Energy Metabolism of Human Leukemic Lymphocytes and Granulocytes

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With the technical assistance of Clarence Ellis

MANY DIVERGENT RESULTS have been published concerning the energy metabolism of human leukemic leukocytes. This subject has been extensively reviewed by Seelich. Leukemic lymphocytes have variously been reported to have low or absent aerobic glycolysis, low to high respiration, and low to high anaerobic glycolysis. Leukemic granulocytes have been reported to have high aerobic glycolysis and high respiration, while other workers appear to demonstrate the opposite. There has also been considerable uncertainty about the metabolic differences of primitive blast cells from acute leukemias compared with more mature leukocytes from chronic lymphocytic and chronic granulocytic leukemia.

We believe that the major causes for the discrepancies in the literature have been methodologic—involving injurious modes of leukocyte isolation, suspension of leukocytes in synthetic media rather than plasma, the use of leukocyte homogenates rather than intact cells, lack of pure lymphocyte or granulocyte preparations, and often failure to check all pertinent metabolic parameters at the conclusion of each experiment (e.g., pH and glucose). The metabolic experiments reported herein followed extensive earlier studies carried out in collaboration with Dr. Dean Burk, in which all of the above methodologic problems have been investigated. The present study was undertaken to attempt to define the energy metabolism of cells taken from patients with acute and chronic leukemias, to observe the ability of leukemic cells to adapt metabolically and functionally to drastic changes in the environment, and to try to reconcile the apparently conflicting data of earlier reports. It was further intended to compare the metabolism of leukemic granulocytes with leukemic lymphocytes.

MATERIALS AND METHODS

Isolation of Leukemic Leukocytes. Fifty ml. of heparinized whole blood (20 U./ml.) was removed from patients having acute and chronic lymphocytic and granulocytic leukemia. Bovine fibrinogen was dissolved in the patients' plasma and immediately added to whole blood to give a final fibrinogen concentration of approximately 9 mg./ml. This mixture was placed in a graduate cylinder at room temperature for 30-45 minutes while the red cells settled out. (Preliminary studies showed that these concentrations of heparin and...
fibrinogen did not alter cell metabolism.) The leukocyte-plasma layer was aspirated, and glucose (2 mg./ml.) and NaHCO₃ (1 mg./ml.) were added. Leukocytes were enumerated in a counting chamber and differential blood counts were performed on cover slips stained with Wilson’s stain. Additional amounts of glucose and NaHCO₃ were added when leukocyte counts exceeded 150,000 cells/mm³. At no time during the isolation were the cells centrifuged, washed, or placed in contact with foreign plasma, unless otherwise specified.

Patients were selected for these studies if their peripheral leukocyte count was greater than 25,000 cells/mm³ and if more than 90 per cent of these cells were of the leukemic series. Thus, studies on acute leukemia were performed on blood containing more than 90 per cent blast cells; in chronic lymphocytic leukemia the predominant cell was the small lymphocyte; in chronic granulocytic leukemia the leukocytes were a mixture of mature and immature granulocytes.

**Manometric Studies.** Two ml. aliquots of leukocytes in plasma were pipetted into special matched manometer flasks designed by Warburg. Of each pair of matched vessels, one had its trough filled with 0.5 ml of water and the other with a bicarbonate buffer to which carbonic anhydrase had been added to facilitate equilibration and to maintain the CO₂ content at 5 per cent. (These trough compartments are suspended above the main portion of the vessel and they do not come into contact with the cells.) Bicarbonate buffer was prepared by mixing one volume of 3.0 M potassium carbonate with 3 volumes of 3.0 M potassium bicarbonate and adding carbonic anhydrase (1 mg./ml.). The flask containing water in the trough was gassed with 5 per cent CO₂/air. In this manner both respiration and glycolysis were measured at physiologic CO₂ levels. Knowing the O₂ consumption from the vessel containing buffer in the trough and assuming a respiratory quotient of 0.8, the CO₂ from respiration and from glycolysis can be calculated from the gas pressure change of the reference vessel containing water in the trough. Anaerobic glycolysis was determined in manometer flasks gassed with 95 per cent N₂/5 per cent CO₂. Manometric readings were determined every 5–15 minutes for 3 hours.

