Gluconeogenesis from Alanine in Patients with Progressive Malignant Disease

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ABSTRACT

We have studied by tracer technique the conversion of the carbon skeleton of alanine to glucose in patients with progressive malignant disease. These data have been compared to similar studies done in patients with chronic undernutrition from other causes. The results show increased conversion of alanine to glucose in the overnight fasting state as compared to the control group. Whereas the percentage increases are comparable to those found with pyruvate-glucose cycling in such subjects, the total amount of carbon conversion is considerably less (alanine carbon, 5.6 mmol/hr versus pyruvate carbon, 39 mmol/hr). Exogenous glucose resulted in good suppression of alanine-to-glucose conversion as it does in normal subjects. It did, however, result in increased glucose-to-alanine conversion, increased alanine levels, and increased flux of alanine from the circulation. Although these latter data may not have specificity for the patient with advanced cancer, a strong dependence for carbohydrate and protein metabolism is suggested. We conclude that uncontrolled gluconeogenesis from alanine is probably not significant in terms of energy expenditure in the patient with uncontrolled cancer.

INTRODUCTION

It has been suggested that an increase in the process of gluconeogenesis may be present in patients with progressive malignant disease and weight loss (4). Two groups of investigators have found increased Cori cycle activity in such subjects (5, 10), but glucose formation from amino acids might be of greater significance in terms of overall body economy (8). For this reason, we have studied the conversion of the carbon skeleton of alanine to glucose by tracer technique in a group of patients with cancer who had systemic manifestations of anorexia and weight loss.

Since our subjects are not in a state of total starvation but, rather, limited caloric intake, we also sought information as to whether glucose administration would result in suppression of gluconeogenesis as it does in normal people (3). From the viewpoint of overall energy expenditure, the cost of increased gluconeogenesis would appear to be appreciable only if this process persisted throughout the 24-hr interval (13).

Our data are compared to similar studies in a group of control subjects who were, for the most part, similarly chronically undernourished (11). They confirm an increased glucose formation from alanine in the overnight fasting state but normal suppression of this cycle with small amounts of carbohydrate.

MATERIALS AND METHODS

The patient material is described in Table 1. All patients were in the advanced stages of their disease and had lost more than 20% of their normal body weight. None were receiving chemotherapy at the time of the study. All were hospitalized at the General Clinical Research Center and were given a diet of their choosing prior to the studies. The initial tracer studies were done after a 14-hr overnight fast. In the experiments with low-dose exogenous glucose administration, infusions of glucose at a constant rate of 55 mmol/hr were started, 1.5 hr prior to the administration of tracer (L. P., Study 1; C. C. and J. C.). The high-dose exogenous glucose administration studies were carried out during periods of parenteral alimentation through a central venous line. Amino acids, including alanine (except L. P., Study 2), were being infused simultaneously with the glucose.

Procedure. It was necessary to do 2 tracer studies on each patient in order to define the kinetics of conversion of alanine and glucose. [U-14C]Alanine was usually the first tracer given by single injection, and the disappearance of labeled alanine, as well as the appearance of the 14C label in glucose, was determined. Usually several days later, a single injection of [U-14C]glucose was given, and the disappearance of labeled glucose, as well as the appearance of the 14C label in alanine, was found. The patients all had Cournand needles placed in the brachial artery prior to the injection of tracer, and blood samples were drawn from this at 2, 5, 10, 15, 30, 60, 90, 120, 150, and 180 min. The amount of tracer given varied from 15 to 25 μCi.

Laboratory Determinations. The concentration of alanine was determined by derivatization and subsequent gas chromatography as described by Clarke et al. (2). Glucose concentration was done by the glucose oxidase method. The isolation of glucose and alanine for counting purposes was done as follows. The protein-free filtrates were introduced in quantitative fashion into a Bio-Rad AG 50W-X8-H+ cation-exchange column, 100 to 200 mesh. Anions, sugars, and neutral compounds were eluted from this column with 3 ml of water. The retained amino acids were eluted with 5 ml of 2 N NH4OH into tubes containing 1 μmol of α-aminoacidic acid, an internal standard added to adjust for mechanical losses. These tubes were then evaporated in a vacuum at 37°C just to dryness, and the residue was redissolved in 0.5 ml of 0.1 N HCl. This preparation was then used to determine the concentration of alanine by derivatization and gas chromatography, as well as for the further separation of amino acids for counting. The water wash from the cation column was then used to separate glucose and other neutral molecules from weakly charged anions by passage through an anion column, Bio-Rad AG 1-X8-formate, 100 to 200 mesh. Glucose passes through this...
column with the first water wash of 6 ml. The charged anions may then be removed with gradient formic acid elution. We have had 100% recovery of radioactive glucose as isolated by this means. Small amounts of glycerol, however, may be eluted with the glucose.

