Lack of Effect of L-Methionine Ingestion on $^{14}$CO$_2$ Excretion from L-Histidine (Imidazole-2-$^{14}$C) in Folic Acid and Vitamin B$_{12}$ Deficient Humans

By Hannes B. Staehelin, H. S. Winchell and N. Kusubov

A diet containing one to two per cent L-methionine normalizes increased urinary excretion of formiminoglutamic acid (FIGLU) and decreased $^{14}$CO$_2$ excretion from L-histidine (imidazole-2-$^{14}$C) in folic acid and vitamin B$_{12}$ deficient rats. If such findings could also be found in man, and if they reflect biochemical effects of methionine on monocarbon fragment metabolism rather than competition between amino acids for intestinal absorption and cell membrane transport, then this finding could provide further insight into the metabolic abnormalities attendant to B$_{12}$ and folic acid deficiency in man. Herbert and Sullivan have demonstrated a suppression in the amount of FIGLU excretion in folic acid and B$_{12}$ deficient human subjects following a loading dose of 20 Gm. histidine when 20 Gm. of methionine a day were ingested prior to the loading dose. There appear to be no observations in the present literature concerning the effect of methionine ingestion on the metabolism of the imidazole number two carbon atom of histidine to CO$_2$ following its parenteral administration in human subjects with folic acid or B$_{12}$ deficiency. The present study was undertaken to provide such data.

Materials and Methods

Measurement of excretion of $^{14}$CO$_2$ in the breath subsequent to the intravenous administration of histidine (imidazole-2-$^{14}$C) was identical to that previously published (specific activity 57.8 μCi./mM., Nuclear Chicago Corp., Chicago, Ill.). Each patient received 11–15 μCi. of histidine (0.03–0.04 mg.) intravenously at the initiation of each study.

All patients showed megaloblastic changes in the bone marrow aspirate. The pertinent peripheral red blood cell determinations for these patients at the time of hospital admission are as follows.

<table>
<thead>
<tr>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>H.B.</td>
<td>17</td>
<td>5.3</td>
<td>1.53</td>
<td>112</td>
</tr>
<tr>
<td>J.B.</td>
<td>12</td>
<td>3.2</td>
<td>0.75</td>
<td>160</td>
</tr>
<tr>
<td>R.T.</td>
<td>27.5</td>
<td>8.9</td>
<td>2.17</td>
<td>127</td>
</tr>
<tr>
<td>H.V.</td>
<td>30</td>
<td>10.9</td>
<td>2.57</td>
<td>116</td>
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</tbody>
</table>

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L-METHIONINE INGESTION

Three patients, H.B., J.B., and R.T. had folic acid deficiency. H.B. and J.B. had dietary folic acid deficiency and additionally, H.B. had severe alcoholic hepatic cirrhosis. The breath \(^{14}\)CO\(_2\) excretion in H.B. and J.B. following injection of L-histidine (imidazole-2-\(^{14}\)C) was characteristic of folic acid deficiency. The reticulocytosis (H.B. 8.5\%, J.B. 5\%) and elevation of hematocrit (H.B. 25\%, J.B. 21\%) and hemoglobin concentration (H.B. 7.5 Gm. %, J.B. 6.0 Gm. %) within two weeks after initiation of a normal hospital diet alone further supported this diagnosis. However, serum folic acid concentration was not measured in these two patients. R.T. had a malabsorption syndrome and a consequent serum folic acid concentration of 0.7 ng./ml. Despite the low serum folic acid level in this patient the breath excretion of \(^{14}\)CO\(_2\) subsequent to administration of histidine (imidazole-2-\(^{14}\)C) was within low normal limits. An additional patient, H.V., was found to have vitamin B\(_{12}\) deficiency, with a serum folate level of 7.6 ng./ml. Intestinal absorption of \(^{57}\)Co-Cyanocobalamin was 1.33 per cent (normal 20–30%) as measured by use of the whole body counter. This patient subsequently responded with a reticulocytosis of 13.2 per cent within one week following parenteral administration of 1000 \(\mu\)g. vitamin B\(_{12}\). \(^{14}\)CO\(_2\) excretion in this patient (H.V.) was within normal limits as is characteristically seen in vitamin B\(_{12}\) deficiency.

RESULTS

Oral ingestion of L-methionine (400 mg./kg. body weight to H.B., R.T. and H.V., and 800 mg./kg. body weight to J.B.) given in three equal doses prior to repeat administration of L-histidine (imidazole-2-\(^{14}\)C) failed to alter the pattern of appearance of \(^{14}\)CO\(_2\) in the breath (Table 1). H. V. and R.T. received the last oral dose of methionine three hours prior to the study. H.B. and J.B. received the last oral dose of methionine 12 hours and six hours prior to the study, respectively.

