly employed in peptide synthesis, the molar concentration of glutamic acid was in effect only one-half that of glycine. Moreover, the liver preparation presumably contains glutamic acid dehydrogenase (28) and a replacement of the isotopic nitrogen in glutamic acid by ordinary nitrogen would be expected to result from the action of this enzyme. In this case the N\textsuperscript{15} concentration in glutathione would not be a true measure of the rate of entrance of glutamic acid into the tripeptide. The reversible deamination of glutamic acid under these conditions becomes evident from an experiment.

**Table IV**

*Formation of Glutathione in Pigeon Liver Homogenates*

<table>
<thead>
<tr>
<th>Isotopic additions</th>
<th>Relative isotope concentrations in glutathione</th>
</tr>
</thead>
<tbody>
<tr>
<td>C\textsuperscript{14}-Glycine and N\textsuperscript{15}-glutamic acid</td>
<td>C\textsuperscript{14}</td>
</tr>
<tr>
<td></td>
<td>1.40</td>
</tr>
<tr>
<td>&quot; and N\textsuperscript{15}-ammonium chloride</td>
<td>1.76</td>
</tr>
</tbody>
</table>

* Specific activity of glycine moiety calculated for a specific activity of 100 in the glycine added.
† Atom per cent excess N\textsuperscript{15} in glutamic acid moiety calculated for 100 atom per cent excess N\textsuperscript{15} in glutamic acid or NH\textsubscript{4}Cl added. Each flask contained 1.6 gm. of liver, amino acids, and buffer as described in the experimental part; total volume per flask, 20 ml.; incubated for 1 hour in oxygen at 37°.

with N\textsuperscript{15}H\textsubscript{4}Cl in addition to C\textsuperscript{14}-glycine and non-isotopic glutamic acid. Glutamic acid was isolated after hydrolysis of glutathione and found to contain roughly the same isotope concentration as in the experiment with added N\textsuperscript{15}-glutamic acid, showing that amination of ketoglutaric acid must have occurred.

It is also possible that the introduction of N\textsuperscript{15} from labeled ammonia observed here is the result of a reversible deamination of the glutamyl residue in glutathione itself. Nevertheless it appears likely that the process under investigation involves the renewal of both the glutamyl-cysteine and cysteinylglycine linkages in the glutathione molecule.

**SUMMARY**

1. Incubation of liver slices and homogenates in the presence of C\textsuperscript{14}-glycine or N\textsuperscript{15}-glutamic acid results in the formation of labeled glutathione, demonstrating the synthesis of the tripeptide under *in vitro* conditions.
2. N-Acetylglycine is utilized for glutathione formation in liver slices but not in liver homogenates. This eliminates the acetyl derivative as an intermediate in the synthetic process.

3. Adenosine triphosphate markedly accelerates the aerobic synthesis of glutathione in liver homogenates. This stimulation by adenosine triphosphate was observed also under anaerobic conditions but could not always be reproduced.

4. A method for the synthesis of glycine labeled by isotopic carbon is described.

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Konrad Bloch


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