Serine and Aspartic Acid Metabolism in Leukemic Leukocytes: Correlation to Effectiveness of Therapy

By N. V. Dimitrov, J. Hansz, M. A. Toth, and B. Bartolotta

Leukocytes from patients with acute granulocytic leukemia (AGL), acute monocytic leukemia (AMoL) and blastic crisis of chronic granulocytic leukemia (BC) were incubated with serine-U-C\(^4\) or aspartic acid-U-C\(^4\). The metabolism was followed by determination of radioactivity of CO\(_2\), lipids, proteins, organic acids, and nucleotides. Leukemic leukocytes in AGL, AMoL, and BC exhibit high serine utilization compared to lymphocytic leukemias. Significant changes in the metabolic pattern of the leukocytes from patients with chronic granulocytic leukemia (CGL) preceded the onset of blastic crisis. Serine utilization remained abnormal in two patients with AGL after complete remission was achieved. Low conversion of serine to CO\(_2\) in AMoL was associated with achievement of complete remission and long survival time. Aspartic acid was most actively metabolized by leukemic cells in BC. The results of these studies indicate that the metabolic pattern of serine and aspartic acid varies with the type of leukemic cell. There is evidence that mature polymorphonuclear (PMN) leukocytes in the blood of leukemic patients may possess an abnormal metabolism.

The two major functions of the amino acids are participation in protein synthesis and as constituents in the intermediary metabolism of the cell. The relationship between the intra- and extracellular amino acid pool is maintained by transport mechanisms that vary for each individual amino acid and type of cell. The leukocyte is considered a metabolically active cell possessing its own transport mechanisms.\(^1\) This applies much more to the various leukemic cells that have an abnormal proliferative capacity and compete actively with the normal cells of the body to maintain their metabolic requirement. Most of the research concerning the relationship between amino acids and leukocytes (normal and leukemic) has been directed toward the nutritional requirements of the cells\(^6\) and their content of free amino acids.\(^2\) Thus, exogenous or intracellularly synthesized amino acids have been shown to be nutritional requirements for certain types of leukemic cells.

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Such a requirement is the result either of a failure of the cell to synthesize the amino acid in an amount needed for the maintenance of normal metabolism\textsuperscript{7,8} or of an increased demand for certain amino acids necessary for protein synthesis. A higher rate of incorporation of various amino acids into proteins by leukemic leukocytes has been previously reported.\textsuperscript{11-13} The content of free amino acids in normal and leukemic leukocytes has also been investigated.\textsuperscript{2-4,9} McMenamy et al.\textsuperscript{9} established a high leukocyte:plasma ratio for most of the amino acids in leukemic patients. Abnormally high levels of glutamic acid and proline and low levels of ornithine have been shown as characteristic for leukemic cells without regard to type of leukemia.\textsuperscript{2,3,9} The activity of several enzymes involved in the amino acid metabolism of normal and leukemic leukocytes has also been investigated.\textsuperscript{8,14-17} The aforesaid investigations dealt with limited parameters of amino acid metabolism of leukemic cells and failed to provide information about the entire anabolism and catabolism of the amino acids. An attempt to investigate the amino acid metabolism in this direction was made by Stjernholm.\textsuperscript{18} He reported that the ketogenic amino acids leucine and isoleucine are catabolized by normal leukocytes to CO\textsubscript{2} and lipids at lower rate than some of the leukemic leukocytes. The same amino acids are converted to the corresponding \(\alpha\)-ketoacids more readily by leukemic cells than by normal leukocytes. This condition, which mimics the pattern of "maple syrup urine disease" suggests the presence of reduced levels of the decarboxylase.

The metabolic pattern of asparagine in various lymphoproliferative disorders was also previously reported.\textsuperscript{19} It has been demonstrated that the rate of total utilization of asparagine and its conversion into various metabolic products depends upon the type of cell and stage of the disease.

All these studies have contributed little to the entire understanding of amino acid metabolism in leukemic cells and its relationship to the leukemic process. In this study the utilization of serine and aspartic acid by leukemic leukocytes was investigated in an effort to determine the metabolic pattern of these amino acids and to correlate it with the response to therapy.

**Materials and Methods**

Patients with various types of acute and chronic leukemia, and normal individuals served as blood donors for this study. The experiments were performed before and after the course of chemotherapy. Two patients with acute granulocytic leukemia were studied when complete remission was achieved.

