Alpha-Glycerophosphate and Lactic Dehydrogenases of Hematopoietic Cells from Leukemic Mice

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SUMMARY

Bone marrow cells, lymphocytes, lymph nodes, solid lymphoid tumors, spleen and ascites cells from mice implanted with leukemia have markedly decreased α-glycerophosphate and increased lactic dehydrogenase activities. A striking parallel between the ratio of these dehydrogenases and the progression of the leukemia was found. These data, in conjunction with related findings, suggest that the α-glycerophosphate cycle, in contradistinction to its probable role in normal cells, may not mediate a significant portion of the hydrogen transport in leukemic cells.

In glycolysis, DPNH formed by glyceraldehyde phosphate dehydrogenase is reoxidized by the lactic or glycerol-P oxidation-reduction enzyme systems, as follows:

\[ \text{DPNH} + H^+ + \text{Pyruvate} \rightleftharpoons \text{DPN} + \text{Lactate} \] (1)

\[ \text{DPNH} + H^+ + \text{DHAP} \rightleftharpoons \text{DPX} + \text{Glycerol-P} \] (2)

Reaction (2) combined with the direct mitochondrial oxidation of glycerol-P

\[ \text{Glycerol-P} + \frac{1}{2}O_2 \rightarrow \text{DHAP} + H_2O \] (3)

constitute the glycerol-P cycle in which hydrogen from the extramitochondrial pool of DPNH is made available to the respiratory chain (2, 4, 7, 11). This cycle, first described from insect flight muscle and later from mammalian brain (6, 9), is also active in hematopoietic tissues such as bone marrow, spleen, and isolated leukocytes. These findings suggest that in hematopoietic cells a significant share of DPNH is reoxidized by the glycerol-P cycle. The question arises, however, as to the importance of the glycerol-P system relative to the lactate pathway in normal and malignant hematopoietic cells. The excessive production of lactate by many malignant tissues is well known. Furthermore, in a lymphosarcoma a high lactate and low level of glycerol-P has been reported (2).

Thus, to gain a better understanding of the functional interrelationships of these competing hydrogen transport systems we compared their activities in normal and malignant hematopoietic cells. In this paper we report the activities of the extramitochondrial soluble lactic and glycerol-P dehydrogenases from normal and leukemic mice.

MATERIALS AND METHODS

The acute lymphatic leukemia, B82, and its tumor form, B82T, carried in C58 mice or F1 hybrids thereof were kindly supplied by Dr. Joseph H. Burchenal of the Sloan-Kettering Institute. In initial studies, Leukemia L1210 in DBA/2 mice or in F1 hybrids were generously given by Dr. Abraham Goldin, National Institutes of Health. In subsequent experiments, L1210 was transplanted by intraperitoneal injection of ascites fluid from a stock originally supplied by Dr. Burchenal. Lymphoma Nos. 1 and 2 were obtained through the courtesy of Dr. G. Burroughs Mider, National Institutes of Health.

Marrow cells were aspirated from the femur, tibia, and humerus. Isolated lymphocytes were obtained by teasing excised mesenteric, retroperitoneal, and popliteal lymph nodes. Homogenates of these nodes, spleen, and solid tumors were centrifuged for 10 minutes at 40,000 \( \times \) g. The residues were resuspended in 4 per cent polyvinyl pyrrolidone containing 0.05 M K phosphate buffer, pH 7.4, and centrifuged as before. The combined supernatants or dilutions thereof were used in the enzyme assays.

Lactic and glycerol-P dehydrogenase activities

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1 The following abbreviations are used: DPNH and DPN for reduced and oxidized diphosphopyridine nucleotide; glycerol-P for α-glycerophosphate; DHAP for dihydroxyacetone phosphate.
were determined by measuring the initial rates of DPNH oxidation at 340 m|\mu|. The lactic dehydrogenase reaction system contained: 0.1 ml. of 0.003 M DPNH; 1.0 ml. of 0.1 M Na phosphate buffer, pH 7.4; 0.1 ml. of 0.01 M pyruvate; 0.1 ml. of diluted enzyme preparation; and water to 8.0 ml. The glycerol-P reaction system contained: 0.1 ml. of 0.003 M DPNH; 1.0 ml. of 0.1 M triethanolamine buffer, pH 7.5; 0.1 ml. of 0.011 M DHAP; 0.1 or 0.2 ml. enzyme preparation; and water to 3.0 ml. The rates of endogenous oxidation of DPNH by the tissue preparations used in this study were negligible.

