In vivo oxidation of the methyl group of hepatic 5-methyltetrahydrofolate

M LUMB, I CHANARIN, R DEACON, J PERRY

From the Haematology Section, Medical Research Council Clinical Research Centre, Northwick Park Hospital, Harrow, Middlesex

SUMMARY Methionine given parenterally to rats caused rapid disappearance of methyltetrahydrofolate from the liver and a corresponding rise in tetrahydrofolate and formyl-tetrahydrofolate concentrations. When \([1^4C]H_4\text{folate}\) was given, methionine caused an increased \([1^4C]0\), excretion, indicating that oxidation of the methyl group had occurred. Methionine was more effective than S-adenosylmethionine at causing oxidation, but serine was ineffective.

The lowest dose of methionine to produce an effect was 0.5 \(\mu\)mol, which is less than the daily dietary intake in a rat. The data suggest that the concentration of methylfolate in rat livers is controlled by the concentrations of methionine.

Folic acid functions in the transfer of single carbon units. These are formyl \((\text{CHO} \rightarrow)\) required for purine synthesis, methylene \((\text{CH}_2)\) required for thymidine synthesis, and methyl \((\text{CH}_3)\) required for methionine synthesis. 5-methyltetrahydrofolate \((5\text{-CH}_3\text{H}_4\text{PteGlu, methylfolate})\) is also the form in which natural folates are absorbed from the gut into portal blood. It is also the dominant analogue in plasma and the only form of folate in the cerebrospinal fluid. The manner in which methylfolate is formed and metabolised is central to understanding the interaction between cobalamin and folate, and these aspects have been reviewed.¹

5-Methyl-tetrahydrofolate is formed by reduction of 5,10-methylene-tetrahydrofolate. The enzyme is 5,10-methylene-tetrahydrofolate reductase, and in vitro the reaction is noticeably shifted in the direction of methylfolate synthesis. On the basis of in vitro studies the reverse reaction, involving oxidation of methylfolate, is regarded as unlikely.

The methyl group of \(5\text{-CH}_3\text{H}_4\text{PteGlu}\) is transferred to homocysteine by methionine synthetase in the de novo synthesis of methionine. This is said to be the only means for the disposal of the methyl group of methylfolate. Failure of the methionine synthetase reaction, which occurs in cobalamin deficiency or in cobalamin inactivation by nitrous oxide, may lead to accumulation of \(5\text{-CH}_2\text{H}_4\text{PteGlu}\) and subsequent "trapping" of folate. This has been termed the methylfolate trap hypothesis.²³

When these ideas were put forward,³ it was also noted that methionine had considerable effects on the distribution of folate analogues in rat livers. The authors found that 1% methionine added to the rat diet and fed for 24 hours before sacrifice considerably increased the amount of tetrahydrofolate and formyl-tetrahydrofolate at the expense of methyltetrahydrofolate. This occurred in both control and experimental animals. The mechanism of this redistribution was not established, although it was conjectured that methionine might provide a methyl group acceptor via S-adenosylhomocysteine derived from demethylation of S-adenosylmethionine.

The effect of methionine in lowering \(\text{CH}_3\text{H}_4\text{PteGlu}\) and increasing \(\text{H}_4\text{PteGlu}\) and \(\text{CHO}\text{H}_4\text{PteGlu}\) concentrations in liver has been confirmed most recently by Brody et al., who showed that 300 \(\mu\)mol of methionine in the rat virtually removed all hepatic methylfolate within 30 minutes. They too did not comment on how this occurred. They used cobalamin-inactivated rats so that the methyl group could not have been passed on to homocysteine.⁴

S-adenosylmethionine in vitro inhibits the activity of the enzyme methylene tetrahydrofolate reductase and so inhibits further synthesis of methylfolate. On this basis the concentration of S-adenosylmethionine has been assumed to be the main regulatory factor in the rate of methylfolate synthesis.