Blood was drawn into heparin (30 U/10 ml) and 5\% dextran (Sigma, mol wt 200,000) solution in 4:1 proportion for acceleration of red blood cell sedimentation. The mixture was allowed to sediment for 30–40 min. The white blood cell suspension was decanted and centrifuged for 5 min at 100 \(\times\) g. The supernatant was discarded and the sedimented leukocytes were suspended in 1 ml distilled water for hemolysis of the remaining erythrocytes. After shaking for 30 sec, 40 ml of 0.9\% NaCl solution was added and the suspension was centrifuged at 60 \(\times\) g for 5 min. The supernatant was discarded and the sedimented leukocytes were suspended in Krebs-Ringer bicarbonate buffer (KRB) containing 80 mg/100 ml glucose. The final preparations contained 7–16 platelets/100 leukocytes. All preparations were made in siliconized glassware at room temperature.

The incubation and chemical determination of the metabolic products were performed using methods described elsewhere.\textsuperscript{19,20} The cells were incubated in siliconized 50 ml
Erlenmeyer flasks containing a center well. Uniformly labeled $^{14}$C-serine or $^{14}$C-aspartate (2.5 μCi) was added to the cells suspended in KRB. For each isotope a corresponding cold amino acid was added in an amount equal to the average serum level of a normal person. The radioactive material was obtained from the New England Nuclear Corp., Boston, Mass. It was assayed by paper chromatography and was found to be radiochemically pure. The incubations were carried out with 100-200 × 10⁶ cells (25 × 10⁶ per 1 ml serum). The flasks were closed with serum caps and flushed for 1 min with 95% O₂ and 5% CO₂. The flow of the gas mixture was less than one half liter/min, which had no toxic effect on the cells. Incubation mixtures were agitated at 37°C for 2 hr. After incubation, 2 ml of NCS solubilizer, a basic reagent from the Amersham-Searle Co, Des Plaines, Ill., was injected into the center well. The reaction was terminated by injection of 1 ml of 35% perchloric acid ($\text{HClO}_4$) into the main compartment containing the cells. The flasks were then allowed to stand for 3 hr at room temperature to complete the diffusion of CO₂ into the NCS solution. After addition of 15 ml of water the incubation mixture was centrifuged for 10 min at 10,000 g. The sediment was washed three times by suspension in 10 ml of 1% HClO₄ followed by centrifugation. The amino acids from the combined supernatant solutions were separated by a Dowex-50 (H⁺ form) column and eluted with 1.5 N ammonia. After the eluate was evaporated to dryness the amino acids were dissolved in 0.2 ml of 50% ethanol and chromatographed (20 μl) on Whatman 3MM paper strips in saturated phenol-water solvent. The chromatograms were monitored for $^{14}$C with a paper strip scanner model RSC-363 (Baird Atomic, Cambridge, Mass.). The identity of the amino acids was determined by spraying ninhydrin onto duplicate strips with known amino acids. Thin layer chromatography was used for further identification of the amino acids (n-butanol/acetic acid/water [8:2:2]). The radioactivity was determined with a liquid scintillation counter after the identified spots were scraped from the silica gel plate. Coincidence of radioactivity with the amino acids was also determined with an amino acid analyzer (Spinco Model 120) provided with a split divider for $^{14}$C counting. Some of the radioactive amino acids (glycine and glutamate) were eluted from the paper and, after addition of cold amino acid (carrier) recrystallized to constant specific activity. The glycine : serine and glutamate : aspartate ratios were determined using the specific activity of each amino acid, obtained from paper chromatography and thin layer chromatography. As a control of the method some samples were run on the amino acid analyzer.

The fraction of the original eluate obtained after separation of the amino acids contained organic acids and nucleotides. The presence of nucleotides was determined by means of [unspecified method].