At the completion of the experiment, which was performed in triplicate vessels, the pH was determined with a Beckman pH meter and aliquots were saved for determination of high energy phosphate compounds and for dry weight. Dry weight was obtained by centrifuging the cells, washing twice with cold 0.9 per cent NaCl, drying in an oven, and weighing the residue. The error due to the presence of NaCl is negligible; the error due to large numbers of platelets is significant in the case of CGL. Respiration (QO₂), aerobic glycolysis (Q⁺), were expressed in the conventional manner, as microliters of O₂ or CO₂ consumed or produced per mg dry weight of cells per hour.

**Plasma Glucose and High Energy Phosphate.** Glucose was determined by the glucose oxidase method. High energy phosphage was estimated by the Seits procedure, which depends upon charcoal absorption of ADP and ATP, but not of AMP or other mononucleotides. A standard curve was determined in each experiment using purified ATP. Spot checks of the method compared favorably with the Luciferase assay.

**Alterations in the Environment of Cells.** Glucose-free plasma was prepared by incubating the patients’ plasma with Brewer’s yeast at 37 C. for 60 minutes, then centrifuging out the yeast. No detectable quantities of glucose remained in the plasma following this procedure. Leukocytes were centrifuged out of the plasma suspension at 100 r.p.m. for 10 minutes and resuspended in previously prepared glucose-free plasma. Glucose (2 mg./ml.) was then added back to half of the vessels by tipping in from the sidearm of the manometer flask.

An alternative method for evaluating the effect of glucose deficiency was to add 2-deoxy-D-glucose (5 mg./ml.) in place of glucose. An alternative method for studying anaerobiosis was to tip in sodium azide (5 × 10⁻⁵M) from the flask sidearm after gassing with 5 per cent CO₂/air.

Exchange of plasma environment was performed by slow-speed centrifugation (100 r.p.m. for 10 min.) of leukocytes followed by resuspension of the cells in previously prepared plasmas taken from other patients having the same and different types of leukemia.

Phagocytosis was studied by adding starch particles or polystyrene latex particles to
granulocyte suspensions and examining the cells for evidence of particle ingestion after 3 hours of incubation.

**RESULTS**

**Lymphocytes**

The data from the present studies are reported graphically in Figure 1 and summarized in Table 1. Cells taken from both acute and chronic lymphocytic leukemic patients were characterized by relatively high $Q_{O_2}$ (ca.5), by a high rate of anaerobic glycolysis $Q^{N_2}_{A}$ (ca. 20), and by negligible aerobic glycolysis $Q^{A}_{A}$ (ca. 1-2). That this aerobic glycolysis was indeed negligible is illustrated in Table 2, which records the results of studies performed at various stages in the illness of a patient with acute lymphocytic leukemia. The only observations in which the $Q^{A}_{A}$ was significant were made at times in the patient’s disease when he had 20-25 per cent contamination from cells of the granulocytic series. All of the aerobic glycolysis in those experiments was attributable to the presence of these contaminating cells as determined by calculating the amount of glycolysis known to be contributed by granulocytes (see following section).

Figure 2 shows the effect of glucose-free plasma on lymphocyte respiration. The presence of glucose resulted in respiratory rates and ADP-ATP concentrations approximately the same as those obtained in the absence of glucose. Likewise, 2-deoxy-D-glucose effected either no change or slight inhibition of lymphocyte respiration, and this was paralleled by the ADP-ATP concentration.

Lymphocytes demonstrated a high rate of anaerobic glycolysis ($Q^{N_2}_{A}$) and cells incubated in this way over 3 hours showed no change in ADP-ATP concentration, compared with aerobic control vessels incubated for 3 hours (Fig. 3) or zero time controls. Three-hour incubation of lymphocytes anaerobically in the absence of glucose resulted in ADP-ATP levels too low to detect. The effect of the respiratory inhibitor azide was to stimulate glycolysis to levels obtained under anaerobic conditions. Furthermore, some oxygen uptake by the cells continued, despite the presence of the inhibitor. Similar results were obtained using cyanide as a respiratory inhibitor.

Lymphocytes isolated from their own plasma and then resuspended in plasma taken from other patients showed various metabolic responses depending upon the individual plasma. An occasional normal or CGL plasma caused a profound stimulation of aerobic glycolysis. The dry weights of lymphocytic and granulocytic leukemic cells as determined in these studies are listed in Table 3.