DISCUSSION

In folic acid deficiency, and possibly in vitamin B\(_{12}\) deficiency, the number of formimino carbon atoms from FIGLU which are metabolized through the monocarbon pool to CO\(_2\) per unit time is fixed by the availability of reduced forms of folic acid. When large quantities of histidine are absorbed from the intestinal tract and delivered to sites of metabolism in subjects with a limited ability to metabolize FIGLU, only a small fraction of the FIGLU arising from the metabolism of such histidine can be metabolically accom-

Table 1.—Time at Which Maximum Rate of \(^{14}\)CO\(_2\) Occurred (T \(_{\text{max}}\)), and Cumulative Percent of Administered \(^{14}\)C Which Was Excreted in Breath as \(^{14}\)CO\(_2\) During Initial 60 Min. After I.V. Administration of Histidine (Imidazole-2-\(^{14}\)C), Before and After Ingestion of L-methionine in Patients with Folic Acid or vitamin B\(_{12}\) Deficiency

<table>
<thead>
<tr>
<th>Patient</th>
<th>Weight (Kg.)</th>
<th>(T_{\text{max}}) (min.)</th>
<th>(^{14})CO(_2) Excretion in 60 min. (%)</th>
<th>(^{14})CO(_2) Excretion in 60 min. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>L-Methionine Ingestion</td>
<td>L-Methionine Ingestion</td>
<td>L-Methionine Ingestion</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Before</td>
<td>After</td>
<td>Before</td>
</tr>
<tr>
<td>H.B.</td>
<td>78</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>J.B.</td>
<td>66</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>R.T.</td>
<td>60</td>
<td>40</td>
<td>35</td>
<td>0.82</td>
</tr>
<tr>
<td>H.V.</td>
<td>75</td>
<td>36</td>
<td>32</td>
<td>1.21</td>
</tr>
</tbody>
</table>

* \(^{14}\)CO excretion too small for accurate determination (i.e., indeterminate \(T_{\text{max}}\) and cumulative 60 min. \(^{14}\)CO\(_2\) excretion < 0.10%).
modated and a large quantity of the excess FIGLU is excreted in the urine. When tracer quantities of histidine labeled with $^{14}$C at the imidazole number two carbon atom site are administered parenterally to such subjects we would expect that the fraction of the $^{14}$C label excreted in the urine on FIGLU would be large and the fraction excreted as $^{14}$CO$_2$ in the breath would be proportionately smaller. When sufficiently small quantities of histidine are absorbed from the intestinal tract and delivered to sites of metabolism in subjects with a limited ability to metabolize FIGLU such that the amount of reduced folic acid available is sufficient to metabolize much of the FIGLU arising from the metabolism of histidine, little excess FIGLU accumulates and little FIGLU is excreted in the urine. When tracer quantities of histidine labeled with $^{14}$C at the imidazole number two carbon position is administered parenterally under these latter conditions we would expect little excretion of $^{14}$C labeled FIGLU in the urine and proportionately greater $^{14}$CO$_2$ excretion in the breath. Thus, any perturbation which decreases absorption of histidine into the body of such patients would result in a decrease in total FIGLU excretion in the urine, as well as an increase in $^{14}$CO$_2$ excretion in the breath and a decrease in $^{14}$C labeled FIGLU excretion in the urine following parenteral administration of histidine (imidazole-2-$^{14}$C). It is to be expected that large oral doses of methionine would result in decreased delivery of histidine to its site of metabolism by virtue of its established inhibition of intestinal absorption and cell membrane transport of histidine. Herbert and Sullivan discussed this mechanism as a possible explanation of the methionine effect on FIGLU excretion. In addition to this the results of Brown et al. can also be explained on the basis of methionine inhibition of intestinal absorption and cell membrane transport of histidine alone, and a direct effect of methionine on histidine metabolism need not be invoked.

Since the rate-limiting step in the metabolism of the imidazole number two carbon atom site of histidine in the normal nonfolic acid deficient nonvitamin $B_{12}$ deficient animal is not known, the above arguments are not applicable. Indeed, in the normal animal with sufficient stores of folic acid and vitamin $B_{12}$ the availability of large quantities of methionine as a monocarbon fragment donor might be expected to suppress the entrance of other monocarbon fragments (such as the imidazole number two carbon atom of histidine) into the monocarbon pool. This is consistent with findings in normal rats. It is proposed that in the present studies the failure to demonstrate alteration in $^{14}$CO$_2$ excretion following the intravenous administration of histidine (imidazole-2-$^{14}$C) to folic acid and vitamin $B_{12}$ deficient subjects was due to failure of methionine given in this dosage and for this duration to significantly alter body histidine pool size. The delay between the administration of the last methionine dose and the injection of histidine may have contributed to the lack of effect of methionine in patients H.B. and J.B. However, we did not increase the methionine dose or prolong the duration of treatment because of apparent induction of undesirable side effects by methionine in these patients.

**Summary**

Three human subjects with folic acid and one with vitamin $B_{12}$ deficiency
failed to show any effect of ingestion of L-methionine (400–800 μg./kg. body weight) on the production of 14CO2 from parenterally administered high specific activity L-histidine (imidazole-2-14C). It is suggested that the inhibitory effect of methionine on intestinal absorption and cell membrane transport of histidine can explain previously published effects of methionine on the oxidation of the imidazole-2 carbon atom of L-histidine and that a direct effect of methionine on histidine metabolism need not be invoked.

REFERENCES
Brief Report: Lack of Effect of L-Methionine Ingestion on $^{14}$CO$_2$ Excretion from L-Histidine (Imidazole-2- $^{14}$C) in Folic Acid and Vitamin B$_{12}$ Deficient Humans

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