The Aerobic Carbohydrate Metabolism of Leukocytes in Health and Leukemia

II. The Effect of Various Substrates and Coenzymes on Glycolysis and Respiration*

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In the previous paper (1), data were presented on the aerobic carbohydrate metabolism of homogenates of leukocytes isolated from normal (N), chronic myelocytic leukemic (CML), and chronic lymphatic leukemic (CLL) bloods. It was shown that with glucose and hexose diphosphate (HDP) as substrates, oxygen consumption, glucose utilization, and lactic acid production were significantly higher in N than in CML and CLL homogenates.

There have been few reports on the effect of varying substrate and coenzyme conditions in leukocyte material. Maninelarena (5) reported the respiratory and glycolytic activities of guinea pig exudate leukocytes with various substrates and inhibitors. Using intact, washed cells suspended in Ringer's solution and conventional manometric technic, he typically found an O₂ consumption around 0.46 μl/10⁶ cells/hr and glycolytic rate of 0.89 μl CO₂/10⁶ cells/hr. Homologous serum benefited respiration. Added glucose, hexose diphosphate, and fructose increased glycolysis; succinate increased respiration. Glycolysis was decreased by fluoride and iodoacetate. KCN and malonate decreased respiration. Reports of various authors have shown that leukocyte respiration is decreased by saponin (7), potassium arsenite (12), and thiouracil (11) and is stimulated by menadione (10) and ascorbic acid (6).

This paper will report the effects on O₂ consumption, glucose utilization, and lactic acid production of diversification of added substrates and cofactors. The results show additional biochemical differences between normal and leukemic leukocytes and further support the view that classification of tissues on the basis of in vitro metabolic rates requires qualifying statements concerning the incubation medium employed.

MATERIALS AND METHODS

Materials and methods have been described in detail elsewhere (1). The basic incubation system, referred to below as the "complete" system, was as follows (final concentrations in parentheses): KH₂PO₄-KHPO₄ buffer, pH 7.4 (0.01 is), ATP-Na₁ (0.0011 is), DPN⁻ (0.0007 is), cytochrome c³ (4 × 10⁻⁴ is), glucose (0.0053 is), HDP (0.0058 is), MgCl₂ (0.0038 is), and H₂O q.s. 2.8 ml. Tissue aliquot was usually 0.5 ml.

Pyruvate⁴ and succinate⁵ were used as substrates in several experiments. Pyruvate was made by dilution and neutralization of redistilled pyruvic acid. Solutions of sodium succinate were made up directly. Both were kept in 0.01 is stock solutions. 0.8-M solutions additions to incubations contained 20 μM and gave final concentrations of 0.01 is.

RESULTS

Effect on different substrates.—Typical experiments showing the effects of different substrate conditions on oxygen consumption, glucose utilization, and lactic acid production in N, CML, and CLL leukocyte homogenates are shown in Table 1.

If the activity of the "complete" system (containing as substrates 15 μM each of glucose and HDP) is assigned a comparative value of 100, the effects of different substrates can be expressed as percentages of the "complete" system. Charts 1, 2, and 3 show these results graphically, indicating the several substrate combinations and the comparisons with the glucose–HDP system. Results shown are the means of four to six experiments.

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On a percentage basis, variations in substrates affected \( O_2 \) consumption of N homogenates more profoundly than of CML and CLL material. The differences shown in Chart 1 between N and CML and N and CLL are statistically significant in the incubations containing succinate (\( P = 0.01 - 0.001 \)). Differences between CML and CLL in the succinate-containing systems are probably not significant (\( P = 0.1 - 0.2 \)), except in the pyruvate+succinate system where the CML vs. CLL \( P \) value is 0.001. In the presence of glucose, HDP, or pyruvate or all three, respiration remained unaltered. The decrease in \( O_2 \) uptake on omission of glucose and HDP was especially pronounced in the N material. The striking finding is the relatively greater respiratory stimulation of succinate in the normal and the insensitivity of leukemic homogenate respiration to substrate variation.

Percentile variation of lactic acid production under varying substrate conditions was similar in N, CML, and CLL, all differences among the three being statistically insignificant. Endogenous lactic acid production in the absence of added substrate is low in all three tissues, indicating that "aerobic glycolytic rates" depend strictly on availability of substrate. The presence of glucose or HDP alone yields almost as much lactic acid as both together. These points are illustrated in Table 2, where calculations are shown of the ratio lactic acid production/\( O_2 \) consumption (both activities expressed in terms of glucose equivalents)\(^5\) with glucose and HDP present and with no added substrates present. It is seen that the preponderance of aerobic glycolysis over respiration is directly related to the availability of glycolyzable substrate. Note (a) the similarity of the ratios of N and CML in the presence of substrate, both being twice CLL and (b) the disparity of the ratios in the absence of added substrate, that of CML being higher than N and CLL.