**Granulocytes**

Data from these studies are summarized in Table 1 and Figure 4. Metabolism of leukemic granulocytes was marked by a high rate of aerobic glycolysis, low respiration, and a large $Q^{A}_{A}$ value. Anaerobic glycolysis was approxi-
Fig. 1.—Metabolic quotients of cells from acute and chronic lymphocytic leukemia. Each point represents one experiment, the lines define the mean, and the shaded areas depict two standard errors from the mean.

approximately the same for both types of acute and chronic leukemias. The metabolic effects of anaerobiosis or of a glucose-free environment are illustrated by the experiments represented in Figures 5 and 6. Granulocytes from acute and chronic leukemia increased their glycolysis anaerobically, possessing the same amount of ADP-ATP at the end of 3 hours of incubation as incubated aerobic controls and the same as time zero preincubation samples. In the absence of glucose, respiration rose markedly and the ADP-ATP again remained constant. Anaerobically, in the absence of glucose, ADP-ATP levels fell to zero by the end of the three hour incubation. 2-Deoxy-D-glucose (3–5 mg./ml.) added to cells simulated the metabolic effects of glucose-free plasma, with inhibition of glycolysis, concomitant stimulation of respiration, and no change in ADT-ATP levels. If much higher concentrations of 2-deoxy-D-glucose were used, inhibition of respiration ultimately occurred, resulting in a fall in high energy phosphate. Respiratory inhibitors azide and cyanide simulated the conditions of anaerobiosis except that some oxygen consumption persisted. This azide-insensitive respiration was found with mature chronic granulocytic leukemia and primitive acute granulocytic leukemic cells.

Suspension of leukemic granulocytes in foreign plasma was sometimes accompanied by marked alterations in cellular metabolism. Figures 7 and 8, showing the effects of normal plasma compared to patients’ own plasma, demonstrate a marked decrease in Q^O_2 accompanied by a rise in QO_2 due to the

* ADP-ATP levels could have fallen to zero much sooner than 3 hours, for shorter incubation periods were not measured.
<table>
<thead>
<tr>
<th></th>
<th>( \frac{Q_0}{Q_{39}} )</th>
<th>( \frac{Q_{O_2}}{Q_{A}} )</th>
<th>( \frac{ATP^{+}-P}{\mu g./mg.} )</th>
<th>( \frac{Q_{N_2}}{Q_{A}} )</th>
<th>( \frac{ATP^{+}-P}{\mu g./mg.} )</th>
<th>( Q_{O_2} )</th>
<th>( \frac{ATP^{+}-P}{\mu g./mg.} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chronic lymphocytic leukemia (29)</td>
<td>5.10±0.46</td>
<td>1.56±0.72</td>
<td>0.90</td>
<td>1.60±0.60</td>
<td>18.3±2.8</td>
<td>1.54±0.52</td>
<td>5.4±0.51</td>
</tr>
<tr>
<td>Acute lymphocytic leukemia (8)</td>
<td>4.81±1.30</td>
<td>2.16±1.14</td>
<td>1.8</td>
<td>0.82±0.42</td>
<td>20.80±5.20</td>
<td>0.78±0.52</td>
<td>4.47±1.00</td>
</tr>
<tr>
<td>Chronic granulocytic leukemia (35 *)</td>
<td>2.11±0.19</td>
<td>10.40±1.14</td>
<td>17.9</td>
<td>1.45±0.71</td>
<td>25.5</td>
<td>1.88</td>
<td>4.2</td>
</tr>
<tr>
<td>Acute granulocytic leukemia (16)</td>
<td>3.58±0.80</td>
<td>15.60±5.00</td>
<td>15.9</td>
<td>1.39±0.18</td>
<td>20.00±0.88</td>
<td>1.12±0.34</td>
<td>5.05±1.05</td>
</tr>
</tbody>
</table>

\( Q_{O_2} \) = microliters of oxygen consumed per mg. dry weight of cells per hour.

\( Q_{A}^{O_2} \) = microliters of CO\(_2\) produced by acid interacting with bicarbonate buffer per mg. dry weight of cells per hour. This is determined in an aerobic atmosphere.