The validity of these views are of importance in understanding folate and one-carbon unit metabolism and how cobalamin deficiency produces its effects. The purpose of this study was to explore how methionine and related compounds changed the concentrations of
methylfolate in rat livers, and in particular to obtain evidence for the in vivo oxidation of the methyl group of methylfolate to formate and carbon dioxide.

**Material and methods**

Methionine, 5-deoxy-5'-methylthioadenosine, glycine, serine and betaine were obtained from Sigma. Sodium formate was obtained from Koch Light. S-adenosylmethionine was generously donated by Dr G Stramentinoli of Bioresearch, Milan.

Male Sprague-Dawley rats weighing between 80 and 120 g were used. Animals were given the compounds in a standard volume of 0.5 ml in water by intraperitoneal injection. Thirty minutes later, animals were killed, livers removed and heated for five minutes in 20 volumes of 1% ascorbic acid (pH7) at 95°C. Samples were then cooled, homogenised, and stored at −20°C.

**Identification of folates**

Liver homogenates were treated with γ-glutamyl carboxypeptidase prepared from pig kidney. Homogenate (0.1 g) was adjusted to pH4-5 and incubated with 1-5 ml of enzyme preparation for five hours at 37°C, then diluted to 10 ml in 0.1M sodium phosphate buffer (pH 5-7).

After centrifugation at 3000 × g for 30 minutes the supernatant was applied to a column of DEAE cellulose (Whatman DE52). Folate monoglumates were separated using a non-linear salt gradient. The mixing chamber contained 150 ml of 0.1M potassium phosphate (pH 6.0), to which was added 0.2M 2-mercaptoethanol. The reservoir contained 350 ml of 1.0M potassium phosphate (pH 6.0) with 0.2M 2-mercaptoethanol. Fractions (5 ml) were collected, all the folates eluting before fraction 50. Folates were identified by elution position and measured by microbiological assay with Lactobacillus casei (NCIMB 8081). 5-CHO-H₄PteGlu was measured using Pediococcus cerevisiae (NCIMB 7837). As 5-formyl- and 5-methyl- H₄PteGlu elute very close together and 5-methyl- H₄PteGlu is inactive for P cerevisiae, P cerevisiae assay was used as a measure of 5-formylfolate.

Amounts of each folate were expressed as the percentage of the total folate.

[14C]O₂ PRODUCTION

5-[14C]H₃-H₄PteGlu specific activity 60 mCi/mmol, was obtained from Amersham Internation. Ten Sprague Dawley rats were exposed to N₂O/O₂ (1/1) in a specially constructed perspex chamber. After four days half the rats were given an intraperitoneal injection containing 300 μmol of methionine and 2 μCi 5-[14C]H₃-H₄PteGlu alone. The animals were then placed in individual chambers through which nitrous oxide and oxygen continued to be passed. Expired carbon dioxide was collected by passing the outflow from the chambers through two vessels connected in series, each containing 50 ml of 2M potassium hydroxide. The efficiency of this system was checked by passing the outflow of the second vessel through a solution of barium hydroxide (50 g/l). No precipitate was formed. One ml samples of the potassium hydroxide were taken every hour into 10 ml NE260 (Nuclear Enterprises) and counted on a LKB Betarak liquid scintillation counter. Quench corrections were made by the channels ratio method using an external standard.

**Results**


Rats given [14C]H₃-H₄PteGlu exhaled [14C]O₂, reaching a peak at two hours after the dose of methylfolate. When this was accompanied by an injection of 300 μmol of methionine the total [14C]O₂ excretion was greater (fig 1), reaching a peak at three hours, and this difference was significant at the 5% level.