### Table 1.—Serine Metabolism

<table>
<thead>
<tr>
<th>Type of Leukemia</th>
<th>No. of Patients</th>
<th>CO₂</th>
<th>Lipids</th>
<th>Proteins</th>
<th>Organic Acids</th>
<th>Nucleotides</th>
<th>Glycine:Serine Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>AGL</td>
<td>9</td>
<td>3.33±0.82</td>
<td>8.04±1.85</td>
<td>41.55±12.13</td>
<td>5.49±3.80</td>
<td>0.28±0.08</td>
<td></td>
</tr>
<tr>
<td>AMoL</td>
<td>9</td>
<td>3.64±0.62</td>
<td>7.72±4.29</td>
<td>55.50±10.36</td>
<td>10.09±2.53</td>
<td>0.20±0.11</td>
<td></td>
</tr>
<tr>
<td>BC</td>
<td>10</td>
<td>5.17±1.86</td>
<td>7.19±3.03</td>
<td>25.55±5.44</td>
<td>34.97±9.15</td>
<td>0.63±0.16</td>
<td></td>
</tr>
<tr>
<td>AcLL</td>
<td>7</td>
<td>0.58±0.14</td>
<td>2.90±0.35</td>
<td>12.78±1.75</td>
<td>14.72±2.88</td>
<td>0.09±0.05</td>
<td></td>
</tr>
<tr>
<td>LySa</td>
<td>5</td>
<td>0.27±0.12</td>
<td>1.97±0.21</td>
<td>7.24±1.76</td>
<td>16.45±2.28</td>
<td>0.10±0.06</td>
<td></td>
</tr>
<tr>
<td>CCL</td>
<td>8</td>
<td>0.60±0.21</td>
<td>2.37±0.88</td>
<td>10.15±5.08</td>
<td>2.86±1.83</td>
<td>0.11±0.04</td>
<td></td>
</tr>
<tr>
<td>Normal PMN</td>
<td>9</td>
<td>0.55±0.16</td>
<td>0.52±0.20</td>
<td>1.86±0.64</td>
<td>0.32±0.12</td>
<td>0.08±0.03</td>
<td></td>
</tr>
</tbody>
</table>

*μmol/10⁸ cells/hr.
†Abbreviations for types of leukemia: AGL, acute granulocytic leukemia; AMoL, acute monocytic leukemia; BC, blastic crisis in chronic granulocytic leukemia; AcLL, acute lymphocytic leukemia; LySa, lymphosarcoma with leukemic blood picture; CCL, chronic granulocytic leukemia.

Unutilized serine.
\$±1 SD.
of absorption spectra and high voltage electrophoresis. Further investigation of this fraction was not performed.

Lipids were extracted from the original sediment in three steps with acetone, ether and chloroform : methanol (65:35). The specific lipid composition is the subject of a special investigation.

The remaining residue was primarily protein. The protein fraction was hydrolyzed by means of the proteolytic enzyme Pronase (Calbiochem, Los Angeles, Calif.) in order to determine the radioactivity of some of the individual amino acids incorporated in the proteins. The amino acids from the hydrolysate were identified by paper chromatography.

The radioactivity of all metabolic products was determined with a Beckman liquid scintillation spectrometer LS 150. The calculations were expressed as \( \mu M \) of metabolic product or \( \mu M \) of amino acid (serine or aspartic acid) incorporated into the given fraction using the standard conversion formula:

\[
\begin{align*}
\text{Cold amino acid} & \quad x \text{ \( \mu \) moles} \\
1^4\text{C amino acid} & \quad y \text{ \( \mu \) moles} \\
\text{Incubation specific radioactivity} & \quad 5\mu\text{Ci}/(x+y) \text{ \( \mu \) moles} \\
\text{Working constant} & \quad 5\mu\text{Ci}/\text{\( \mu \) mole} \\
\text{Conversion: } 2.22 \times 10^6 \text{ dpm} & = 1 \mu\text{Ci} \\
Z \times 10^6 \text{ dpm} & = \mu\text{moles} \\
Z \times 10^6 \text{ DPM} & = \mu\text{moles} \\
\text{CO}_2 = Z \times 10^6 \text{ DPM} \times \text{cells} \times \text{hr} \times 31 & = \mu\text{moles}/\text{cells}/\text{hr}
\end{align*}
\]

The other metabolic products are calculated in the same manner.†

**RESULTS**

The metabolic pattern of serine varies with the type of leukemic cell. The conversion of serine to metabolic products is presented in Table 1. An active production of \( \text{CO}_2 \) was noted in AGL, AMoL, and BC, whereas leukocytes from the lymphocytic leukemias and CGL produced \( \text{CO}_2 \) at the rate of normal polymorphonuclear (PMN) leukocytes. High conversion of serine to lipids was observed in the former groups, while moderate conversion was present in lymphocytic leukemias and CGL. All leukemic groups showed higher lipid production than the normal PMN leukocytes. Labeled sphingolipids were identified only in ALL and CGL. A detailed investigation of these metabolic products is in progress. All types of leukemic cells incorporated serine into proteins at a higher rate than normal PMN leukocytes. The most active conversion of serine to protein was seen in AGL and AMoL. The incorporation of serine into the fraction of organic acids and nucleotides was increased in all types of leukemia but most significantly in BC. The qualitative determination of the individual nucleotides and organic acids was not completed at this point of the investigation. The ratio of glycine to nonutilized serine was high