Since the initial column fraction which contains amino acids usually has radioactivity in amino acids other than alanine, it is necessary to isolate alanine on another column for the determination of specific activity. This was a 130-x 0.63-cm column of Technicon Chromcheck, type B [8% cross-linked, 17 ± 1 (S.D.) μm in diameter]. A sample of the fraction containing amino acids was reconstituted with 0.05 N HCl with a cysteic acid marker added. Buffers were pumped through the column by the use of a programmer. The temperature of the column was set at 40° with cold water circulating through the column jacket. The first buffer is 0.2 M lithium citrate, pH 2.3, which is necessary to isolate alanine on another column for the determination of specific activity. This was a 130-x 0.63-cm column of Technicon Chromcheck, type B [8% cross-linked, 17 ± 1 (S.D.) μm in diameter]. A sample of the fraction containing amino acids was reconstituted with 0.05 N HCl with a cysteic acid marker added. Buffers were pumped through the column by the use of a programmer. The temperature of the column was set at 40° with cold water circulating through the column jacket.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Diagnosis</th>
<th>Age (yr)</th>
<th>Sex</th>
<th>Wt (kg)</th>
<th>15-hr overnight fast glucose infusion</th>
<th>Plasma alanine (μmol/liter)</th>
<th>Plasma glucose (mmol/liter)</th>
</tr>
</thead>
<tbody>
<tr>
<td>D. H.</td>
<td>Lymphoma</td>
<td>63</td>
<td>F</td>
<td>50</td>
<td>206</td>
<td>201</td>
<td>4.8</td>
</tr>
<tr>
<td>C. B.</td>
<td>Cancer of hypopharynx, metastatic</td>
<td>57</td>
<td>M</td>
<td>36</td>
<td>201</td>
<td>5.7</td>
<td></td>
</tr>
<tr>
<td>L. P.</td>
<td>Cancer of colon, metastatic</td>
<td>75</td>
<td>F</td>
<td>52</td>
<td>200</td>
<td>6.1</td>
<td></td>
</tr>
<tr>
<td>I. K.</td>
<td>Cancer of lung, metastatic</td>
<td>52</td>
<td>F</td>
<td>45</td>
<td>244</td>
<td>5.5</td>
<td></td>
</tr>
<tr>
<td>J. C.</td>
<td>Cancer of lung, metastatic</td>
<td>47</td>
<td>F</td>
<td>51</td>
<td>152</td>
<td>6.6</td>
<td></td>
</tr>
<tr>
<td>C. C.</td>
<td>Cancer of breast, metastatic</td>
<td>41</td>
<td>F</td>
<td>40</td>
<td>166</td>
<td>7.4</td>
<td></td>
</tr>
<tr>
<td>S. M.</td>
<td>Lymphoepithelioma</td>
<td>75</td>
<td>M</td>
<td>45</td>
<td>177</td>
<td>8.2</td>
<td></td>
</tr>
<tr>
<td>I. P.</td>
<td>Cancer of pancreas, metastatic</td>
<td>58</td>
<td>F</td>
<td>32</td>
<td>355</td>
<td>10.8</td>
<td></td>
</tr>
</tbody>
</table>

* Numbers in parentheses, rate of exogenous glucose infusion (mmol/hr).

Conversion data require a slightly more sophisticated mathematical approach, since these curves contain not only the rate of appearance of a labeled metabolite in the circulation but also its disappearance, and the curves are in essence the convolution of the 2 processes described above.

In our system, we first determine the fraction of one substance that will be converted to the other. Let \( s_{AG}(\tau) \, d\tau \) be the fraction of substance observed at \( t_0 \) in Form A that will be first observed in Form G in the interval \((t_0 + \tau, t_0 + \tau + d\tau)\). Once the conversion has occurred, the subsequent motion of the converted substance will be identical to its disappearance curve \([f_{AG}(t)]\). That is,

\[ s_{AG}(\tau) = \int_{t_0}^{t_0 + \tau} f_{AG}(t) \, dt \]

where \( s_{AG}(\tau) \) is the fraction of substance observed at \( t_0 \) that will be first observed in Form G in the interval \((t_0 + \tau, t_0 + \tau + d\tau)\).
C. Waterhouse et al.