When the substrate is pyruvate (in excess) and with DPN present, little lactic acid is formed unless glucose is added, probably indicating the need for some reaction sequence which will convert DPN to DPNH\(_2\), the necessary coenzyme for the transformation of pyruvic to lactic acid. This point is receiving further study.

Glucose disappearance diminished in the absence of HDP, indicating the probable role of HDP as a primer of phosphorylation. The percentile effect of HDP omission was about the same in normal and leukemic homogenates. Even with HDP present, added succinate decreased the disappearance of glucose, suggesting some connection between increased oxidation due to succinate and the initial phosphorylation of glucose, possibly via the adenylic system. We cannot explain why glucose utilization is lower when succinate alone is added to glucose+HDP than it is when succinate and pyruvate are added.

Effect of variation in added ATP, DPN, and cytochrome c.—Comparisons were made of metabolic activity in the absence of added ATP, DPN, and cytochrome c and in the presence of these substances, alone and in various combinations. Reduced DPN\(^6\) (DPNH\(_2\)) was added in certain experiments. Typical results for N, CML, and CLL homogenates with various substrates are shown in Table 1.

### Table 1

<table>
<thead>
<tr>
<th>Additions</th>
<th>N</th>
<th>CML</th>
<th>CLL</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>( O_2 )</td>
<td>Glucose</td>
<td>Lactic</td>
</tr>
<tr>
<td>None</td>
<td>0.22</td>
<td>0.31</td>
<td>0.08</td>
</tr>
<tr>
<td>Gluc+HDP</td>
<td>0.47</td>
<td>2.28</td>
<td>3.63</td>
</tr>
<tr>
<td>Gluc</td>
<td>0.47</td>
<td>1.79</td>
<td>2.58</td>
</tr>
<tr>
<td>HDP</td>
<td>0.44</td>
<td>*</td>
<td>3.63</td>
</tr>
<tr>
<td>Succinate</td>
<td>0.88</td>
<td>*</td>
<td>0.97</td>
</tr>
<tr>
<td>Gluc+HDP+Succ</td>
<td>0.87</td>
<td>2.13</td>
<td>3.61</td>
</tr>
<tr>
<td>Gluc+Succ</td>
<td>0.89</td>
<td>1.65</td>
<td>3.49</td>
</tr>
<tr>
<td>HDP+Succ</td>
<td>0.98</td>
<td>*</td>
<td>3.52</td>
</tr>
<tr>
<td>Pyruvate</td>
<td>0.46</td>
<td>*</td>
<td>0.80</td>
</tr>
<tr>
<td>Py+Succ</td>
<td>0.84</td>
<td>*</td>
<td>0.58</td>
</tr>
<tr>
<td>Gluc+HDP+Py</td>
<td>0.47</td>
<td>2.25</td>
<td>3.61</td>
</tr>
</tbody>
</table>

* No glucose added.

\( ^5 \) One mm glucose is equivalent to 2 mm lactic acid and 6 mm of oxygen.

\( ^6 \) Purchased from Sigma Chemical Co., St. Louis, Mo.
CLL homogenates (with glucose and HDP as substrates) are shown in Table 3.

If the activity in the "complete" system (containing 3 μM at ATP, 0.04 μM of cytochrome c, and 2 μM of DPN) is assigned a relative activity of 100 per cent, percentile effects of various deletions and additions can be shown graphically (Chart 4). Results shown are the means of four to six experiments.

It is seen that metabolic activity is markedly affected by the presence or absence of these particular cofactors. Oxygen uptake is decreased by omission of all three cofactors, the percentile effect on N being greater than on CML and CLL. The omission of DPN only or the replacement of DPN by reduced DPN causes a depression in oxygen consumption similar in degree to that following omission of DPN, ATP, and cytochrome c, in each case the N effect being greater. This would indicate that the homogenates require DPN for maximal oxygen consumption and are unable to derive it by oxidation of DPNH₂. ATP omission (but with DPN and cytochrome c present) causes a small decrease in O₂ uptake, again more striking with N homogenates. Omission of cytochrome c causes a greater lowering of O₂ consumption in the leukemic material than in the normal, producing, in fact, in CML and CLL a greater decrease than when cytochrome c, ATP, and DPN were all omitted. These results suggest that, when glycolysis is brisk, the depression of respiration due to

