THE METABOLISM OF CHOLINE AND ITS CONVERSION TO 
GLYCINE IN THE RAT

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(Received for publication, February 12, 1953)

There is considerable evidence (1–3) to suggest that a major pathway of choline metabolism in the animal is via a series of oxidative steps,

\[
\text{Choline} \rightarrow \text{betaine aldehyde} \rightarrow \text{betaine}
\]

and that it is betaine which serves as the immediate donor of methyl groups in transmethylation reactions. In view of an earlier finding that betaine was readily demethylated to glycine in the rat (4), it was considered of interest to compare injected isotopic choline and betaine as precursors of the glycine moiety of hippuric acid in the intact animal. The present finding that betaine and choline were indeed efficient precursors of glycine led to the inclusion of dimethylethanolamine among the compounds studied, in view of the fact that du Vigneaud et al. (5) had suggested that this compound might be “the principal demethylation product of choline” in the rat. The technique employed was based on that of Shemin (6) who had compared a variety of substances with respect to their capacities to yield glycine under these circumstances.

An alternative fate of choline is suggested by the findings of de la Huerga and Popper (7) who recovered as much as two-thirds of the choline nitrogen, fed to patients with hepatobiliary disease, as urinary trimethylamine and trimethylamine oxide. A 2-carbon residue might well arise from this reaction. Alternatively glyoxylic acid might arise from the glycine oxidase-catalyzed oxidation of sarcosine (8) derived in turn from the partial demethylation of betaine. Reamination of such a 2-carbon fragment might regenerate glycine, and indeed glyoxylic acid has been observed to serve as a precursor of glycine carbon in the rat (9). Choline labeled with C\textsuperscript{14} in the ethanolic residue as well as with N\textsuperscript{15} has been therefore employed. Glycine arising from such choline by the latter mechanisms would exhibit greater dilution with respect to N\textsuperscript{15} than to C\textsuperscript{14}, whereas in glycine formed via demethylation of betaine, both isotopes would be equally diluted.

One of the reported effects of aminopterin poisoning is an inhibition of choline oxidase activity. This has been demonstrated in a variety of tissue

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preparations derived from aminopterin-poisoned animals, including livers of rats (10) and monkeys (11) as well as chick bone marrow (12) and monkey kidney (11). In addition oxidation of betaine aldehyde as well as transmethylation from betaine to homocysteine is profoundly depressed in livers of aminopterin-poisoned rats (10). These defects, in so far as they occur in the intact aminopterin-poisoned animal, should result in a lower yield of glycine from choline and betaine. We have therefore compared the isotope abundances in hippuric acid excreted by normal and by severely aminopterin-poisoned rats receiving isotopic choline or betaine.

EXPERIMENTAL

Preparations of Isotopic Compounds—Choline-N15 was prepared from \((\text{CH}_3)_2\text{N}^{15}\) and ethylene chlorohydrin (13). Trimethylamine hydrochloride was prepared from N15HCl and paraformaldehyde (14). M.p. of choline picrate, 243°; N15, 1.82 and 3.02 atom per cent excess in two preparations.

Choline-C14 labeled equally in the 2 carbons of the ethanolic residue was purchased from Tracerlab. Doubly labeled choline was prepared by dilution of the C14-labeled material with choline-N15. Specific activity, 306 \(\times 10^5\) c.p.m. per mM.

Betaine-N15 chloride was prepared by the methylation of glycine-N15 with dimethyl sulfate (15). Glycine-N15 was prepared according to Schoenheimer and Ratner (16) by Dr. E. J. Bien, to whom the authors are indebted for the sample employed. M.p. of betaine picrate, 180–181°; N15, 3.13 and 1.70 atom per cent excess in two preparations.

Dimethylethanolamine-N15 was prepared by the pyrolysis of carefully dried choline chloride-N15 under reduced pressure (17). Active thermal decomposition was observed to commence when the bath temperature reached 300°. The product was redistilled at atmospheric pressure. B.p. of dimethylethanolamine, 132°; m.p. of dimethylethanolamine gold chloride double salt, 195° with decomposition; N15, 2.94 atom per cent excess.

Aminopterin was kindly contributed for these experiments by Dr. T. Jukes of the Lederle Laboratories Division, American Cyanamid Company. The authors wish to express their gratitude to Mr. Frank J. Rennie and Mrs. Eleanor Schroeder for assistance in the isotope analyses.