\[ i_{\alpha\beta}(r) \, dr = \left[ \int_{0}^{t} s_{\alpha\beta}(r') \delta(r - r') \, dr' \right] \, dr, \]  
(A)

where \( i_{\alpha\beta}(r) \) is the radioactive flow rate from alanine to glucose at time \( r \) and \( i_{\alpha\beta}(0) \) is the radioactive flow rate in glucose at time \( r \) when glucose is injected at time 0. Integration of Equation A then gives

\[ S_{\alpha\beta} = i_{\alpha\beta0}/i_{\alpha\beta0}, \]  
(B)

where upper case letters correspond to the integral from time 0 to \( \infty \). Note that \( S_{\alpha\beta} \), the fraction of observed A reaching observed G for the first time, is a pure number, i.e., dimensionless. To obtain the cold flow rate from A to G, one multiplies \( i_{\alpha\beta} \cdot S_{\alpha\beta} \) where \( i_{\alpha\beta} \) equals the throughput of alanine through the system, more commonly called the IDR of alanine in the steady state.

The total throughput of alanine or glucose must, when considering 2 substances, be the sum of the contribution from both substances. Thus, 2 equations are obtained which can be solved for the direct input of glucose or alanine in the system,

\[ i_{\alpha0} = i_{\alpha0}^A + i_{\alpha0}^G S_{\alpha\beta} \quad \text{and} \quad i_{\alpha0} = i_{\alpha0}^A + i_{\alpha0}^G S_{\alpha\beta}, \]  
(C)

where \( i_{\alpha0} \) is the direct arrival rate of glucose into the system, i.e., rate of entry of unlabeled glucose molecules into the circulation, and \( i_{\alpha0}^A \) is equivalent for alanine.

Actual data from J. C. (overnight fast) are shown in a sample calculation. The general format for radioactivity is

\[ \text{Pump rate for plasma (ml/hr) - radioactive density (dpm/ml) at time t} \]

\[ \text{injected dose of radioactivity (dpm)} \]

The above formula gives the number of doses of radioactivity passing through the circulation at time \( t \). The integral for the radioactive curves is done by computer, using the trapezoidal rule. The final slope for these curves is determined from the last 3 or 4 points of the curve. In some conversion curves, when the final downslope of the curve has not been established during the time interval for study, it is necessary to use deconvolution to determine the S curves or the fraction of one substance converted to the other.

Integral of radioactive flow rate of alanine when alanine is given,

\[ i_{\alpha0} = 180 \times 10^3 \int_{0}^{t} \text{dpm/ml in } A(t) \, dt/61.56 \times 10^6 = 1.350 \text{ doses} \]

Initial bolus not seen = 1; \therefore \text{total integral} = 2.350 \text{ doses}

Integral of radioactive flow rate of glucose when alanine is given,

\[ i_{\alpha0} = 180 \times 10^3 \int_{0}^{t} \text{dpm/ml in } G(t) \, dt/61.56 \times 10^6 = 12.120 \text{ doses} \]

Integral of radioactive flow rate of glucose when glucose is given,

\[ i_{\alpha0} = 180 \times 10^3 \int_{0}^{t} \text{dpm/ml in } G(t) \, dt/67.08 \times 10^6 = 20.528 \text{ doses} \]

Initial bolus not seen = 1; \therefore \text{total integral} = 21.528

\[ \int \text{dpm/ml in } A(t) \, dt/67.08 \times 10^6 = 0.235 \text{ dose} \]

Cold flow rate of alanine (mmol/hr)

\[ = \text{pump rate (ml/hr) - concentration of alanine in mmol/ml} \]

\[ = 180 \times 10^3 \int_{0}^{t} \delta(t) \, dt/61.56 \times 10^6 = 27.36 \text{ mmol/hr} \]

Cold flow rate of glucose (as above), \( 180 \times 10^3 \cdot 4.86 \times 10^3 = 874.8 \text{ mmol/hr} \)

Alaneen throughput, \( i_{\alpha0} \) or IDR, or flux from plasma

\[ = \text{Cold flow (mmol/hr)/radioactive flow (doses)} \]

\[ = 27.3 \div 2.350 = 11.64 \text{ mmol/hr} \]

Glucose throughput \( i_{\alpha0} \) or IDR or flux from plasma = \( 874.8 \div 21.528 = 40.6 \text{ mmol/hr} \)

Fraction of alanine converted to glucose, \( S_{\alpha\beta} \)

\[ i_{\alpha0}/i_{\alpha0} = 12.120/21.528 = 0.563 \]