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* Condition: three carbons for serine and four carbons for aspartic acid.
† Condition: one carbon.
1 Lipids are considered condition two carbons because serine is metabolized via two-carbon compounds for formation of neutral fats, phospholipids, and sphingolipids. Aspartic acid is metabolized only via acetyl-Co A (two carbons). For proteins, and organic acids and nucleotide fractions, the whole molecule was used for calculation, it being considered as an incorporation of the amount of amino acid (serine, aspartic acid) into the fraction.
Table 2.—Serine Metabolism Respiratory CO2

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age</th>
<th>Type of Leukemia</th>
<th>Blasts (%)</th>
<th>CO2 mmol/10^6 cells/hr.</th>
<th>Agent*</th>
<th>Response</th>
<th>Survival Time (wk)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S.L.</td>
<td>M</td>
<td>44</td>
<td>AGL</td>
<td>88</td>
<td>5.31</td>
<td>CAR, VP</td>
<td>Resistant</td>
<td>3</td>
</tr>
<tr>
<td>W.W.</td>
<td>M</td>
<td>31</td>
<td>AGL</td>
<td>70</td>
<td>4.41</td>
<td>VMP</td>
<td>Resistant</td>
<td>7</td>
</tr>
<tr>
<td>H.B.</td>
<td>M</td>
<td>47</td>
<td>AGL</td>
<td>81</td>
<td>5.93</td>
<td>VAMP</td>
<td>Resistant</td>
<td>11</td>
</tr>
<tr>
<td>B.H.</td>
<td>F</td>
<td>69</td>
<td>AGL</td>
<td>73</td>
<td>4.58</td>
<td>VAMP</td>
<td>Resistant</td>
<td>3</td>
</tr>
<tr>
<td>C.E.</td>
<td>F</td>
<td>57</td>
<td>AGL</td>
<td>78</td>
<td>2.51</td>
<td>VAMP</td>
<td>Resistant</td>
<td>15</td>
</tr>
<tr>
<td>S.B.</td>
<td>F</td>
<td>61</td>
<td>AGL</td>
<td>72</td>
<td>3.96</td>
<td>CAR, VMC</td>
<td>Remission</td>
<td>26</td>
</tr>
<tr>
<td>I.S.</td>
<td>F</td>
<td>53</td>
<td>AGL</td>
<td>65</td>
<td>4.47</td>
<td>CAR, VMC</td>
<td>Remission</td>
<td>21</td>
</tr>
<tr>
<td>I.S.</td>
<td>F</td>
<td>68</td>
<td>AGL</td>
<td>77</td>
<td>2.16</td>
<td>CAR, VMP</td>
<td>Partial Remission</td>
<td>20</td>
</tr>
<tr>
<td>M.S.</td>
<td>M</td>
<td>38</td>
<td>AMoL</td>
<td>98</td>
<td>3.18</td>
<td>VAMP</td>
<td>Resistant</td>
<td>3</td>
</tr>
<tr>
<td>J.M.</td>
<td>M</td>
<td>30</td>
<td>AMoL</td>
<td>100</td>
<td>4.08</td>
<td>CAR, VMP</td>
<td>Resistant</td>
<td>10</td>
</tr>
<tr>
<td>G.R.</td>
<td>M</td>
<td>49</td>
<td>AMoL</td>
<td>93</td>
<td>3.71</td>
<td>CAR, VMC</td>
<td>Resistant</td>
<td>14</td>
</tr>
<tr>
<td>J.R.</td>
<td>F</td>
<td>42</td>
<td>AMoL</td>
<td>95</td>
<td>4.58</td>
<td>VAMP</td>
<td>Resistant</td>
<td>3</td>
</tr>
<tr>
<td>M.M.</td>
<td>M</td>
<td>35</td>
<td>AMoL</td>
<td>98</td>
<td>0.71</td>
<td>VAMP, CAR</td>
<td>Complete Remission &gt; 60</td>
<td></td>
</tr>
<tr>
<td>S.L.</td>
<td>M</td>
<td>41</td>
<td>AMoL</td>
<td>100</td>
<td>0.94</td>
<td>CAR, MPC</td>
<td>Complete Remission &gt; 52</td>
<td></td>
</tr>
<tr>
<td>W.W.</td>
<td>M</td>
<td>36</td>
<td>AMoL</td>
<td>96</td>
<td>0.90</td>
<td>CAR, VMC</td>
<td>Complete Remission &gt; 37</td>
<td></td>
</tr>
<tr>
<td>P.G.</td>
<td>M</td>
<td>53</td>
<td>BC</td>
<td>68</td>
<td>6.10</td>
<td>CAR, MPC</td>
<td>Resistant</td>
<td>15</td>
</tr>
<tr>
<td>M.B.</td>
<td>F</td>
<td>58</td>
<td>BC</td>
<td>51</td>
<td>8.61</td>
<td>VAMP</td>
<td>Resistant</td>
<td>10</td>
</tr>
<tr>
<td>V.V.</td>
<td>F</td>
<td>67</td>
<td>BC</td>
<td>62</td>
<td>2.38</td>
<td>VAMP</td>
<td>Resistant</td>
<td>18</td>
</tr>
<tr>
<td>B.W.</td>
<td>F</td>
<td>55</td>
<td>BC</td>
<td>49</td>
<td>3.78</td>
<td>VAMP</td>
<td>Resistant</td>
<td>12</td>
</tr>
<tr>
<td>C.W.</td>
<td>M</td>
<td>29</td>
<td>BC</td>
<td>42</td>
<td>4.04</td>
<td>CAR, VMP</td>
<td>Partial Remission</td>
<td>17</td>
</tr>
<tr>
<td>A.A.</td>
<td>M</td>
<td>59</td>
<td>BC</td>
<td>58</td>
<td>5.88</td>
<td>CAR, VMC</td>
<td>Partial Remission</td>
<td>11</td>
</tr>
<tr>
<td>M.G.</td>
<td>M</td>
<td>52</td>
<td>CGL</td>
<td>1</td>
<td>0.33</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>W.S.</td>
<td>M</td>
<td>61</td>
<td>CGL</td>
<td>1</td>
<td>0.72</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F.H.</td>
<td>F</td>
<td>48</td>
<td>CGL</td>
<td>0</td>
<td>0.74</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>F</td>
<td>67</td>
<td>CGL</td>
<td>0</td>
<td>0.81</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal PMN (9 individuals)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.55 ± 0.16</td>
</tr>
</tbody>
</table>