Administration to Animals—Adult male rats of the Sherman strain, weighing initially 200 to 300 gm., were maintained on ground Purina laboratory chow (Ralston Purina Company, St. Louis, Missouri). Each rat received a single injection of neutralized isotopic test material together with sodium benzoate after a 24 hour fast. The dose of sodium benzoate was in all cases 0.35 mM per 100 gm. of body weight. In initial experiments, in accord with the practice of Shemin (6), the isotopic test materials were injected at the level of 0.35 mM per 100 gm. However, choline
was found to be toxic at this dosage level, giving a mortality of over 50 per cent. Consequently, in later experiments the isotopic materials were all administered at the level of 0.10 mM per 100 gm. of body weight. The animals were fasted during the 24 hour urine collections subsequent to their injections.

**Aminopterin Poisoning**—Aminopterin was added to the ground diet in the amount of 4 mg. per kilo of diet, as suggested by Williams (18). The rats were offered this diet *ad libitum* for 10 days. Several animals died toward the end of this period. Evidences of intoxication included marked anorexia, diarrhea, and loss of 10 to 20 per cent of body weight, despite the fact that the diet was stated to contain vitamin B₁₂. At autopsy gastrointestinal atony and adrenal enlargement were noted. Williams (18) has recommended the analysis of the liver for ascorbic acid as a guide to aminopterin intoxication. Such analyses have been performed (19–21), and an average of 256 γ per gm. of wet weight of liver was found in four normal animals. In seven aminopterin-poisoned rats, an average of 193 γ was found. However, when non-poisoned rats were pair-fed with aminopterin-poisoned animals, an average of 204 γ of ascorbic acid was found per gm. of liver. This assay has not proved to be very useful or specific in our hands.

**Isolation of Hippuric Acid**—Individual rats were kept in separate metabolism cages, and the urine, preserved with toluene, was collected for the 24 hours following injection. The urine was filtered, acidified to pH 3.0 with H₂SO₄, and continuously extracted with ether for 8 to 12 hours. The ether was evaporated and the residue was twice recrystallized from hot water after decolorization with active charcoal. M.p. 188–190°;

\[ C₇H₇O₇N. \text{ Calculated, N 7.8; found, N 7.5 to 7.8} \]

**DISCUSSION**

Shemin (6) has compared the concentration of N¹⁸ in urinary hippuric acid with that of glycine which was injected intraperitoneally into rats, together with sodium benzoate, at a dosage level of 0.35 mM per 100 gm. of body weight. He found that glycine, under these circumstances, underwent a dilution of 2.8-fold. In the present series of experiments under the same circumstances (Table I), an average dilution \((n_o/n_i)\) of 3.7-fold was found. From the conventional isotope dilution equation (22)

\[ A = a (n_o/n_i - 1) \]

where \(a\) is the quantity of isotopic material injected and \(A\) is the quantity of non-isotopic diluent, it may be calculated that 0.35(3.7 - 1) = 0.94 mM of non-isotopic glycine, per 100 gm. of rat, mixed with the injected glycine prior to entering into hippuric acid formation. When isotopic glycine was
injected at the lower dosage, urinary hippuric acid was considerably poorer in isotope, a dilution of 8.5-fold having occurred, indicating a contribution by the rat of 0.1(8.5 - 1) = 0.75 mM of non-isotopic glycine per 100 gm. The values for the contribution of non-isotopic glycine at these dosage levels do not differ significantly.

When betaine was injected (Table I), the dilution of isotope was somewhat greater than when glycine was injected. For comparison it may be noted that the nitrogen of injected serine in Shemin's experiments (6) was diluted about twice as much as that of injected glycine prior to its incorporation into hippuric acid. Serine is generally held to be a major precursor of glycine, and from the present results it would appear that betaine is a glycine precursor of efficiency comparable to serine. This result is in support of our earlier study (4) in which the conversion of betaine to glycine was investigated in the rat.

From the observed isotope dilution \( \frac{n_o}{n_i} \) as choline goes to hippuric acid, it would appear that choline is also an excellent precursor of glycine in the body of the rat. The values obtained at each of two dosage levels are of the same order of magnitude as those procured in the betaine experiments.

