THE METABOLISM OF GLYCINE BY FOLIC ACID-DEFICIENT
CHICK LIVER HOMOGENATES*

BY JOHN R. TOTTER, BARBARA KELLEY, PAUL L. DAY, AND
RAYMOND R. EDWARDS

(From the Department of Biochemistry, School of Medicine, Little Rock, and the
Institute of Science and Technology, University of Arkansas,
Fayetteville)

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Recent work has made it clear that folic acid is intimately concerned
in glycine metabolism. It was shown that a deficiency of glycine pro-
duced by feeding sodium benzoate (1) was partially prevented by this
vitamin. Likewise, growth in rats fed toxic levels of glycine is improved
by folic acid (2, 3). Holland and Meinke (4) have found that both glycine
and folic acid seem to improve the ability of such organisms as Streptococcus
faecalis to grow with very low levels of serine in the medium. Likewise
there is some evidence that porphyrin metabolism is influenced by folic
acid both in animals and in microorganisms (1, 5, 6). It is known that
the α-methylene carbon of glycine is incorporated into porphyrin (7)
and that the carboxyl carbon of glycine does not appear in the porphyrin
portion of the hemoglobin molecule (8). These experiments taken to-
gether suggest that folic acid is necessary for the breakdown of glycine
into a 1-carbon intermediate or incorporation of this carbon into serine
and other substances such as purines, pyrimidines, and porphyrin. Evi-
dence that folic acid is concerned in the production of pyrimidines and
purines has been reviewed in a recent paper by Prusoff et al. (9), but direct
evidence that the vitamin action is exerted through its influence on glycine
metabolism is lacking. It seemed likely to us that the best approach to
the solution of this problem was through the use of C14-labeled glycine.
The results of the first of these studies is presented here.

For the present experiments chicks were used, since it is possible to
induce an uncomplicated dietary deficiency of folic acid in this species.
The radioactive glycine1 used contained C14 in the carboxyl group and
exhibited an activity of about 10 µc. per mg.

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1 The glycine containing C14 in the carboxyl group was generously supplied by Dr.
Martin D. Kamen of the Mallinckrodt Institute for Radiology, Washington Univer-
sity, St. Louis, Missouri.
Day-old white rock chicks were obtained from a hatchery and housed in a metal brooder maintained at constant temperature. Food and water were given ad libitum. The deficient diet was similar to that commonly employed for folic acid assays with chicks and had the same composition as that used by Keith et al. (10). Control birds received the deficient diet supplemented with 200 γ of folic acid per 100 gm. of diet.

After 3½ weeks on the diet the chicks were sacrificed and 2 gm. samples of liver from each bird were homogenized in a Potter-Elvehjem apparatus with an equal volume of KHCO₃-KCl buffer as described by Winnick et al. (11). 0.3 ml. aliquots of the homogenates were incubated with 0.1 ml. of solution containing 0.0257 mg. of carboxyl-labeled radioactive glycine (specific activity about 10 μc. per mg). 0.1 ml. of folic acid solution containing either 2 or 10 γ of folic acid and the same amount of Na₂HPO₄ was added to one set of duplicate tubes. An equal amount of Na₂HPO₄ solution of the same concentration was added to duplicate control tubes; the total volume in all cases was 0.5 ml. Incubations were carried out at 38° in conical centrifuge tubes supported in a horizontal position and under an atmosphere of 95 per cent O₂ with 5 per cent CO₂. After 60 to 90 minutes the proteins were precipitated with trichloroacetic acid and extracted with alcohol, alcohol-ether, and ether, to obtain the phospholipides. The technique described by Winnick and coworkers (11) was used throughout. The trichloroacetic acid supernatants from the deficient and control chicks were saved separately and each group pooled for the isolation experiments described below. The samples containing added folic acid were not saved separately since the effect of the vitamin in vitro seemed to be very small.

An aliquot of the phospholipide solution from each experiment was evaporated on a counter plate and counted directly. A colorimetric phosphorus determination (12) was made on a similar aliquot after wet ashing with sulfuric acid. The protein residue was dried in vacuo, weighed, powdered, and counted. Corrections for self-absorption due to protein were calculated from a curve constructed by plotting counts against weight for two of the most radioactive protein samples obtained in the experiment. The same correction curve, which agrees closely with a published curve (13), was used for correcting all other sample counts, except those of the phospholipides in which the correction for sample weight was negligible.