* Abbreviations for therapeutic agents: V, vincristine; A, methotrexate (amethopterin); C, Cytoxan; P, prednisone; M, 6-mercaptopurine; CAR, cytosar (cytosine arabinoside).

in AGL and BC, the latter being exceedingly high. When CO2 production by various leukemic cells was compared to response to therapy and survival time (Table 2), low conversion of serine to CO2 before initiation of therapy in AMoL was associated with achievement of complete remission and longer survival time of the patient. Further comparison revealed that the blast cells appeared to convert serine more actively to CO2; immature granulocytes in CGL metabolized serine at the rate of normal PMN leukocytes.

Periodic determination of serine utilization (Fig. 1) indicated that significant changes in the metabolic pattern of the leukocytes from patients with CGL preceded the onset of blastic crisis. The increase of serine utilization appeared
SERINE AND ASPARTIC ACID METABOLISM IN LEUKOCYTES

Fig. 2.—Total serine utilization by leukemic leukocytes from two patients with acute granulocytic leukemia before and after treatment.

when the morphological distribution of the leukocytes was not altered and no blasts were noted in the peripheral blood. The appearance of increased number of blasts (blastic crisis) was associated with a drastic increase of serine utilization which did not change when the number of blasts decreased after therapy. Total utilization of serine was determined in two patients with AGL after complete remission was achieved. When morphologically normal PMN leukocytes were the predominant cells in the peripheral blood and all blasts disappeared, the utilization of serine remained abnormal (Fig. 2).