**RESULTS**

In Table 1, glycerol-P and lactic dehydrogenase activities of isolated lymphocytes and lymph nodes from normal and leukemic mice are compared. Nonmalignant lymphoid tissues had appreciable glycerol-P dehydrogenase activity. The ratios of the lactic to glycerol-P enzymes were less than 10. This relationship between the two dehydrogenases changed remarkably in leukemic preparations. The specific activity of glycerol-P dehydrogenase decreased 85 per cent. On the other hand, lactic dehydrogenase increased over twofold. Thus, the ratios of the competing enzymes in the leukemic cells reached values of 100, more than 10 times their normal value.

**TABLE 1**

<table>
<thead>
<tr>
<th>Preparation</th>
<th>Lactic (amoles DPNH/ml/min/mg protein)</th>
<th>Glycerol-P (amoles DPNH/ml/min/mg protein)</th>
<th>Ratio of LAD:G-PD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isolated lymphocytes:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>0.66</td>
<td>0.083</td>
<td>8</td>
</tr>
<tr>
<td>Leukemia B82</td>
<td>1.43</td>
<td>0.014</td>
<td>100</td>
</tr>
<tr>
<td>Lymph nodes:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>1.51</td>
<td>0.167</td>
<td>9</td>
</tr>
<tr>
<td>Leukemia B82</td>
<td>2.58</td>
<td>0.085</td>
<td>95</td>
</tr>
</tbody>
</table>

Lymph nodes from leukemic mice were obtained 14 days after implantation of B82. The nodes, spleen, and liver of these animals were considerably enlarged. Histological sections of the nodes showed that much of the cytological architecture had been lost. Sinuses and primary and secondary follicles were obliterated with infiltrating leukemic lymphocytic cells. Experimental procedures are described in the text.

As shown in Table 2, analogous changes in the relative activities of the dehydrogenases were found in bone marrow. Glycerol-P and lactic dehydrogenase activities in the normal marrow cell were approximately one-third those found in the normal lymphocyte. The ratios of the two enzymes from these nonmalignant hematopoietic cells were essentially the same. This ratio increased from about 10 in the normal marrow to 45 in the marrow infiltrated with leukemic cells. Lactic dehydrogenase activity increased several-fold, whereas glycerol-P dehydrogenase activity decreased. For comparison, values for the leukemia L1210 ascites cell are included in Table 2. Enzymic activities of the ascites cell were somewhat lower than those of the leukemic marrow cell, but the ratios were similar.

**TABLE 2**

<table>
<thead>
<tr>
<th>Preparation</th>
<th>Lactic (amoles DPNH/ml/min/10^6 cells)</th>
<th>Glycerol-P (amoles DPNH/ml/min/10^6 cells)</th>
<th>Ratio of LAD:G-PD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bone marrow:</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Normal</td>
<td>8.50</td>
<td>0.80</td>
<td>11</td>
</tr>
<tr>
<td>Leukemia L1210</td>
<td>25.80</td>
<td>0.53</td>
<td>45</td>
</tr>
<tr>
<td>Ascites:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leukemia L1210</td>
<td>9.08</td>
<td>0.19</td>
<td>48</td>
</tr>
</tbody>
</table>

Bone marrow cells were suspended in either Krebs-Ringer phosphate or 5 per cent polyvinyl pyrrolidone containing 0.05 M Na phosphate buffer, pH 7.4. Cells were obtained from 7 to 10 days after transplantation. A representative differential count of marrow cells from normal mice was, in per cent: stem cell, 8; granulocytic series, 48; lymphocytic series, 0; and erythrocytic series, 44. A representative differential count of marrow cells from leukemic mice was, for these cell types: 8; 6; 84; and 2, respectively. Ascites cells were harvested from 7 to 11 days after transplantation. Experimental procedures are described in the text.