Fraction of glucose converted to alanine, \( S_{\alpha\beta} \)

\[ i_{\alpha0}/i_{\alpha0} = 0.100 \]

Alaneen converted to glucose mmol/hr

\[ \int_{0}^{t} \delta/61.56 \times 10^6 \times 0.6 = 6.6 \text{ mmol/hr} \]

Glucose converted to alanine (mmol/hr)

\[ \int_{0}^{t} \delta/61.56 \times 10^6 \times 0.563 = 4.06 \text{ mmol/hr} \]

Direct input into circulating alanine and/or glucose, \( i_{\alpha0} \) or \( i_{\alpha0}^A \)

\[ 11.64 = i_{\alpha0}^A + i_{\alpha0} \cdot 0.100 \]

\[ 81.2 = 2 i_{\alpha0}^A + i_{\alpha0} \cdot 0.563 \]

Note that in the above equations equivalence of carbon atoms in the 2 substances is necessary. \( i_{\alpha0}^A \) and \( i_{\alpha0} \) are multiplied by 2 to give alanine equivalence. See Table 2, Footnote a.

\[ i_{\alpha0}^A = 4 \text{ mmol/hr} \]

\[ i_{\alpha0} = 39 \text{ mmol/hr} \]

\textbf{Results}

The alanine disappearance curves as shown in Chart 1 are similar to those of the control subjects. Determination of the flux of alanine into plasma or, equivalently, the IDR, is shown in Table 2. The mean for the group of subjects with malignant disease was 11.7 mmol/hr as compared to a mean of 10.9 mmol/hr for the controls, i.e., no significant difference.

The data for alanine-to-glucose conversion in the overnight fasting state are shown both in the tracer curves themselves (Chart 2) and in the figures from kinetic analysis (Table 2). Over twice as much carbon from alanine was converted to glucose in the group with malignant disease as was found in the control subjects. In terms of quantity, the contribution of alanine was still relatively small, i.e., 4 to 5% of total new glucose production.

The radioactive glucose disappearance curves were similar to those previously (12) described for similar subjects. The
Unfortunately, the curves of incorporation of $^{14}$C into alanine when glucose was given were not satisfactory in 3 instances, and only gross comparison can be made with the control subjects, with 2.4 mmol in the latter group and 2.9 mmol in the group with cancer (not statistically different).

The studies done with exogenous glucose loads do not lend themselves well to statistical analysis because of the varying amounts of glucose given and the coexistent infusion of amino acids in the last 3 experiments. However, the main reason for doing these studies was to determine whether alanine-to-glucose conversion was normally suppressed by the administration of glucose. It appears evident that this was true (Table 3).

### Table 2

**Alanine glucose kinetics after overnight 14-hr fast**

<table>
<thead>
<tr>
<th>Patient</th>
<th>IDR (mmol/hr)</th>
<th>Direct input (mmol/hr) in circulating</th>
<th>Interconversion (mmol/hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Alanine</td>
<td>Glucose</td>
<td>A—G</td>
</tr>
<tr>
<td>D. H.</td>
<td>10.9</td>
<td>37.0</td>
<td>35.7</td>
</tr>
<tr>
<td>C. B.</td>
<td>10.7</td>
<td>42.6</td>
<td>41.5</td>
</tr>
<tr>
<td>L. P.</td>
<td>16.3</td>
<td>43.1</td>
<td>41.4</td>
</tr>
<tr>
<td>I. K.</td>
<td>12.0</td>
<td>38.8</td>
<td>37.4</td>
</tr>
<tr>
<td>J. C.</td>
<td>11.5</td>
<td>40.6</td>
<td>39.0</td>
</tr>
<tr>
<td>C. C.</td>
<td>10.1</td>
<td>33.4</td>
<td>32.4</td>
</tr>
<tr>
<td>S. M.</td>
<td>10.6</td>
<td>35.3</td>
<td>33.0</td>
</tr>
<tr>
<td>Mean ± S.</td>
<td>11.7 ± 0.8</td>
<td>38.6 ± 1.3</td>
<td>37.2 ± 1.4</td>
</tr>
<tr>
<td>Control (7)</td>
<td>10.9 ± 0.8</td>
<td>31.4 ± 1.9</td>
<td>30.7 ± 1.9</td>
</tr>
</tbody>
</table>

* Since 2 mmol of alanine are necessary for the formation of 1 mmol of glucose, equivalence in this column requires that the alanine-to-glucose (A—G) figures be divided by 2.