\( Q_{N_2}^{O_2} \) = microliters of CO\(_2\) produced by acid interacting with bicarbonate buffer in nitrogen atmosphere.

\( ATP^{+}-P \) = high energy phosphate from ADP and ATP expressed as micrograms of phosphorus per mg. dry weight of cells.

* = number of experiments carried out under aerobic conditions (fewer anaerobically or without glucose).
Table 2.—Aerobic Glycolysis of Leukocytes from a 13-year-Old Male with Acute Lymphocytic Leukemia (studied in conjunction with Drs. Burk and Stambuk). The patient died on November 16, 1958.

<table>
<thead>
<tr>
<th>Date</th>
<th>HGB</th>
<th>WBC/mm³</th>
<th>Differential Count</th>
<th>Aerobic Glycolysis (Q₂O₂)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lymph %</td>
<td>(Blasts)</td>
<td>PMN %</td>
<td>Observed</td>
<td>Calc./100% PMN</td>
</tr>
<tr>
<td>----------</td>
<td>----------</td>
<td>--------</td>
<td>-----------</td>
<td>----------------</td>
</tr>
<tr>
<td>IV-10-57</td>
<td>10.6</td>
<td>13,200</td>
<td>92</td>
<td>(80)</td>
</tr>
<tr>
<td>XI-31-57</td>
<td>12.4</td>
<td>61,000</td>
<td>76</td>
<td>(76)</td>
</tr>
<tr>
<td>1-14-58</td>
<td>11.0</td>
<td>41,000</td>
<td>77</td>
<td>(47)</td>
</tr>
<tr>
<td>11-21-58</td>
<td>9.5</td>
<td>113,000</td>
<td>93</td>
<td>(90)</td>
</tr>
<tr>
<td>X-24-58</td>
<td>6.9</td>
<td>93,000</td>
<td>94</td>
<td>(94)</td>
</tr>
</tbody>
</table>

addition of normal plasma. Metabolic changes produced by foreign plasma in our other studies were qualitatively and quantitatively variable.

Granulocytes taken from patients with chronic granulocytic leukemia showed normal phagocytosis of starch and latex particles as measured by microscopic examination of supra-vital and stained cell preparations. Phagocytosis was accompanied by an increase in cellular respiration and glycolysis. Figure 9 shows the effect of 2-deoxy-D-glucose, which blocked phagocytosis as well as the normal increase in respiration and glycolysis. Phagocytosis and ameboid motility were unaffected by anaerobiosis or by azide, but were markedly inhibited when cells were incubated in glucose-free plasma. Chelating agents, such as EDTA, also interfered with phagocytosis and the normal metabolic response to this process. Three patients being studied sequentially made the transition to the blastic phase of acute granulocytic leukemia. One of these showed a striking increase in Q₀₂ together with a fall in Q₂₂, a second showed a moderate decrease in Q₂₂, and the third was unchanged metabolically as the transition occurred (Table 4).

**DISCUSSION**

**Lymphocytes**

Far more agreement exists in the literature on the metabolism of leukemic lymphocytes than that of granulocytes. Very low to absent aerobic glycolysis for leukemic lymphocytes has been reported by a number of authors. Remmel reported very low values for Q₂₀₂, Q₂₂, and Q₂₂ for lymphocytes, in disagreement with the results reported in this paper. Glover found that the more mature the lymphocyte (or granulocyte), the higher its Q₂₀₂ and the lower its Q₂₂—findings also not substantiated in this report. One explanation for the large discrepancies in absolute values for lymphocyte metabolism reported by the above authors stems from their utilization of the value 5.1 million cells per 1 mg. dry weight, a determination derived from normal cells by Bird and often used to calculate metabolic quotients for leukemic cells. In contrast, our studies have shown the value to be 22.2 million cells per 1 mg. dry weight for chronic lymphocytic leukemia cells and 12.2 million for acute lymphocytic
Fig. 2.—The effect of glucose on respiration of acute leukemia lymphocytes in glucose free plasma. High energy phosphate was measured at the end of the incubation.