**TABLE 3**

<table>
<thead>
<tr>
<th>Tumor</th>
<th>Lactic (amoles DPNH/ml/min/mg protein)</th>
<th>Glycerol-P (amoles DPNH/ml/min/mg protein)</th>
<th>Ratio of LAD:G-PD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leukemia B82T</td>
<td>2.95</td>
<td>0.023</td>
<td>128</td>
</tr>
<tr>
<td>Lymphoma No. 2</td>
<td>2.55</td>
<td>0.020</td>
<td>98</td>
</tr>
<tr>
<td>Lymphoma No. 1</td>
<td>3.48</td>
<td>0.004</td>
<td>870</td>
</tr>
</tbody>
</table>

The dehydrogenase activities of solid lymphoid tumors are shown in Table 3. In activity, the enzymes resemble those found in lymph nodes infiltrated with leukemic cells. The highest specific activities for lactic dehydrogenase were found in these tumors. Also, the ratio of the dehydrogenases was high, especially in Lymphoma No. 1 where a value of 870 was obtained. In this tumor, glycerol-P dehydrogenase was barely detectable. The dehydrogenase activities of spleen are
shown in Table 4. In the normal animal, lactic dehydrogenase of spleen had half the activity, per mg. protein, as compared with that in lymph nodes. Glycerol-P dehydrogenase was less active. Although the lactic to glycerol-P dehydrogenase ratio in the nonmalignant spleen was relatively high, the ratio in spleen infiltrated with leukemic cells increased several-fold and attained values well above 100. As with the other hematopoietic tissues, these elevated levels resulted from increases in lactic and decreases in glycerol-P dehydrogenase activities. Some heterogeneity in degree of infiltration was noted in the different leukemias. This was reflected in variation of enzymic activity. It is perhaps noteworthy that the higher ratios were correlated with the acute leukemias, L1210 and B82.

### Table 4

**Glycerol-P and Lactic Dehydrogenase Activities of Spleen**

<table>
<thead>
<tr>
<th>Source</th>
<th>Lactic (µmoles DPNH/ml/min/mg protein)</th>
<th>Glycerol-P (µmoles DPNH/ml/min/mg protein)</th>
<th>Ratio of ( \text{LAD-GPD} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>0.72</td>
<td>0.018</td>
<td>40</td>
</tr>
<tr>
<td>Leukemia B82</td>
<td>1.55</td>
<td>0.012</td>
<td>129</td>
</tr>
<tr>
<td>Leukemia L1210</td>
<td>1.59</td>
<td>0.010</td>
<td>159</td>
</tr>
<tr>
<td>Lymphoma No. 1</td>
<td>0.98</td>
<td>0.009</td>
<td>109</td>
</tr>
</tbody>
</table>

Experimental details are described in the text.

The course of the leukemia, from time of implantation to death of the animals, was monitored hematologically and by measurements of dehydrogenase activities. Leukocyte counts of the peripheral blood and glycerol-P dehydrogenase activities of spleen are described in Chart 1. The white blood cells increased very slowly, from about 4,500 to 6,000 per cu. mm., during the first 4 days after the implant. Thereafter, the counts advanced sharply, increasing from 8,000 on the 5th day to 25,000 on the 7th day. By the 8th day, immediately prior to death, leukocyte counts were over 60,000. Coinciding with these hematological events were changes in glycerol-P dehydrogenase. During the initial phase, the dehydrogenase activity declined slowly. When the leukocyte count mounted rapidly, glycerol-P dehydrogenase activity fell very fast, decreasing by the 8th day to one-fourth that of normal spleen.

Concurrent measurements of lactic dehydrogenase are shown in Chart 2. The activity climbed continuously as the leukemia progressed, increasing from 0.45 µmoles DPNH/ml/min/mg protein to 1.5 just before death.

The striking parallel between these enzymic activities and the progression of the leukemia is readily seen by comparing the ratios of lactic to glycerol-P dehydrogenase with the white blood cell counts, as plotted in Chart 3. In the beginning of the disease, as the number of leukocytes gradually increased, the ratio increased commensurably from 33 to 50. The cell count and the dehydrogenase ratio mounted markedly and simultaneously in the advanced stage of the leukemia. In a terminal condition, the ratio in spleen was 225, approximately 7 times greater than that of normal spleen.

Changes in the progression of the leukemia brought about by chemotherapeutic agents, such as amethopterin, are reflected in the activities of both dehydrogenases. On the 7th day after implantation, when the white blood cell count was 25,000, amethopterin (10 mg/kg) was injected subcutaneously in a group of mice. By the next day, the leukocyte counts of untreated mice had climbed to 60,000, and the animals succumbed soon thereafter. In contrast, the counts of those receiving the drug fell to 18,000 on the 8th day, remained at this level on the 9th day, but on the 10th day, prior to death, the counts rose again, reaching values of 65,000. As shown in Chart 1, glycerol-P dehydrogenase activity, which was relatively low at the time of treatment, did not fall further, as did that of the controls. Instead, on the...
9th day, activity almost doubled. Then, coinciding with the sharp increase in leukocytes on the 10th day, the enzymic activity dropped to its lowest level.

