A KINETIC ANALYSIS OF THE GLYCOLYTIC RATE AND CERTAIN GLYCOLYTIC ENZYMES IN NORMAL AND LEUCEMIC LEUCOCYTES*

By WILLIAM S. BECK

(From the Atomic Energy Project and the Department of Medicine, University of California Medical Center, Los Angeles, California)

(Received for publication, January 21, 1955)

It is generally agreed that human leucocytes have high aerobic glycolytic rates (1) and previous studies (2, 3) have shown the rates in homogenates of isolated normal leucocytes to be significantly higher than in leucemic cells. The present studies seek to explain this metabolic difference in terms of possible differences between the enzymic mechanisms of glycolysis in normal and leucemic leucocytes.

Throughout the huge literature on glycolysis, it is often stated that the glycolytic rate of a tissue is determined by a single, rate-limiting step in the sequential chain of reactions although its identity is unknown. Nor is it known whether the rate-limiting step is the same in all tissues or whether it remains the same in pathological variants showing altered glycolytic rates. Some evidence has implicated the cell adenosine triphosphate-adenosine diphosphate ratio (4) as the determinant of glycolytic rate, a ratio at least partially controlled by the balance between adenosinetriphosphatase and hexokinase, but the data are inconclusive. Presumably, that ratio would influence the activity of adenylic acid-linked enzymes, one of which might then become rate-limiting. Burton (5) and Hinshelwood (6) have emphasized the possible hazards of the "master reaction" concept by pointing out that in a sequence of irreversible first order reactions

\[ A \xrightarrow{k_1} B \xrightarrow{k_2} C \]

where \( A, B, \) and \( C \) are the reactants and \( k_1 \) and \( k_2 \) are the velocity constants of the reactions, the rates of change in the concentrations of the reactants at time \( t \) may be expressed by the equations

\[ \frac{dA}{dt} = k_A A, \quad \frac{dB}{dt} = k_A A - k_B B, \quad \frac{dC}{dt} = k_B B \]

The rate of production of \( C \) thus depends upon \( k_A A \) and \( k_B B \) and not upon either alone unless one is considerably greater than the other.

* Based on work performed under contract No. AT-04-1-GEN-12 between the Atomic Energy Commission and the University of California at Los Angeles.
An enzyme could function as pace-maker in a multienzyme system for many reasons, among which are spatial location, pH or thermodynamic conditions, low apoenzyme concentration, coenzyme or activator deficiency, low coenzyme or substrate affinity or diffusibility, and presence of inhibitors. Thus, although the functional capacity, i.e. $V_{\text{max}}$, of an individually examined enzyme reaction may be demonstrably high, its actual performance as a link in a metabolic chain may be low or limiting for all or any of these reasons.

In a stimulating essay (7), Dixon wrote "it is scarcely practicable to work out the complete kinetics of a chain of enzyme reactions quantitatively" and indeed little has been done along these lines in single tissues and with systematic consideration of the above factors. Leucocytes are well suited for a study of this type because of their high aerobic glycolytic rate, low oxygen consumption, availability in "pure culture," ease of enumeration and morphological study, and variety of pathological analogues. A number of glycolytic enzymes and coenzymes have been studied, and an attempt is made here to compare them in normal and leucemic cells and to correlate their properties with those of the over-all glycolytic process as measured under apparently optimal conditions.

**Materials and Methods**

**Clinical Material**—Data were obtained on the leucocytes of forty-eight normal subjects, forty-five patients with chronic myelocytic leucemia, and thirty-five patients with chronic lymphocytic leucemia. The myelocytic leucemia patients had leucocyte counts averaging 103,000 per c.mm. with a range of 66,500 to 330,000. All differential counts showed cellular immaturity, myelocytes, metamyelocytes, and band forms predominating. The average percentage of myeloid cells was 95 per cent. The patients with lymphocytic leucemia had counts ranging from 37,750 to 430,000 per c.mm. with a mean of 123,000 and an average lymphocyte percentage of 97. Although the leucocyte populations of single bloods did contain more than one morphological form, one cell type predominated. Within the three clinical groups studied, leucocyte differential patterns were essentially similar.

**Isolation of Leucocytes and Preparation of Homogenates**—Details of these procedures have been published previously (2). Special care was used to obtain erythrocyte-free preparations. Prior to homogenization, six cell counts were done on the saline suspensions. The standard deviation of the mean of the suspension counts was computed and expressed as a percentage. The mean standard deviation of a series of 187 suspensions was found to be 8.1 per cent, and the standard deviation of this mean was 2.1 per cent.

Suspensions were homogenized for 4 minutes in ice-cold, glass McShan-
Erway homogenizers (8). Microscopically, at least 80 per cent of the cells appeared to have been disrupted. Nuclei and other subcellular components appeared intact. In testing these homogenates, it was shown that 10 minutes centrifugation at 600 × g eliminated nuclei and unbroken cells. The resulting supernatant fraction contained 60 to 80 per cent of total homogenate nitrogen. In some experiments, homogenates were prepared in the Nelco blender and the French pressure cell (9).

Reagents—Stock solutions of ATP (Pabst), DPN (90 per cent, Sigma), cytochrome c (Nutritional), and fructose 1,6-diphosphate (Schwarz) were neutralized and kept frozen. Bovine fibrinogen (Armour), glucose (Merck), sodium pyruvate (Eastman), nicotinamide (Eastman), cysteine hydrochloride (Nutritional), and DPNH (90 per cent, Sigma) were freshly prepared. Calcium salts of DHA-P, G 3-P, and α-GP (Delta) were converted to sodium salts with Amberlite IR-120 cation exchange resin. G-3-P dioxane bromide addition compound (Concord) and G-3-P and DHA-P obtained from cyclohexylammonium salts of their dialkyl acetal derivatives (the gifts of Dr. Clinton Ballou) were used in some experiments. Reagent triose phosphates were assayed enzymatically, chromatographically, and by alkali-labile phosphorus determinations.