In contrast to choline and betaine, dimethylethanolamine is a relatively

| Dose \( \mu \text{m per 100 gm.} \) | Isotopic precursor, \( \frac{n_o}{n_i} \) |
|-----------------|-----------------|-----------------|-----------------|
| Glycine | Betaine | Choline | Dimethylethanolamine |
| 0.35 | 3.24 | 6.95 | 7.78 |
| 5.46 | 2.38 | 3.62 | |
| 0.10 | 8.54 | 13.0 | 18.9 |
| 8.45* | 11.9 | 51.8 |
| 11.4* | 10.4  | 77.3 |
| 12.1* | 23.1 | 105 |
| 0.10 (aminopterin) | 10.4 | 11.2 | 13.5 |
| 23.1 | 28.1 |

* The food consumption of these rats was restricted to that consumed ad libitum by the aminopterin-poisoned animals.
inefficient precursor of glycine for hippuric acid synthesis. When admin-
istered in comparable dosage levels, the nitrogen of betaine or choline under-
went 10- to 20-fold dilution prior to its appearance in hippuric acid, whereas
the nitrogen of dimethylethanolamine was diluted 50- to 100-fold.

When the hippuric acid excreted by aminopterin-poisoned rats was stud-
ied after injection of glycine, betaine, or choline at a level of 0.1 mm per
100 gm., isotope dilutions were observed which did not deviate markedly
from the values obtained with normal animals. Whereas in isolated experi-
ments higher dilutions occurred after choline and betaine injection in the
poisoned than in the normal rats, overlapping values were also obtained.
These unexpected results are taken to mean that in the intact rat on the
diet employed and at the level of aminopterin intoxication achieved no

table II
Per Cent of Injected Material Available for Hippuric Acid Synthesis

<table>
<thead>
<tr>
<th>Dose</th>
<th>Glycine</th>
<th>Betaine</th>
<th>Choline</th>
<th>Dimethylethanolamine</th>
</tr>
</thead>
<tbody>
<tr>
<td>mM per 100 gm.</td>
<td>per cent</td>
<td>per cent</td>
<td>per cent</td>
<td>per cent</td>
</tr>
<tr>
<td>0.35</td>
<td>100</td>
<td>45</td>
<td>40</td>
<td>10</td>
</tr>
<tr>
<td>0.10 (aminopterin)</td>
<td>100</td>
<td>74</td>
<td>60</td>
<td></td>
</tr>
</tbody>
</table>

great inhibition had occurred either in the oxidation of choline or in the
demethylation of betaine.

A clearer understanding of the meaning of these data may be gained by
the calculation of the percentage of each injected test substance which was
actually transformed into glycine and thereby made available for hippurate
synthesis. The method of calculation will be seen from the accompanying
example. From Equation 1 it has been shown that about 0.94 mm of body
glycine was available for hippurate synthesis per 100 gm. of rat in the first
series of experiments. It is assumed that 100 per cent of injected glycine
is available for this purpose. Since, in this series (dosage, 0.35 mM per
100 gm.) the value of \( n_0/n_i \) for betaine was 6.95, again by Equation 1, 0.94
= \( a(6.95 - 1) \), whence \( a = 0.16 \) mm of glycine formed from isotopic be-
taine per 100 gm. of rat. Of the 0.35 mm of betaine per 100 gm. injected,
\( (0.16/0.35)100 = 45 \) per cent was thus transformed into glycine available
for conjugation with benzoic acid. Results of these computations for the
means of each group of experiments are given in Table II.

At the higher level of dosage, 45 per cent of the injected betaine and 40
per cent of the injected choline were transformed into available glycine
during the period of hippurate formation. Analogous computations from the data of Shemin (6) reveal that 40 per cent of injected serine is converted into glycine for the same purpose under comparable conditions. From this it appears that choline and betaine serve as precursors of glycine, with an efficiency approximating that of serine.

At the lower dosage level (0.10 mM per 100 gm.) 74 and 60 per cent of injected betaine and choline respectively contributed to the glycine moiety of hippuric acid. The reason for the increased efficiency of utilization is not known, but may be related to diminished dissipation of betaine and choline by other routes. Only 10 per cent of injected dimethylethanolamine was used for hippuric acid synthesis at this dosage level.

### Table III

**Conversion of Choline-N\(^{15}\)C\(^{14}\) (Ethanolamine-Labeled) to Glycine**

Choline labeled with N\(^{15}\) as well as with C\(^{14}\) in its 2-carbon residue has been injected together with benzoic acid into three rats. The concentrations of both isotopes in the injected choline \(n_o\) and \(c_o\) have been compared with the corresponding concentrations in the glycine moiety of the excreted hippuric acid \(n_i\) and \(c_i\).