The metabolic pattern of aspartic acid (Table 3) differed significantly from that of serine. Leukemic leukocytes from patients with blastic crisis in CGL converted aspartic acid more actively to CO₂ compared to AGL and AMoL. The same ratio of incorporation was found for the protein fraction. The lipid fraction did not show significant changes. Increased incorporation of aspartic acid was noted in the fraction of organic acids and nucleotides by leukocytes from patients with AGL and BC. More glutamic acid was converted in blastic crisis producing a higher ratio glutamic:aspartic acid. ALL, LySA, CGL, CLL,

Table 3.—Aspartic Acid Metabolism*

<table>
<thead>
<tr>
<th>Type of Leukemia</th>
<th>No. of Patients</th>
<th>CO₂ (mumoles/10⁸ cells/hr.)</th>
<th>Lipids (mumoles/10⁸ cells/hr.)</th>
<th>Proteins (mumoles/10⁸ cells/hr.)</th>
<th>Organic Acids and Nucleotides (mumoles/10⁸ cells/hr.)</th>
<th>Gln/Asp† Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>AGL</td>
<td>8</td>
<td>9.42 ± 1.17†</td>
<td>0.63 ± 0.30</td>
<td>3.42 ± 0.42</td>
<td>11.21 ± 2.55</td>
<td>0.51 ± 0.07</td>
</tr>
<tr>
<td>AMoL</td>
<td>9</td>
<td>4.40 ± 0.86</td>
<td>0.34 ± 0.14</td>
<td>1.72 ± 0.42</td>
<td>3.71 ± 0.97</td>
<td>0.12 ± 0.06</td>
</tr>
<tr>
<td>BC</td>
<td>10</td>
<td>33.54 ± 4.26</td>
<td>0.38 ± 0.13</td>
<td>10.40 ± 1.22</td>
<td>11.69 ± 2.03</td>
<td>0.42 ± 0.12</td>
</tr>
<tr>
<td>ALL</td>
<td>9</td>
<td>0.36 ± 0.13</td>
<td>0.15 ± 0.07</td>
<td>0.34 ± 0.08</td>
<td>3.86 ± 0.38</td>
<td>—</td>
</tr>
<tr>
<td>LySA</td>
<td>5</td>
<td>0.44 ± 0.22</td>
<td>0.08 ± 0.01</td>
<td>0.47 ± 0.09</td>
<td>4.06 ± 1.20</td>
<td>—</td>
</tr>
<tr>
<td>CGL</td>
<td>9</td>
<td>1.87 ± 0.68</td>
<td>0.42 ± 0.28</td>
<td>0.85 ± 0.35</td>
<td>3.73 ± 0.95</td>
<td>—</td>
</tr>
<tr>
<td>CLL</td>
<td>6</td>
<td>0.40 ± 0.17</td>
<td>0.09 ± 0.02</td>
<td>0.38 ± 0.11</td>
<td>4.35 ± 0.43</td>
<td>—</td>
</tr>
<tr>
<td>Normal PMN leukocytes</td>
<td>9</td>
<td>0.52 ± 0.20</td>
<td>0.22 ± 0.09</td>
<td>0.32 ± 0.12</td>
<td>4.16 ± 0.65</td>
<td>—</td>
</tr>
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* mmoles/10⁸ cells/hr.
† The amount of glutamic acid was sufficient for determination of the ratio Gln/Asp only in the first three types of acute leukemia.
‡ Unutilized aspartic acid.
§ ± 1 SD.
and normal PMN leukocytes experienced identical metabolic activity, with the exception of slightly increased CO₂ in CGL.

When the patterns of serine and aspartic acid metabolism were compared, three major differences were noted. (1) Whereas CO₂ production from serine was almost equally distributed among AGL, AMoL, and BC, CO₂ produced from aspartic acid differed significantly in the above mentioned groups. The most active leukemic cell appeared to be that in blastic crisis of CCL. (2) The protein fraction from serine and aspartic acid in these three types of leukemia appeared to be different. Incorporation of aspartic acid into the protein was far less than that of serine. (3) When the ratio of incorporated serine into organic acid and nucleotides to CO₂ production (from serine) was compared to that of aspartic acid, a significant difference was noted in BC. The ratio obtained for serine was approximately 23 times higher than for aspartic acid.

**DISCUSSION**

The rapid development of research concerning antileukemic therapy during the last 2 decades has stimulated the metabolic investigation of the leukemic leukocytes. The varying effects of one and the same drug or a combination of drugs on morphologically identical cells emphasized the necessity for a more accurate determination of the type of leukemic cell. Attempting to determine the metabolic pattern seemed to be an appropriate approach to the investigation of this problem.