**EFFECT OF METHIONINE OF FOLATE ANALOGUES IN LIVER**

The distribution of CH₃-H₄PteGlu, CHO-H₄PteGlu, and H₄PteGlu in rat livers is shown in table 1 and fig 2. Methionine (0.5 μmol) produced a fall in the methylfolate concentrations from 45% to 36%, and the maximum decline to 2.6% was reached with a 50 μmol dose. The fall in the CH₃-H₄PteGlu concentra-

![Fig 1](http://jcp.bmj.com/)

Two groups of five rats breathing N₂O/O₂ (1/1) for three days were given an injection of [14C]H₃-H₄PteGlu. One group (0) was given 300 μmol methionine intraperitoneally at the same time. Exhaled [14C]O₂ was collected into potassium hydroxide.
Table 1  Effect of parenteral methionine on percentage of folate analogues present in rat liver

<table>
<thead>
<tr>
<th>Dose of methionine (μmol)</th>
<th>CH₃-H₄PteGlu</th>
<th>CHO-H₄PteGlu</th>
<th>H₄PteGlu</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean* (SD)</td>
<td>Mean (SD)</td>
<td>Mean (SD)</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>Range</td>
<td>Range</td>
</tr>
<tr>
<td>0</td>
<td>16</td>
<td>16</td>
<td>16</td>
</tr>
<tr>
<td>0.5</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>1.0</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>4.0</td>
<td>6</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>8.0</td>
<td>6</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>16.0</td>
<td>6</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>50.0</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>100.0</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>300.0</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
</tbody>
</table>

*Value is significantly less than in group not given methionine.

EFFECT OF S-ADENOSYL METHIONINE ON FOLATE ANALOGUES IN LIVER

S-adenosylmethionine was far less effective on a molar basis than methionine in producing a fall in methylfolate analogues (table 1 and fig 3), implying oxidation of the methyl groups to formyl and carbon dioxide (fig 1).

EFFECT OF 5'-METHYLTHIOADENOSINE ON FOLATE ANALOGUES IN LIVER

This compound, derived from decarboxylated S-adenosylmethionine via synthesis of polyamines, is metabolised back to methionine with release of a formate unit. At a dose of 16 μmol 5'-methylthioadenosine produced a fall of 30% in methylfolate concentration.
In vivo oxidation of the methyl group of hepatic 5-methyltetrahydrofolate

Table 2 Effect of parenteral S-adenosylmethionine on percentage of folate analogues present in rat liver

<table>
<thead>
<tr>
<th>Dose of S-adenosylmethionine (µmol)</th>
<th>CH₃-H₄PteGlu</th>
<th>CHO-H₄PteGlu</th>
<th>H₄PteGlu</th>
</tr>
</thead>
<tbody>
<tr>
<td>(n =)</td>
<td>(Mean (SD)</td>
<td>Range)</td>
<td>(Mean (SD)</td>
</tr>
<tr>
<td>0</td>
<td>16</td>
<td>45.0 (7.0)</td>
<td>36.4-56.7</td>
</tr>
<tr>
<td>3</td>
<td>6</td>
<td>32.3* (6.5)</td>
<td>25.1-42.9</td>
</tr>
<tr>
<td>9</td>
<td>3</td>
<td>28.0* (4.7)</td>
<td>21.7-35.0</td>
</tr>
<tr>
<td>194</td>
<td>3</td>
<td>23.7* (5.6)</td>
<td>18.9-29.9</td>
</tr>
</tbody>
</table>

*Value is significantly less than in group not given S-adenosylmethionine.

Discussion

Kutzbach and Stokstad suggested that the concentration of methylfolate in liver is regulated by control of the rate of its synthesis. S-adenosylmethionine inhibited the activity of methylenetetrahydrofolate reductase which reduces methylene—H₄PteGlu to methyl—H₄PteGlu. Our data suggest that the concentration of methylfolate in liver is more finely attuned to that of methionine. Excess methionine is toxic and its increase leads to rapid conversion of the methyl group of methylfolate to more oxidised forms, and presumably to the end of methionine synthesis. This oxidation occurs in both B₁₂-deficient and B₁₂-inactivated animals (unpublished observations) as well as in healthy animals. The amount of methionine producing this effect can be as little as 0.5 µmol, which is well below the normal daily intake in a rat. S-adenosylmethionine was less effective than methionine and suggests that S-adenosylmethionine concentrations are less important than methionine in regulating methylfolate metabolism. Indeed, the activity of S-adenosylmethionine was related to the amount of methionine regenerated.