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Choline, (n_o)</th>
<th>Hippurate, (n_i)</th>
<th>Choline, (c_o)</th>
<th>Hippurate, (c_i)</th>
<th>(c_o/c_i)</th>
<th>(c_o/c_i)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>1.90</td>
<td>0.101</td>
<td>18.7</td>
<td>1.31 (\times) 10(^6)</td>
<td>6.34 (\times) 10(^6)</td>
<td>20.7</td>
</tr>
<tr>
<td>Aminopterin-poisoned</td>
<td>3.11</td>
<td>0.111</td>
<td>28.0</td>
<td>3.06 (\times) 10(^6)</td>
<td>1.12 (\times) 10(^6)</td>
<td>27.3</td>
</tr>
<tr>
<td>Pair-fed</td>
<td>3.02</td>
<td>0.249</td>
<td>12.1</td>
<td>3.06 (\times) 10(^6)</td>
<td>2.16 (\times) 10(^6)</td>
<td>14.3</td>
</tr>
</tbody>
</table>

Du Vigneaud et al. (5) have shown that in the rat dimethylethanolamine serves as a methyl acceptor and consequently as a precursor of choline. Assuming the series of reactions

\[
\text{Dimethylethanolamine} \rightarrow \text{choline} \rightarrow \text{betaine} \rightarrow \text{glycine}
\]

to represent the only pathway whereby these precursors can contribute nitrogen to glycine, on the basis of the present data the relative rates of the several steps can be estimated. During the interval of hippurate synthesis, in these rats, 74 per cent of injected betaine was demethylated to glycine, \((60/74)\times 100 = 81\) per cent of injected choline was oxidized to betaine, and \((10/60)\times 100 = 17\) per cent of injected dimethylethanolamine was methylated to choline. Whereas the primary demethylation of choline cannot be ruled out as occurring to a minor extent, the present results as well as those of others (1, 23) favor the view that the major fate of choline involves oxidation to betaine, followed by loss of methyl groups.
The effect of aminopterin poisoning on these processes may also be considered in relation to the values in Table II. Whereas the contributions of both choline and betaine to glycine of hippuric acid are perhaps somewhat smaller than those in the corresponding unpoisoned animals, it is noteworthy that the conversion of choline to betaine, \((47/58)100 = 81\) per cent, does not differ appreciably from the corresponding value in normal rats. It would appear that under these conditions no demonstrable inhibition of choline oxidase activity has been achieved in the intact animal.

To gain insight into the mechanism whereby choline is converted into glycine, experiments have been conducted with doubly labeled choline, 

\[\text{(CH}_3\text{)}_2\text{N}^{15}\text{C}^{14}\text{H}_2\text{C}^{14}\text{H}_2\text{OH}\]

Experiments have been conducted upon normal and aminopterin-poisoned rats and also upon rats whose food intake was restricted to equal that consumed ad libitum by the poisoned animals. In each case the abundance of each isotope in choline was compared with that in the glycine moiety of hippuric acid, providing two ratios of dilution, the one with respect to N\(^{15}\), \((n_0/n_i)\), the other with respect to C\(^{14}\), \((c_0/c_i)\) (Table III). When, in each experiment, these two ratios were in turn compared with each other, the value of the expression \((c_0/c_i)/(n_0/n_i)\) did not deviate markedly from unity. From this it is concluded that in the transformation of isotopic choline to glycine the carbon skeleton and the nitrogen atom undergo equal dilution. This finding implies that the transformation occurs without rupture of the C—C—N linkages.

**SUMMARY**

Betaine, choline, and dimethylethanolamine have been studied as precursors of glycine in the intact rat by the expedient of injecting each of these materials labeled with N\(^{15}\) together with sodium benzoate and measuring the isotope abundance in the urinary hippuric acid.

Both betaine and choline are precursors of glycine under these circumstances and serve in this capacity with an efficiency comparable to that of serine. Dimethylethanolamine is a far less efficient precursor of glycine.

The relative rates *in vivo* of the several steps in the postulated reaction sequence dimethylethanolamine \(\rightarrow\) choline \(\rightarrow\) betaine \(\rightarrow\) glycine have been estimated. The methylation of dimethylethanolamine proceeds far more slowly than either the oxidation of choline or the demethylation of betaine under our experimental conditions.

Experiments with doubly labeled choline \((\text{C}^{14}, \text{N}^{15})\) reveal that the ethanolic residue of choline together with the attached nitrogen atom is transformed intact into glycine.

Poisoning with aminopterin did not significantly decrease the oxidation of choline to betaine under the conditions employed.
The findings are interpreted as supporting the contention that choline undergoes an obligatory oxidation to betaine prior to its demethylation to glycine.

BIBLIOGRAPHY

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