**TABLE 2**

<table>
<thead>
<tr>
<th>Glucose+HDP</th>
<th>N</th>
<th>CML</th>
<th>CLL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ratios computed in terms of glucose equivalents</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Additions</td>
<td>N</td>
<td>CML</td>
<td>CLL</td>
</tr>
<tr>
<td>Glucose+HDP</td>
<td>29.2</td>
<td>31.0</td>
<td>14.6</td>
</tr>
<tr>
<td>None</td>
<td>3.5</td>
<td>10.0</td>
<td>1.6</td>
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</table>
cytochrome c deficiency is relatively greater than if glycolysis is sluggish. DPNH₂ seems actually to inhibit O₂ consumption in N homogenates, since activity was 100 per cent with 1 μM of DPN present but was 23 per cent lower with 1 μM each of DPN and DPNH₂ present, a significant difference. This did not occur with CML and CLL. The results show that O₂ uptake was the same in N, CML, and CLL with 1 μM of DPN as with 2 μM. Glucose utilization requires added DPN and ATP, the requirement for DPN being proportionately the same in N, CML, and CLL and that for ATP being greater in N than in CML and CLL. Table 3 shows that if 6 μM at ATP is added instead of 3 μM, glucose utilization is less than maximal (in CML). Omission of cytochrome c had little effect on glucose utilization. Substitution of DPNH₂ for DPN lowered glucose utilization, simulating the effect of DPN omission. One μM of DPN with or without 1 μM of DPNH₂ was as effective in supporting glucose utilization as was 2 μM of DPN.

The diminution in lactic acid production after omission of ATP, DPN, and cytochrome c was greater in CML and CLL than in N. Interestingly, when all three of these cofactors are omitted, metabolic depression of N homogenate is reflected chiefly in O₂ and glucose uptake and least in lactic acid production, whereas leukemic material shows its greatest lability in lactic acid production and least in glucose utilization and oxygen consumption. DPN omission causes a large decrease in lactic acid production in CML and CLL but only a slight decrease in N. ATP and cytochrome c omission cause only slight decreases in lactic acid production. CLL reacts differently than CML in its response to DPN/DPNH₂ manipulation. Substitution of DPNH₂ for DPN lowers lactic acid production in CML and CLL (to a greater extent than in N) but addition of DPN (1 μM) or DPN (1 μM)+DPNH₂ (1 μM) depresses only CLL and not CML or N. One μM of DPN adequately supports O₂ uptake and glucose utilization in CLL but appears to be inadequate for maximal lactic acid production. This could pos-

**TABLE 3**

**EFFECT OF VARIATION IN ADDED COENZYMES ON O₂ CONSUMPTION, GLUCOSE UTILIZATION, AND LACTIC ACID PRODUCTION IN N, CML, AND CLL LEUKOCYTE HOMOGENATES**

Typical experiments. All incubations contained homogenate, 0.05 ml.; phosphate buffer, pH 7.4, 0.15 ml.; glucose, 15 μM; HDP, 15 μM; MgCl₂, 15 μM plus additions. Boldface indicates “complete” system.

Results are expressed in mM/10¹⁰ cells/hour.

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>CML</th>
<th>CLL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>O₂</td>
<td>Gluc.</td>
<td>Lactic</td>
</tr>
<tr>
<td></td>
<td>cons.</td>
<td>util.</td>
<td>prod.</td>
</tr>
<tr>
<td>DPN</td>
<td>0.07</td>
<td>0.29</td>
<td>1.76</td>
</tr>
<tr>
<td>DPNH₂</td>
<td>0.10</td>
<td>0.30</td>
<td>1.55</td>
</tr>
<tr>
<td>ATP</td>
<td>0.11</td>
<td>0.63</td>
<td>1.70</td>
</tr>
<tr>
<td></td>
<td>0.05</td>
<td>0.44</td>
<td>1.42</td>
</tr>
<tr>
<td></td>
<td>0.11</td>
<td>0.46</td>
<td>1.51</td>
</tr>
<tr>
<td></td>
<td>0.11</td>
<td>0.39</td>
<td>1.51</td>
</tr>
<tr>
<td></td>
<td>0.11</td>
<td>0.35</td>
<td>1.51</td>
</tr>
</tbody>
</table>

**CHART 4.**—Effect of variation in added ATP, DPN, and cytochrome c on O₂ consumption, glucose utilization, and lactic acid production in N, CML, and CLL leukocyte homogenates. Arbitrary activity of 100 per cent is assigned to system containing ATP 3 μM; cytochrome c, 0.04 μM; and DPN, 2 μM. Graph shows comparative activity of systems containing only additions indicated: DPN (1) and DPNH₂ (1) refer to addition of only 1 μM of these substances. Incubation conditions given in the legend for Table 3. Bar graphs show means of four to six experiments. All activities calculated in terms of 10¹⁰ cells per hour.
sibly be related to the above-described differences in the ratios of glycolysis to respiration between CLL and both CML and N, CLL showing a smaller ratio.