Fig. 3.—Aerobic and anaerobic glycolysis of leukemic lymphocytes in glucose free plasma taken from the same patient studied in Figure 2. Aerobic glycolysis in the absence of glucose was too low to measure.

leukemic cells. When data of other authors are recalculated using these measurements, the metabolic quotients become similar to those reported herein and to those of Luganova and Burk. It is not feasible to compare data ob-
Table 3.—Dry Weight of Leukemic Cells
(X 10^6/mg. ± 2 Standard Errors)

<table>
<thead>
<tr>
<th>Leukemic Type</th>
<th>Weight (mg./mg.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chronic granulocytic leukemia</td>
<td>6.6±1.1</td>
</tr>
<tr>
<td>Chronic lymphocytic leukemia</td>
<td>22.4±3.0</td>
</tr>
<tr>
<td>Acute granulocytic leukemia</td>
<td>10.3±1.1</td>
</tr>
<tr>
<td>Acute lymphocytic leukemia</td>
<td>12.2±5.0</td>
</tr>
</tbody>
</table>

tained from intact leukocytes in autologous plasma with measurements made in synthetic media or using cell homogenates.

The data presented in this paper describe the essential features of energy metabolism of intact human lymphocytes from acute and chronic leukemia. Under aerobic (physiologic) conditions, lymphocytes derive their energy virtually exclusively from respiration, not from aerobic glycolysis. The small degree of aerobic glycolysis demonstrated in some of the studies was attributed to granulocytic contamination, according to calculations based on the percentage of contaminating granulocytes and their known glycolytic quotients. The lack of aerobic glycolysis of intact lymphocytes in chronic lymphocytic leukemia was also noted for lymphoblasts taken from patients with acute lymphoblastic leukemia—cells which also obtain energy primarily from respiration. Both types of lymphocytic cells have equally high rates of anaerobic glycolysis, demonstrating a powerful restraint of glycolysis by the presence of oxygen (Pasteur effect).

Perhaps the most remarkable feature of lymphocyte metabolism is that lymphocytes from acute and chronic leukemia (as well as normal lymphocytes), which ordinarily derive their energy from respiration, can completely compensate for this loss of oxidative phosphorylation by the high rate of glycolysis found in an anaerobic atmosphere. These cells maintain approximately the same ADP-ATP levels under anaerobic as under aerobic conditions. In glucose-free plasma, lymphocytes continue to oxidize endogenous substrates (amino acids, respiratory cycle intermediates, etc.) so that there is little change in ADP-ATP. In contrast to granulocytes, lymphocytes do not increase O2 uptake via the hexose-monophosphate shunt.18 Leukemic lymphocytes, including the most primitive blast cells, retain this cyanide and azide-insensitive respiration.

Attempts were made to induce aerobic glycolysis of chronic lymphocytic leukemia cells by isolating them and incubating them with the plasma taken from patients having other forms of leukemia or with normal plasma. In some of these studies aerobic glycolysis was produced in association with a concomitant decrease in QO2—again showing the close interrelationship of these processes. Luganova has reported induction of aerobic glycolysis of lymphocytic cells by plasma taken from patients with granulocytic leukemia.15 The nature of these plasma factors responsible for the induction of aerobic glycolysis and the associated decrease in QO2 (Crabtree effect) is unknown. It is known

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that plasma isoantibodies react with leukemic lymphocyte antigens to cause cellular damage, which could result in these metabolic alterations. The Crabtree effect has also been produced by corticosteroids and by normal plasma. It is clear that to study leukocytes in the absence of autologous plasma can greatly distort cellular metabolism.

**Granulocytes**

Many problems complicate the understanding of the metabolic properties of leukemic granulocytes, as pointed out in Seelich's review article. A general consensus prevails in the literature that granulocytes from chronic granulocytic leukemia have a high rate of aerobic glycolysis compared with respiration. No such agreement exists, however, concerning the aerobic glycolysis of immature granulocytes or myeloblasts. An earlier publication by Kempner recorded a case of acute granulocytic leukemia (myeloblastic) in which no aerobic glycolysis was found, despite a large anaerobic glycolysis. Kempner recognized the importance of studying a homogeneous population of cells to avoid the metabolic contribution of extraneous cell types and the necessity of providing adequate glucose and buffering when working with high cell concentrations, so that glycolysis would not be completed by the time manometric measurements began. Nevertheless, his study could not avoid the fundamental problem of morphologic classification of acute leukemias; hence the possibility exists that he studied a case of acute lymphoblastic leukemia. Since
Fig. 5.—Glycolysis of leukemic granulocytes (CGL) aerobically and anaerobically. There was no acid formation in the absence of glucose.

