Effect of Blood in the Gut on Measurements of Endogenous Carbon Monoxide Production

By Robert P. Brouillard, Marcel E. Conrad, and Thomas A. Bensinger

Changes induced in measurements of endogenous carbon monoxide (CO) production by blood in the lumen of the gut were studied in five normal volunteers. The study was undertaken because exogenous heme is absorbed by intestinal mucosal cells where the porphyrin ring is split with the release of CO that could contribute to blood CO levels and lead to a fallacious diagnosis of hemolytic disease. Volunteers who consumed 200 ml of their own blood doubled their endogenous production of CO (0.69 versus 0.34 μmoles/kg/hr). This suggested that at least 3% of the ingested heme was degraded and recovered as CO within 2 hr. Measurements of serum bilirubin also showed a significant increase after ingestion of blood. These data indicate that blood in the gastrointestinal tract can interfere with quantification of heme and bilirubin turnover from measurements of either endogenous CO production or bilirubin and suggest that this might occur with the ingestion of meat.

Heme turnover in man can be accurately measured by quantifying endogenous CO production because the degradation of 1 mole of heme results in the production of 1 mole of CO as well as 1 mole each of iron and bilirubin. It has been shown that heme is absorbed directly into the duodenal mucosa where the porphyrin ring is split. This study was undertaken to determine if blood in the lumen of the gut affects measurements of endogenous CO production.

MATERIALS AND METHODS

Endogenous CO production was measured using a previously described closed rebreathing system at 24°C. Blood carboxyhemoglobin levels were measured using a Beckman GC-5 gas chromatograph. The reproducibility on repeat analysis of samples was ±0.02%. Five normal subjects (four male and one female) volunteered for the experiment after informed consent was obtained. The subjects excluded meat from their diets for 24 hr and fasted for 12 hr before study. Smoking was discontinued 24 hr before study. Each subject was placed in the closed rebreathing system and a baseline was obtained for 120–150 min after equilibrium was reached. Each subject was then removed from the apparatus and ingested 200 ml of his own blood within 10 min. CO was remeasured for 120–150 min. Subsequently, the CO space was determined as previously described. Hematologic values listed in Table I were obtained using standard laboratory methods. Statistics were performed using a paired Student’s t test.

RESULTS

All subjects tolerated the procedure without incident. The mean VCO before ingestion of blood was 0.34 ± 0.11 SD and increased to 0.69 ± 0.11 SD μmoles/kg/hr during the 2-hr observation period after the consumption of
Table 1. Hematologic Data on Volunteers

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Hgb (g/200 ml)</th>
<th>Reticulocyte Count (%)</th>
<th>Weight (kg)</th>
<th>Heme Ingested</th>
<th>( V_{\text{CO}} ) (( \mu \text{mol/kg/hr} )) Before</th>
<th>( V_{\text{CO}} ) (( \mu \text{mol/kg/hr} )) After</th>
<th>CO Capacity (ml) Before</th>
<th>CO Capacity (ml) After</th>
<th>Total Bilirubin (mg/100 ml) Before</th>
<th>Total Bilirubin (mg/100 ml) After</th>
</tr>
</thead>
<tbody>
<tr>
<td>P.K.</td>
<td>M</td>
<td>16</td>
<td>2.0</td>
<td>82</td>
<td>1984</td>
<td>0.44</td>
<td>0.78</td>
<td>1742</td>
<td>0.5</td>
<td>0.6</td>
<td>—</td>
</tr>
<tr>
<td>R.P.</td>
<td>M</td>
<td>14.5</td>
<td>2.1</td>
<td>75</td>
<td>1798</td>
<td>0.34</td>
<td>0.66</td>
<td>1423</td>
<td>0.5</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>M.P.</td>
<td>M</td>
<td>15.8</td>
<td>1.0</td>
<td>70</td>
<td>1959</td>
<td>0.21</td>
<td>0.70</td>
<td>1314</td>
<td>0.6</td>
<td>0.9</td>
<td>—</td>
</tr>
<tr>
<td>R.B.</td>
<td>M</td>
<td>14.3</td>
<td>0.9</td>
<td>69</td>
<td>1773</td>
<td>0.45</td>
<td>0.53</td>
<td>1202</td>
<td>0.6</td>
<td>0.7</td>
<td>—</td>
</tr>
<tr>
<td>R.H.</td>
<td>F</td>
<td>12.3</td>
<td>1.1</td>
<td>60</td>
<td>1525</td>
<td>0.25</td>
<td>0.80</td>
<td>1128</td>
<td>0.4</td>
<td>0.6</td>
<td>—</td>
</tr>
</tbody>
</table>

blood \( (p = 0.01) \). Total bilirubin increased from 0.5 to 0.7 mg/100 ml; this was a significant difference \( (p < 0.02) \). The mean "extra CO" recovered over the 2-hr post-ingestion period was 49.1 ± 8 SD \( \mu \)moles and suggested that approximately 3% of the ingested heme had been degraded with subsequent production of CO.

DISCUSSION

Measurement of endogenous CO production is of value because it provides a rapid, noninvasive, and accurate reflection of heme turnover. It is therefore ideally suited to study of dynamic clinical situations involving hemolysis and has been used to evaluate rapidly the effects of treatment on the hemolytic rate. \(^7\) It has been shown that variables such as the phase of the menstrual cycle, presence of liver disease, and certain drugs may affect the \( V_{\text{CO}} \). \(^8\) The effect of heme absorption by the gastrointestinal tract has not been evaluated.

Previous studies have shown that heme is directly absorbed into the duodenal mucosal cell of man. \(^5\) Since the heme is degraded within the intestinal mucosal cell, we believed that this absorbed heme might contribute to endogenous CO production. In the subjects studied, the mean \( V_{\text{CO}} \) doubled during the 2 hr following ingestion of 200 ml of whole blood. Increases in bilirubin concentration were also observed during the post-blood-ingestion period. Although only 3% of the ingested heme was recovered as CO in 2 hr, this "extra CO" doubled the \( V_{\text{CO}} \) and brought the value into an abnormal range that could be mistaken for hemolysis. It indicates that blood in the lumen of the gut contributes to measurements of carbon monoxide production and suggests that gastrointestinal bleeding and the ingestion of meat can be a cause of abnormal values. Although not tested in these experiments, we believe that the heme from both myoglobin and hemoglobin can be degraded in the gut with the release of carbon monoxide and that 8 oz of lean beef contains about the same quantity of heme as was used in our experiments.

We believe from previous experiments on the mechanism of absorption of hemoglobin iron that increased carbon monoxide production in our subjects was largely caused by degradation of heme within the intestinal mucosal cells. \(^5\) Recent data has shown that microsomal heme oxygenase—an enzyme that converts heme to bilirubin, CO, and iron—is present in high concentrations in rat duodenal mucosa. \(^9\) However, certain intestinal bacteria can degrade heme and may also serve as a factor in enhancing carbon monoxide production. \(^10\)

Lynch and Moede \(^11\) showed significant variations in carbon monoxide production in individuals in whom measurements were made at intervals between
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12 hr and 7 wk. Additionally, Lundh et al. showed that the $V_{CO}$ increases after 36 hr of marked caloric restriction. Our studies were performed consecutively with only a brief interruption to permit the ingestion of blood in order to diminish this variable. Therefore, our data does not provide the minimum period of fasting from foods containing heme for valid measurements of autologous heme degradation by the carbon monoxide method. It also does not permit calculation of the total amount of heme degradation that occurred because of the limited period of observation after the ingestion of blood.

REFERENCES

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RP Brouillard, ME Conrad and TA Bensinger