Thymine, uric acid, adenine, xanthine, guanine, uracil, serine, glycine, guanidoacetic acid, and creatine, as carriers, were each added to separate aliquots of the trichloroacetic acid supernatants. Appropriate procedures for the isolation of each substance were used and the recovered compound recrystallized until no further loss in activity resulted. In some cases it
was necessary to remove the trichloroacetic acid by ether extraction and concentrate the residual solution by evaporation in order to provide sufficiently active material to give satisfactory counting rates. After a preliminary count each substance, with the exception of glycine and serine, was treated with 1 ml. of a solution containing 5 mg. of ninhydrin and heated to destroy any amino acids. If any loss of activity occurred, an additional treatment with ninhydrin was made. It was found that when a similar procedure was applied to a mixture of radioactive serine and non-radioactive thymine more than 95 per cent of the counts were removed and

**Table I**

**Effect of Dietary Folic Acid and Folic Acid in Vitro on Uptake of Radioactive Glycine by Chick Liver Homogenate Proteins and Phospholipides**

0.0257 mg. of glycine containing C\(^{14}\) in the carboxyl group and giving about 54,000 c.p.m. was added to 0.3 ml. of 1:1 homogenate. Incubation period 60 to 90 minutes at 39\(^{\circ}\); total volume 0.5 ml; counting efficiency approximately 7.5 per cent; accuracy approximately ±3 per cent.

<table>
<thead>
<tr>
<th>Diet</th>
<th>Folic acid addition to homogenate</th>
<th>No. of samples</th>
<th>C.p.m. per mg.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Average</td>
</tr>
<tr>
<td></td>
<td>Proteins (25-30 mg.)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Deficient</td>
<td>2 or 10</td>
<td>13</td>
<td>0.55</td>
</tr>
<tr>
<td>&quot;</td>
<td>+ 200 γ folic acid</td>
<td>11</td>
<td>0.60</td>
</tr>
<tr>
<td>&quot;</td>
<td>+ 200 γ “ “</td>
<td>8</td>
<td>1.46</td>
</tr>
<tr>
<td>&quot;</td>
<td>+ 200 γ “ “</td>
<td>7</td>
<td>1.64</td>
</tr>
<tr>
<td></td>
<td>Phospholipides (3-4 mg.)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Deficient</td>
<td>2 or 10</td>
<td>6</td>
<td>10.3</td>
</tr>
<tr>
<td>&quot;</td>
<td>+ 200 γ folic acid</td>
<td>6</td>
<td>13.2</td>
</tr>
<tr>
<td>&quot;</td>
<td>+ 200 γ “ “</td>
<td>6</td>
<td>21.2</td>
</tr>
<tr>
<td>&quot;</td>
<td>+ 200 γ “ “</td>
<td>5</td>
<td>33.1</td>
</tr>
</tbody>
</table>

only negligible activity remained. It appears, therefore, that the activities recorded for these compounds are not due to amino acid contaminants.

**RESULTS AND DISCUSSION**

The results of the counts on phospholipide and proteins are given in Table I. It may be seen that the liver homogenates from the deficient chicks showed a much lower degree of incorporation of radioactive carbon than did those from the replete birds. These results have not yet been compared with similar studies on chicks rendered deficient in other members of the vitamin B complex. However, the results given in Table II strongly
suggest that the reduced incorporation of the radioactivity in the deficient chick livers is due in part to a reduced conversion of glycine to serine. In the light of these and other experiments (4) it seems likely that folic acid is required for the conversion of glycine to serine.

The results of the carrier experiments on the trichloroacetic acid extracts are shown in Table II. While the radiochemical purity of the compounds isolated by addition of carriers has not been established as fully as may be desirable, it seems likely that the results are essentially correct. In some cases there were large differences in activity of compounds isolated by identical procedures from the two supernatants. The same probable gross contaminants (serine and glycine) were present in both solutions. Furthermore, when the isolations were repeated with different ratios of carrier to sample, the same total amounts of activity were recovered. Within the limits of error the activity recovered from each of the two solutions was approximately equal to this residual activity. Two compounds, cytosine and hypoxanthine, were unavailable for testing and might be expected to have some activity, but probably not enough to alter the results appreciably.

It may be seen that the thymine, uracil, xanthine, and uric acid of the pyrimidines and purines tested were appreciably radioactive. With the

\[ \text{Table II} \]

**Effect of Folic Acid on Metabolism of Glycine by Chick Liver Homogenates**

0.0257 mg. of glycine giving 54,000 c.p.m. was added to 0.3 ml. of 1:1 homogenate. Total volume 0.5 ml.; efficiency of counting arrangement approximately 7.5 per cent; counting accuracy approximately ±2.2 per cent.