Fig. 6.—Respiration of leukemic granulocytes (CGL) in the presence and absence of glucose. High energy phosphate (ADP-ATP) was determined at the end of a 3-hour incubation. This experiment was part of the experiment depicted in Figure 5.

Morphologic criteria are the primary reference points, this problem is intrinsic to all investigation involving “blastic” forms of leukemia (including the present study).

The most definitive previous studies on leukocyte metabolism are those of Luganova and Seits,8,15 who found a high aerobic glycolysis of myeloblastic cells approaching the anaerobic rate. This incomplete Pasteur effect was also present in chronic granulocytic leukemic cells. These findings are in agreement with the data presented in this report and previously.4
The energy-yielding processes were different for the granulocytic leukemias compared with lymphocytic leukemias, as reflected by a higher rate of aerobic glycolysis, lower respiration, and a much larger $\frac{Q_{o_2}}{Q_{o_2}^n}$ value for granulocytic leukemia cells. Anaerobic glycolysis ($Q_{o_2}^n$) was approximately the same for both types of acute and chronic leukemias.

Granulocytic leukemic cells also differed from lymphocytic cells in the
metabolic response to glucose. Granulocytic cells from acute and chronic leukemias obtain a large portion of energy via the Embden-Myerhof pathway, fermenting glucose to lactic acid aerobically. In the absence of glucose these cells nearly doubled the respiratory rate to keep the ADP-ATP content unchanged. Lymphocytic cells lack significant aerobic glycolysis and fail to increase respiration when glucose is absent. Both types of leukemic cells adapt to anaerobic conditions by increasing anaerobic glycolysis to the extent that ADP-ATP remains undiminished. If anaerobiosis is imposed upon cells in glucose-free plasma, however, the ADP-ATP falls to zero by the end of 3 hours. 2-Deoxy-D-glucose caused the same changes as absence of glucose from the medium. A rise in QO₂ was accompanied by a fall in Qₜ in granulocytic cells, not in lymphocytic cells. Addition of inhibitors, such as azide and cyanide, simulated anaerobiosis. Similar to the data obtained for lymphocytes, there remained some O₂ uptake by mature granulocytes and myeloblasts which was insensitive to inhibition by these agents.

Granulocytes from chronic granulocytic leukemia retain the ability to phagocytize starch particles and polystyrene latex particles. In our studies we have shown increased oxygen uptake during phagocytosis, inhibition of phagocytosis by glucose-free plasma and 2-deoxy-D-glucose, and the lack of inhibition by anaerobiosis and azide—all metabolic characteristics described for normal leukocytes.18,20,21 Figures 7 and 8 show the results of an experiment in which leukemic granulocytes were suspended both in isologous plasma and in plasma taken from a normal subject. Normal plasma depressed Qₜ while QO₂ increased concomitantly. Although the effects of foreign plasmas were variable, it is clear that considerable distortions can be produced. With granulocytic leukemia cells, respiration and aerobic glycolysis are intimately linked; with inhibition of respiration the glycolysis increased, and with inhibition of glycol-
Table 4.—Transition of Chronic Granulocytic Leukemia into Blastic Stage of Acute Granulocytic Leukemia