Serine, which is a nonessential amino acid for the human body, appeared to be essential for the growth of certain leukemic leukocytes. Serine utilization occurs through various metabolic pathways. The conversion of this amino acid to respiratory CO₂ varies significantly according to the type of leukemic leukocyte (Table 1). Thus, the production of CO₂ by leukemic cells from patients with AGL, AMoL and BC is greatly increased compared to the other types of leukemias and normal PMN leukocytes (Table 1). This metabolic activity is of greater importance in AMoL. Low CO₂ production in this type of leukemia indicates good therapeutic response and longer survival time (Table 2). Further studies in the future would be useful to determine the value of this finding. The conversion of serine to glycine was expressed as the ratio glycine:serine. The increased ratio appeared to be unique for blastic crisis in CGL. Simultaneous incubations with glycine did not reveal any significant difference of the utilization between AGL, AMoL, and BC. This could be explained by interference of the transport of glycine across the membrane, whereas the conversion of serine to glycine is entirely an intracellular function. The gradual increase in the rate of serine utilization by leukocytes in CGL could be of some importance. The elevation of total serine utilization when the morphological picture remains unchanged may indicate an imminent blastic crisis (Fig. 1). Calbraith found leukocyte specific activity curves characteristic of those in acute leukemia in patients with polycythemia vera, refractory anemia, and essential thrombocytosis. He suggests that acute granulocytic leukemia “represents an end stage” of various disorders as it does in
CGL. Thus the changes in leukemic cells should be studied in their evolution before bone marrow abnormality is noted.

When the blastic crisis is developed it is accompanied by drastic metabolic changes which remain in spite of the achievement of remission. This combination may be considered as a bad prognostic sign and could explain the malignant course at this stage of the disease. Metabolic changes of lymphocytes during the course of chronic lymphocytic leukemia have been previously reported. Such changes occurred in the terminal stage of the disease when the cells are resistant to therapy and complications have developed.

A fundamental problem in the treatment of leukemia is the determination of when the total leukemic cell kill has been achieved. This is especially true in AcLL and AMoL. In complete remission, when the total leukocyte and differential count is normalized, the isolation of sufficient amount of normal lymphocytes and monocytes for metabolic studies is difficult. If abnormal cells cannot be isolated for control studies, their presence can only be indicated by the detection of the abnormal metabolic products that they produce. Unfortunately, such a method is not available at the moment. However, a more favorable situation exists for patients with AGL since the metabolic changes could be used as an indicator of the restoration of normally functioning polymorphonuclear leukocytes. We have followed two patients with AGL who achieved a complete remission (Fig. 2). Serine utilization remained abnormal in spite of normalization of the peripheral blood picture. The morphologically normal PMN leukocytes had a leukemic metabolic pattern. Smith et al. recently reported high activity of the enzyme pyrophosphate phosphoribosyltransferase in a number of leukemics with normal differential blood smears. Calbraith has followed the kinetic curves of a patient with AGL before and after therapy. The curves remained abnormal when a complete remission was achieved. This may indicate that the evolution of "malignant" clones has not been stopped. Another work from the same laboratory showed a variety of abnormal curves of granulocytes in identical types of leukemia suggesting that the mature neutrophils may arise from leukemic precursors.

Although much of the evidence demonstrated in this study was proposed to differentiate the various types of leukemia, it does suggest that the leukemic cell possesses more than one metabolic defect. The pleomorphism and variability characteristic of leukemic cell behavior could be associated with membrane alterations or enzymatic changes of the cell. Thorough investigation of the metabolic patterns and metabolic pathways may throw more light on this difficult problem. Such an approach could bring to the chemotherapist valuable information regarding choice of drug and control of the disease. The morphological diagnosis does not provide the necessary details that determine the biological activity of the cell. This could produce mismanagement of the disease and failure to achieve long lasting remission or complete cure. The fact that some lymphoproliferative disorders are considered as curable diseases suggests that the abnormal biological mechanisms involved in the malignant process are reversible. Much evidence has been presented that these mechanisms appeared to be metabolic deviations. The preferential utilization of certain amino acids by leukemic cells should be considered as a requirement.
for abnormal proliferation or abnormal cellular behavior. Thus, there is a
possibility that replacement of serine by an analogue or other amino acid
could stop or change the vicious cycle involved in the proliferation of certain
types of leukemic cells. Further investigation is necessary for evaluation of
the amino acids involved in the leukemic process.

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Serine and Aspartic Acid Metabolism in Leukemic Leukocytes: Correlation to Effectiveness of Therapy

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