<table>
<thead>
<tr>
<th>Carrier added</th>
<th>Quantity of carrier per experiment</th>
<th>Specific activity</th>
<th>Per cent activity recovered</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg.</td>
<td>Deficient</td>
<td>Replete</td>
</tr>
<tr>
<td></td>
<td>c.p.m.</td>
<td>c.p.m.</td>
<td></td>
</tr>
<tr>
<td>Glycine</td>
<td>150</td>
<td>150.0</td>
<td>55.5</td>
</tr>
<tr>
<td>Serine</td>
<td>150</td>
<td>85.0</td>
<td>131.0</td>
</tr>
<tr>
<td>Guanidoacetic acid</td>
<td>175</td>
<td>26.8</td>
<td>27.0</td>
</tr>
<tr>
<td>Creatine</td>
<td>160</td>
<td>1.19</td>
<td>4.13</td>
</tr>
<tr>
<td>Uric acid</td>
<td>150</td>
<td>0.32</td>
<td>3.13</td>
</tr>
<tr>
<td>Xanthine</td>
<td>150</td>
<td>0.18</td>
<td>0.80</td>
</tr>
<tr>
<td>Adenine HCl</td>
<td>37.5</td>
<td>0.06*</td>
<td>0.28*</td>
</tr>
<tr>
<td>Guanine</td>
<td>37.5</td>
<td>0.57</td>
<td>1.02</td>
</tr>
<tr>
<td>Thymine</td>
<td>18.7, 37.5</td>
<td>2.41</td>
<td>5.80</td>
</tr>
<tr>
<td>Uracil</td>
<td>75</td>
<td>4.04</td>
<td>5.10</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Residual activity</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Counted as the picrate.
exception of uracil, the activities of those obtained from the livers of folic acid-fed chicks were very much greater than similar compounds from the livers of the deficient birds. These results are in accord with the supposed function of folic acid in the biosynthesis of purines and pyrimidines and provide evidence in support of these concepts.

Adenine and guanine showed only very slight activities and it may be presumed that these compounds, if formed during the incubation, were not primary condensation products. It should be noted that the ratios of activities of uracil were not greatly different from those of serine. Since the glycine probably breaks down to give bicarbonate (and "formate") in an amount approximately equivalent on a molar basis to the serine concentration, it is likely that the ratio shown by the two uracil activities is solely a consequence of the different concentrations of radioactive bicarbonate. On the other hand the activity ratios of thymine, xanthine, and uric acid do not seem to permit such a simple explanation. It therefore appears that folic acid either directly or indirectly promotes the synthesis of this group of compounds.

Examination of the data in Table II reveals that the rate of disappearance of glycine and appearance of serine was much more rapid in the liver homogenates from folic acid-replete birds than in those from the deficient chicks. The increased rate of serine production obtained in these experiments confirms the suggestion of Holland and Meinke (4) that folic acid promotes the conversion of glycine to serine.

The guanidoacetic acid production from glycine by chick liver was found to be fairly rapid and apparently unaffected by the folic acid deficiency as shown by the data in Table II. It is of interest that a second carrier experiment with guanidoacetic acid, carried out on the same supernatants a month later, showed only a fraction of the activity obtained in the first experiment. The activity of glycine carrier isolated at the later date had increased enough to account for the disappearance of the guanidoacetic acid. Since the first isolation of guanidoacetic acid was made some weeks after the incubations, it is entirely probable that the values given for this compound should be higher.

The activity of creatine was higher in the supernatants from folic acid-fed chicks. Since the guanidoacetic acid activity was essentially the same in the two, it is probable that methylation of the guanidoacetic acid must have taken place more rapidly in the livers of the replete birds. Evidence that folic acid may be involved in creatine metabolism has recently been obtained by Dinning and Day (14).

The accelerated disappearance of glycine in the incubations with livers from folic acid-fed chicks deserves comment. If the mechanism for serine formation proposed by Siekevitz and Greenberg (15) is correct, this
observation suggests the possibility that folic acid may be directly concerned with the breakdown of glycine to give "formate." According to the results of Sakami (16) it is quite likely that the methyl groups of choline are also available for "formate" production. If folic acid promotes formation of "formate" by increasing the rate of breakdown of glycine, it could thus possibly exert a sparing action on methyl donors. That folic acid and choline are interrelated has been shown by Schaefer et al. (17). However, evidence has been obtained that folic acid is involved in "formate" metabolism (18, 19). It seems possible that accumulation of "formate" from glycine may reduce the rate of the further breakdown of glycine and that the action of folic acid is to remove "formate" by increasing the rate of its condensation with glycine.

SUMMARY

Liver homogenates from folic acid-deficient and replete chicks were incubated with carboxyl-labeled radioactive glycine and the incorporation of the radioactive carbon into the proteins and phospholipides measured. Proteins and phospholipides of the livers from deficient chicks were found to be only one-half to one-third as active as those from the folic acid-fed chicks.

The addition of folic acid in vitro was found to elevate slightly, but probably not significantly, the uptake of radioactive carbon from glycine. Carrier isolations were conducted on the supernatants after precipitation of the proteins. The results indicate that the livers from folic acid-fed birds are capable of transforming glycine to serine much more rapidly than those from deficient birds. The activities of creatine, uric acid, xanthine, and thymine isolated from livers of replete chicks were found to have much higher activities than those isolated from the livers of deficient chicks. Adenine and guanine were found to have slight or no activity, while uracil was moderately active in both preparations.

BIBLIOGRAPHY

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