| Patient | Date   | Oxygen | Oxygen | Oxygen | Oxygen | Oxygen | Oxygen | Oxygen | ATP-1 | ATP-1 | ATP-1 | ATP-1 | WBC | Blasts (%) |
|---------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|------|-------------|
| 1       | 2/3/61 | 2.0    | 12.5   | 18.7   | 3.5    | 1.17   | 22     | 1.40   | 6.0    | .60    | 5.2    | .30    | 215.0  | 100  |
|         | 3/6/61 | 1.3    | 6.0    | 13.8   | 3.5    | 1.17   | 22     | 1.40   | 6.0    | .60    | 5.2    | .30    | 215.0  | 100  |
|         | 4/3/61 | 2.2    | 7.4    | 10.1   | 3.5    | 1.17   | 22     | 1.40   | 6.0    | .60    | 5.2    | .30    | 215.0  | 100  |
|         | 7/19/61| 5.5    | 2.0    | 1.1    | 3.5    | 1.17   | 22     | 1.40   | 6.0    | .60    | 5.2    | .30    | 215.0  | 100  |
|         | 7/20/61| 6.5    | 1.0    | .5     | 3.5    | 1.17   | 22     | 1.40   | 6.0    | .60    | 5.2    | .30    | 215.0  | 100  |
|         | 7/21/61| 5.0    | 2.8    | 1.7    | 3.5    | 1.17   | 22     | 1.40   | 6.0    | .60    | 5.2    | .30    | 215.0  | 100  |
| 2       | 11/10/60| 3.0    | 16     | 16.0   | 2.34   | 35     | 1.88   |        |        |        |        |        | 12.5   | 0    |
|         | 9/19/61| 1.5    | 5.5    | 11.6   | 1.53   |        |        |        |        |        |        |        | 174.0  | 75   |
|         | 9/20/61| 3.2    | 9.0    | 7.4    | 1.17   |        |        |        |        |        |        |        | 183.0  | 78   |
|         | 10/4/61| 1.0    | 9.8    | 29.3   | 2.34   | 22     | 2.04   |        |        |        |        |        | 15.3   | 70   |
|         | 10/10/61| 2.6    | 6.0    | 6.9    | 1.31   |        |        |        |        |        |        |        | 14.0   | 80   |
| 3       | 9/7/60 | 2.0    | 6.0    | 9.0    |        |        |        |        |        |        |        |        | 64.5   | 0    |
|         | 10/19/60| 2.1    | 10.7   | 15.3   |        |        |        |        |        |        |        |        | 26.0   | 52   |
|         | 10/20/60| 2.0    | 9.8    | 14.7   | 3.50   |        |        |        |        |        |        |        | 29.0   | 54   |
|         | 10/28/60| 3.0    | 10.0   | 10.0   | 3.50   |        |        |        |        |        |        |        | 30.0   | 49   |
ysis the respiration increased. Although lymphocytes also show the former interrelationship they never increase respiration with inhibition of glycolysis. McKinney \(^{22}\) first reported the interaction of respiration and glycolysis in normal leukocytes; it has since been described for leukemic cells by Luganova et al.\(^{15}\) The latter authors pointed out that this interdependence occurs only in cells which demonstrate aerobic glycolysis, such as granulocytes and cancer cells (excluding lymphocytic leukemia cells), and our findings substantiate their hypothesis.

Transition of chronic granulocytic leukemia to the blastic phase of acute granulocytic leukemia was documented in three cases. One of these patients showed a striking increase in $\mathit{QO}_2$ and fall in $\mathit{Q}^\mathit{W}_2$ after the transition; in another, a moderate fall in $\mathit{Q}^\mathit{A}_2$ occurred, and the third showed no appreciable change in metabolism. Although we believe these sequential observations to be of importance, the data are unfortunately too limited to propose any generalizations. Myeloblasts from acute granulocytic leukemia were similar in metabolic properties to more mature counterparts except for somewhat greater $\mathit{QO}_2$ and $\mathit{Q}^\mathit{A}_2$.\(^*\) Despite considerable variation in $\mathit{Q}^\mathit{A}_2$ from case to case, only one exception occurred to the rule of high aerobic glycolysis—a 12-year-old child with myeloblastic leukemia in crisis from whom blood was drawn for study within 24 hours of death. The presence of Auer rods within the cells unquestionably established the diagnosis. The lack of aerobic glycolysis in this exceptional case resembles that of the myeloblastic case reported by Kempner\(^{12}\) and parallels the metabolic pattern of our acute lymphocytic leukemia studies.

We have had the opportunity to perform metabolic studies repeatedly on some patients and to observe them clinically, as well as to view changes in cell morphology. In some instances, patients with undifferentiated "stem-cell" leukemia showed a metabolic response categorized as granulocytic in type. Later in their course, these patients developed unmistakable evidence of acute granulocytic leukemia as manifest by Auer rods and promyelocytes. Another patient with stem-cell leukemia metabolically resembling the lymphoid pattern had been treated for lymphosarcoma previously; the presumptive clinical evidence indicated a transition of a lymphocytic lymphoma into an acute lymphocytic leukemia. Such metabolic classification of leukemic types appear to be highly reliable in the hands of experienced investigators. Since the characteristics of lymphocytes and granulocytes are retained even by the most primitive blast cells, only rare instances of incorrect classification (see above) would be expected by this method. We also noted that chemotherapy with thiopurine or mustard compounds did not grossly alter cellular metabolism unless the drug was incubated in vitro with the leukemic cells.