These results illustrate (a) the need for added ATP, DPN, and cytochrome c for maximal activity in all three tissues; (b) the striking differences in response to various deletions and additions between normal and leukemic material, the normal showing greater lability to environmental change in its O₂ and glucose consumption and the leukemic in lactic acid production; (c) the greater cytochrome c requirement of leukemic homogenates for maximal oxygen uptake and the greater DPN requirement of CLL homogenates for maximal lactic acid production.

**DISCUSSION**

The reported results indicate certain qualitative differences in metabolic behavior between normal and leukemic leukocytes. Glycolysis/respiration ratios vary significantly in the presence and absence of added substrate. CLL material shows a higher ratio of respiration to glycolysis than CML and N, although, in the presence of glucose, it still has a predominantly aerobic glycolytic metabolism. In leukemic cells, there seems to be a greater indifference to substrate, at least in the oxidative cycle, and, conversely, a markedly greater response to addition or deletion of cofactors, particularly DPN and cytochrome c. The relative effects of substrate withdrawal and succinate stimulation are notably smaller in leukemic material.

Roskelley, Mayer, Horwitt, and Salter (8), Greenstein (3), and others found with many normal tissues (but not all) much greater respiratory stimulation on adding succinate than occurred with tumor tissue, suggesting some deficiency in the tumor succinoxidase system—possibly in the effective cytochrome c level. Addition of succinate and cytochrome c caused a much greater percent increase in the O₂ consumption over that with succinate alone in malignant tissue than in normal tissue. This suggested to the authors either a deficiency of cytochrome c in tumor or some shift in the redox equilibrium so that a greater amount of the cytochrome c present is in a reduced state in tumors than in normal tissue. Actual analyses of various tissues by DuBois and Potter (2) showed decidedly lower cytochrome c levels in tumors than in most normal tissues.

Shack (9) found that O₂ uptake of hepatoma slices is limited by the cytochrome system whereas normal liver and other normal tissues had a cytochrome reserve. The results presented in Table 3 show that with glucose and HDP as substrates, omission of cytochrome c causes a greater percentile decrease in O₂ consumption in CML than in N.

The observed DPN dependence recalls a study of Wenner, Spirtes, and Weinhouse (13) in which suspensions of tumor mitochondria were found capable of a “normal” rate of pyruvate oxidation if, in addition to M⁺⁺, ATP, and cytochrome c, an adequate amount of DPN was added. Further studies on the specific role of DPN and ATP in the metabolism of leukemic leukocytes are now in progress in our laboratory.

Of interest is the reduction in lactic acid production in CLL when only 1 μM of DPN is added, and striking differences in lactic acid production between N, CML, and CLL on manipulation of added DPN and DPNH₂. In an effort to clarify these results, measurements are being made of endogenous levels of DPN and DPNH₂ and an attempt is being made to demonstrate a DPNase which could influence the apparent DPN requirement as has been shown in brain tissue (4).

**SUMMARY**

Data have been presented on the oxygen consumption, glucose utilization, and lactic acid production of homogenates of leukocytes isolated from normal (N), chronic myelocytic (CML), and chronic lymphatic leukemic (CLL) blood. Studies were made of the effect of the following substrates in varying combinations: glucose, hexose diphosphate, pyruvate, and succinate. Effects of varying added DPN, DPNH₂, ATP, and cytochrome c were also studied. Data were expressed per 10¹⁵ cells.

Although leukocytes have a predominantly aerobic glycolytic metabolism, the relative preponderance of aerobic glycolysis over respiration depends strictly on the availability of glycolyzable substrates. The glycolysis to respiration ratio is much higher when glucose is present than when it is absent. Of the three tissues, CLL homogenates have the lowest ratio of glycolysis to respiration.

Qualitative as well as quantitative differences in metabolic performance occur on manipulation of substrates. Succinate stimulates oxygen uptake to a greater extent in N than in leukemia material. With pyruvate as the substrate, and with DPN present, lactic acid production is low unless glucose is added.

Omission or manipulation of added cofactors profoundly affected metabolic rates. N, CML, and CLL homogenates required added ATP, DPN, and cytochrome c for maximal activity. Leukemic leukocytes had a greater cytochrome c requirement for maximal oxygen uptake than N cells.
CLL homogenates had a greater than normal DPN requirement for maximal lactic acid production. Normal cells showed a greater lability to cofactor manipulation in their oxygen and glucose consumption, while leukemic cells were most variable in their lactic acid production.

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