### Table 3

**Alanine-glucose kinetics during glucose infusion**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Rate of glucose infusion (mmol/hr)</th>
<th>IDR (mmol/hr)</th>
<th>Direct input (mmol/hr) into circulating</th>
<th>Interconversion (mmol/hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Alanine</td>
<td>Glucose</td>
<td>Alanine</td>
<td>Glucose</td>
</tr>
<tr>
<td>L. P.</td>
<td>55</td>
<td>14.9</td>
<td>66.0</td>
<td>7.5</td>
</tr>
<tr>
<td>C. C.</td>
<td>55</td>
<td>10.6</td>
<td>77.0</td>
<td>7.6</td>
</tr>
<tr>
<td>J. C.</td>
<td>55</td>
<td>13.2</td>
<td>55.1</td>
<td>6.7</td>
</tr>
<tr>
<td>S. M.</td>
<td>0.9</td>
<td>16.9</td>
<td>70.1</td>
<td>4.5</td>
</tr>
<tr>
<td>I. P.</td>
<td>0.6</td>
<td>106</td>
<td>122.0</td>
<td>6.2</td>
</tr>
<tr>
<td>L. P.</td>
<td>1.8</td>
<td>165</td>
<td>155.5</td>
<td>7.0</td>
</tr>
<tr>
<td>S. M.</td>
<td>1.8</td>
<td>165</td>
<td>151.4</td>
<td>151.4</td>
</tr>
<tr>
<td>Mean ± S. E.</td>
<td>12.9 ± 1.3</td>
<td>7.2 ± 0.3</td>
<td>1.0 ± 0.2</td>
<td>2.8 ± 0.7</td>
</tr>
<tr>
<td>Control (5)</td>
<td>11.5 ± 0.3</td>
<td>7.1 ± 1.0</td>
<td>0.72 ± 0.2</td>
<td>2.2 ± 0.4</td>
</tr>
</tbody>
</table>

* Since 2 mmol of alanine are necessary for the formation of 1 mmol of glucose, equivalence in this column requires that the alanine-to-glucose (A—G) figures be divided by 2.

**Study 1.**

**Study 2.**
From overnight fasting conversion levels of 5.5 mmol/hr, 55 mmol of glucose per hr suppressed conversion to 1 mmol/hr, or to about 20%. Control subjects starting at 2.5 mmol/hr fell to 0.72 mmol/hr or to about 30%. Large amounts of glucose infusate (S. M., study 1; H. P., L. P., and S. M., study 2) resulted in further suppression only in the last instance in which we could detect no label in circulating plasma glucose. Inspection of the data in the experiments in which large amounts of glucose were given shows a significant positive correlation of alanine levels with the amount of glucose being infused. In addition, the conversion of glucose to alanine is obviously increased under this condition, and concomitant increase of alanine turnover is demonstrated.

DISCUSSION

Others have presented evidence of the parallelism between pyruvate and alanine metabolism; i.e., when pyruvate levels are high, plasma alanine levels are also high (9). The data here tend to carry this analogy one step further. The presentation of large amounts of exogenous glucose to these subjects resulted in increases of glucose-to-alanine conversion by as much as 2 to 3 times. This undoubtedly was partially responsible for the increased circulating alanine levels and increase in IDR of alanine. Our data do not allow any speculation as to the metabolic fate of the increased alanine turnover. One piece of information which would be of great interest to us is whether such increased turnover of alanine is accompanied by decreased direct entry of alanine into the circulation. The latter would be construed as decreased degradation of body protein. Our data are too sparse to allow an answer to this problem.

The increase in alanine-to-glucose conversion in the overnight fasting state is perhaps comparable to increased Cori cycle activity in patients with malignant disease (10). In view of the increase in glucose flux, the above findings may be a reflection of this phenomenon; i.e., the increased demand for glucose results in increased glycogenolysis as well as increased cycling of both pyruvate and alanine.

The suppression of gluconeogenesis with small amounts of glucose appears to be comparable in control and cancer subjects. This tends to negate the importance of gluconeogenesis in overall energy expenditure. It does, however, confirm other data suggesting normal suppression of free fatty acid levels and turnover that has been found in experimental animals with cancer (1). However, the metabolic fate of the administered glucose may well be different in cancer subjects from that found in control subjects. We have previously reported decreased glucose oxidation in cancer subjects under this condition (12). The alternative metabolic fates of the carbon skeleton would appear to be conversion to other substances, e.g., glycogen, protein, or lipid. All of these processes are energy requiring, and increased conversion in any of these areas might be significant in terms of overall energy expenditure.

REFERENCES

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