Lactic dehydrogenase responded analogously to the effects of amethopterin (Chart 2). On the 1st day after treatment, the enzyme's activity remained at the level of the 7th day rather than increasing, as did that of the untreated mice. Two days later, on the 9th day, lactic dehydrogenase activity decreased to half this level. On the 10th day, concurrent with the elevated white blood cell counts, the enzyme increased to values characteristic of the terminal condition.

The ratios of the two dehydrogenases following administration of the folic acid antagonist is reported in Chart 3. Although it is likely that treatment with amethopterin brought about a shift in cell population to a preponderance of normal cells, these data provide additional evidence suggesting a correspondence between the ratio of the dehydrogenases and the status of the leukemia.

**DISCUSSION**

DPNH, formed during glycolysis in the extramitochondrial portion of the cell, is not oxidized directly by mitochondria (5, 8). Instead, reducing equivalents pass through the cytoplasmic-mitochondrial barrier by oxidation-reduction systems, such as the glycerol-P pathway, described in reactions (2) and (3). Therefore, the appreciable rates of DPNH oxidation with concomitant formation of glycerol-P by a cytoplasmic enzyme from hematopoietic tissues as reported here, plus an unusually high rate of glycerol-P oxidation by mitochondria as described elsewhere, suggest that the glycerol-P cycle is of prime importance in hematopoietic tissues. Thus, our findings that glycerol-P dehydrogenase activity is remarkably curtailed in leukemic cells must be viewed with added significance.

These enzymic studies also provide additional meaning to the recent observations of Boxer and Shonk (1) and Delbruck et al. (3), which were published after our experiments were completed, showing low levels of glycerol-P dehydrogenase in a variety of transplantable tumors. Besides this lowered production of glycerol-P, we have found that mitochondria from hematopoietic tissues infiltrated with leukemic cells have a decreased rate of glycerol-P oxidation, whereas their rates of oxidation of citric acid cycle substrates are not different from those of normal mitochondria. These data provide evidence suggesting that the glycerol-P cycle may not mediate a significant portion of the hydrogen transport in leukemic cells.

* B. Sacktor, manuscript in preparation.
Cytoplasmic lactic dehydrogenase also catalyzes the oxidation of DPNH. However, in contrast to the glycerol-P pathway, hydrogen from the reduced coenzyme remains in the extramitochondrial compartment of the cell and accumulates in lactate. Our findings of increased lactic dehydrogenase activity in leukemic cells in conjunction with the marked decreases in the activity of the competing glycerol-P dehydrogenase may contribute, in part, to the excessive production of lactate typical of malignant cells.

The question arises, however, as to the relevance of these observations with experimental transplantable leukemias in animals to biochemical events in spontaneous leukemic cases in the clinic. Wagner’s report (10) of high concentrations of glycerol-P in human white blood cells suggests an analogy. Further, in preliminary studies, Sacktor, Ellison, and Burchenal have found this identical pattern of decreased glycerol-P dehydrogenase and increased lactic dehydrogenase activities in isolated leukocytes of leukemic patients. In fact, in a case of acute leukemia in a child, glycerol-P dehydrogenase activity was at the limit of detection, and the ratio of lactic to glycerol-P dehydrogenase was over 1,000. These clinical studies are continuing and will be reported in greater detail elsewhere.

These studies demonstrate a striking parallel between the ratio of the dehydrogenases and the progression of the leukemia. On this basis,plus the finding of a decreased oxidation of glycerol-P by mitochondria from leukemic tissues as well as the previously reported incongruous distribution of metabolites in leukemic cells, we are inclined to propose the hypothesis that in leukemic cells the complex of hydrogen transport systems may be balanced differently from that characterizing normal hematopoietic cells. This proposition can be approached experimentally by a variety of technics. Such studies will lead eventually to a better understanding of the functional interrelationships of the hydrogen transport mechanisms in normal and malignant cells.

REFERENCES

B. Sacktor, R. R. Ellison, and J. H. Burchenal, unpublished data.
Alpha-Glycerophosphate and Lactic Dehydrogenases of Hematopoietic Cells from Leukemic Mice

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