It should be mentioned that when the leukocyte counts are extremely high, there is a danger of exhausting the nutrients or of overwhelming the buffer.

\(^*\) Even these differences may be more apparent than real when taking into account that cellular dry weight includes platelets as well as leukocytes. Chronic granulocytic leukemia is often accompanied by marked increase in platelet mass leading to an overestimation of granulocyte weight. This error would not influence the ratio of glycolysis to respiration which is the same for acute and chronic granulocytic leukemia.
In several other experiments it was noted that by the time the cells had been prepared and equilibrated on the manometer bath, there was no further aerobic or anaerobic glycolysis—but it could be restored by providing additional quantities of glucose and buffer. We believe that this may account for some instances in the literature in which no glycolysis was demonstrated in granulocytic leukemias having very high leukocyte counts. In order to obviate this problem, Seits, in his studies, had diluted each preparation to 30,000 cells/mm	extsuperscript{3}; we preferred to perform the present studies using cell concentrations as they were isolated, since these were very close to cell counts existing within the patient. This probably accounts for some of the variability between experiments, although several experiments performed using cell dilutions showed nearly linear metabolic rates between 30,000-150,000 cells/mm	extsuperscript{3}, provided adequate nutrients and buffering were used.

The present studies on energy metabolism of leukemic cells confirm and extend those previously reported. We now have a clear picture of the differences in metabolic processes of leukemic cell types and of the remarkably efficient homeostatic mechanisms which protect these cells from glucose starvation or anaerobiosis. It is also clear that the major differences exist between lymphocytic and granulocytic cells rather than between primitive or mature cells. Our studies, in addition to the work of others, indicate that normal human lymphocytes and granulocytes have a metabolism so similar to that of leukemic counterparts that study of energy metabolism offers no discernible clue to the nature of the leukemic process. It is still possible that differences exist to metabolic inhibitors such as anti-insulin substances (e.g., steroids), and that a small but critical overconsumption of glucose is vital to the leukemic process. We are currently engaged in investigating the relationship between energy metabolism and nucleic acid synthesis to determine whether the adaptive mechanism (e.g., anaerobiosis) is mediated by messenger RNA synthesis coding for glycolytic enzymes.

**SUMMARY**

1. Leukocytes taken from patients having acute lymphocytic leukemia and chronic lymphocytic leukemia are characterized by high respiratory rates and low to absent aerobic glycolysis. Leukemic granulocytes have low respiratory rates and high aerobic glycolysis.
2. Lymphocytes and granulocytes have the capacity for high glycolytic rates under anaerobic conditions.
3. Lymphocyte respiration is independent of glucose concentration in contrast to granulocyte respiration.
4. High energy phosphate levels of lymphocytes and granulocytes are unchanged if these cells are incubated aerobically, either with or without glucose, or anaerobically in the presence of glucose.
5. Aerobic glycolysis can be induced in lymphocytes by the addition of foreign plasma. Foreign plasma may also alter granulocyte metabolism.

**SUMMARIO IN INTERLINGUA**

1. Leucocytos ab patientes con acute leucemia lymphocytic o con chronic leucemia lymphocytic es characterisate per un forte respiration e un debile o absente glycolyse aerobie. Granulocytos leucemic ha un forte respiration e un debile glycolyse aerobie.
2. Lymphocytes e granulocytes ha le capacitate de un forte glycolyse sub conditiones anaeribie.
3. Le respiration de lymphocytes non depende del concentration de glucosa, per contrasto con le respiration del granulocytes.
4. Le nivellos phosphatic a alte energia in lymphocytes e granulocytes non es alterate quando iste cellulas es incubate aerobiemente con o sin glucosa o anaerobiemente in le presentia de glucosa.
5. Glycolyse aerobie pote esser inducite in lymphocytes per le addition de plasma alien. Plasma alien es etiam capace a alterar le metabolismo del granulocytes.

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Energy Metabolism of Human Leukemic Lymphocytes and Granulocytes

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