The data have implications for the methylfolate trap hypothesis, which assumes that the methyl group of methylfolate is not oxidised. Even in the absence of a methionine supplement the B₁₂-inactivated animal is able to dispose of the methyl group of methylfolate by

Table 3 Effect of parenteral 5'-methylthioadenosine on percentage of folate analogues present in rat liver

<table>
<thead>
<tr>
<th>Dose of MTA (µmol)</th>
<th>CH₃-H₄PteGlu</th>
<th>CHO-H₄PteGlu</th>
<th>H₄PteGlu</th>
</tr>
</thead>
<tbody>
<tr>
<td>(n =)</td>
<td>(Mean (SD)</td>
<td>Range)</td>
<td>(Mean (SD)</td>
</tr>
<tr>
<td>0</td>
<td>16</td>
<td>45.0 (7.0)</td>
<td>36.4-56.7</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td>40.0 (4.9)</td>
<td>36.3-45.6</td>
</tr>
<tr>
<td>8</td>
<td>3</td>
<td>28.4* (3.3)</td>
<td>25.1-31.6</td>
</tr>
<tr>
<td>16</td>
<td>3</td>
<td>15.6* (6.2)</td>
<td>8.5-19.3</td>
</tr>
</tbody>
</table>

*Value is significantly less than in group not given methylthioadenosine.
oxidation. This is enhanced considerably by raising the methionine intake.

Accumulation of methylfolate occurs in B_{12}-inactivation, primarily because of one of the central pivots of the methylfolate trap hypothesis. There are other equally important observations, however, that are not accounted for by methylfolate trapping. Firstly, the most important is the failure of B_{12}-deficient or B_{12}-inactivated cells to use H_4PteGlu in a normal manner. Thus H_4PteGlu failed to correct the defect in the dU suppression test in marrow cells from patients with untreated pernicious anaemia. This was confirmed with rat marrow from animals exposed to nitrous oxide. Further H_4PteGlu was not used as substrate for folate polyglutamate synthesis in liver in rats exposed overnight to nitrous oxide.

Secondly, the impairment of both thymidine synthesis in the dU suppression test and of folate polyglutamate synthesis in the B_{12}-inactivated rat was reversed completely if folate carrying a carbon unit at the formate level of oxidation was provided. The most convenient was 5-formyl-tetrahydrofolate, but 10-formyl-tetrahydrofolate and 5,10-methenyl-tetrahydrofolate were equally effective.

Thirdly, the defects due to B_{12}-inactivation were reversed by supplying a compound that yielded formate. The most interesting was 5'-methylthioadenosine, which is derived from S-adenosylmethionine and is reconverted to methionine, yielding a mole of formate for each mole methionine regenerated. This compound was much more effective than methionine itself in reversing the effects of B_{12} deficiency.

We therefore suggest that the effect of B_{12} deficiency is a lack of active formate which is normally derived directly from methionine and indirectly through S-adenosylmethionine and 5'-methylthioadenosine.

These studies reported here are in part fulfilment of the requirements for the Fellowship of the Institute of Medical Laboratory Sciences.

### References


Requests for reprints to: Dr I Chanarin, Clinical Research Centre, Watford Road, Harrow, Middlesex HA1 3UJ, England.
In vivo oxidation of the methyl group of hepatic 5-methyltetrahydrofolate.
M Lumb, I Chanarin, R Deacon and J Perry

*J Clin Pathol* 1988 41: 1158-1162
doi: 10.1136/jcp.41.11.1158

Updated information and services can be found at:
http://jcp.bmj.com/content/41/11/1158

**Email alerting service**

*These include:*

Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

**Notes**

To request permissions go to:
http://group.bmj.com/group/rights-licensing/permissions

To order reprints go to:
http://journals.bmj.com/cgi/reprintform

To subscribe to BMJ go to:
http://group.bmj.com/subscribe/