Aldolase and G-3-PDH were purified from rabbit muscle according to Taylor, Green, and Cori (10) and Cori, Slein, and Cori (11), respectively. Isomerase was purified from rabbit muscle according to Meyerhof and Beck (12). A sample of yeast protein No. 2 (G-3-PDH) was kindly provided by Dr. Edwin Krebs.

Assay Methods—All enzymes were determined at 38°C. Aldolase was measured by a modification (13) of the procedure of Sibley and Lehninger (14). Isomerase was assayed by a method developed in this laboratory (15) and the method of Warburg and Christian (16), DPNase by the cyanide procedure of Colowick, Kaplan, and Ciotti (17), and total pyridine nucleotides in TCA extracts by the fluorometric method of Robinson, Levitas, Rosen, and Perlzweig (18), with methylnicotinamide iodide as the reference standard. Ce+++ was added to the assay system to oxidize reduced tissue pyridine nucleotides. The results are expressed as micromoles of DPN. Nitrogen was measured by the standard micro-Kjeldahl method. Details of the α-GPDH assay are given in Table II.

LDH was assayed spectrophotometrically by measuring ΔΔA420 at 30

1 The following abbreviations are used: ATP, adenosine triphosphate; DHA-P, dihydroxyacetone phosphate; DPN, diphosphopyridine nucleotide; DPNase, diphosphopyridine nucleotidase; DPNH, dihydridophosphopyridine nucleotide; α-GP, α-glycerophosphate; G-3-P, glyceraldehyde-3-phosphate; α-GPDH, α-glycerophosphate dehydrogenase; G-3-PDH, glyceraldehyde-3-phosphate dehydrogenase; LDH, lactic dehydrogenase; TCA, trichloroacetic acid; Tris, tris(hydroxymethyl)aminomethane; HDP, fructose-1,6-diphosphate.

2 Kindly prepared by Dr. David Howton.
seconds in the presence of tissue, excess DPNH, and pyruvate after aging the homogenate for 3 to 5 hours at 4° to promote destruction of endogenous DPN, thus eliminating the small ΔD noted in fresh homogenates in the absence of added substrate. Aging in the presence of nicotinamide preserved this endogenous ΔD. The reaction mixture used in the routine assays contained (final concentration in parentheses) DPNH (6.25 × 10⁻⁵ M), sodium pyruvate (5.5 × 10⁻⁴ M), nicotinamide (0.55 M), phosphate buffer, pH 7.4 (0.033 M), and 0.1 ml. of leucocyte homogenate diluted to contain the equivalent of 5 × 10⁶ cells (added at zero time) per 3 ml. in a 1 cm. silica cell. Data were taken from a Brown recorder attached to a Beckman DU spectrophotometer. In computing results, the ΔD at 60 seconds (2 × 30 seconds ΔD) was converted to micromoles of DPNH oxidized per ml. per minute per 10⁸ cells, with 6.22 as ε₁cm. of DPNH (19). The blank contained all additions except DPNH.

G-3-PDH was assayed spectrophotometrically as ΔD₃₄₀ at 30 seconds in a system containing (final concentration in parentheses) fructose 1,6-diphosphate (0.005 M) or G-3-P (0.0005 M), DPN (0.0001 M), cysteine (0.0004 M), potassium arsenate (0.006 M), KF (0.04 M), Tris buffer, pH 8.5 (0.03 M), and 0.1 ml. of "aged" leucocyte homogenate containing 5 × 10⁶ cells per 3 ml. in a 1 cm. silica cell. When fructose 1,6-diphosphate was used as substrate-precursor, homogenate was added to cuvettes containing all constituents except DPN and was incubated for 30 minutes at 38°. The reaction was initiated by adding DPN. The results were calculated as for LDH. When G-3-P was used as substrate, the system was not preincubated.

In determining Michaelis constants for G-3-PDH, two methods were used to vary the concentration of G-3-P. As shown in Fig. 1, A, when G-3-P was formed by preincubation of tissue with fructose 1,6-diphosphate, the reciprocal of observed velocity (1/v) was a linear function of the reciprocal of duration of preincubation (1/t). Since triose phosphate production by tissue aldolase proceeded linearly with or without added hydrazine for 60 minutes (Fig. 1, B), the concentration of G-3-P could be varied when fructose 1,6-diphosphate was its precursor by varying the duration of preincubation. The actual concentration of G-3-P at the end of preincubation presumably depended upon the relative activities of tissue aldolase, triosephosphate isomerase, and G-3-PDH and, hence, had to be specifically determined in parallel tubes by colorimetric methods described elsewhere (13). Fig. 1, C illustrates an analysis of the G-3-PDH assay system with fructose 1,6-diphosphate as substrate-precursor. When G-3-P was used as substrate, initial concentrations could be varied directly. Fig. 1, D illustrates the linear continuity in a Lineweaver-Burk plot of points obtained by both methods of substrate variation with one homogen-
ate. Since calculations of the Michaelis constant for substrate \( (K_a) \) for G-3-PDH were based on substrate concentrations at zero time, they would be subject to whatever error may result from G-3-P removal, owing to simultaneous isomerase activity, and would tend to be high. The tissue levels and \( K_a \) values of isomerase to be reported below suggest that this source of error would not substantially alter the magnitude of the calculated \( K_a \) values for G-3-PDH.

Aerobic glycolysis was measured in air in a system containing glucose or fructose 1,6-diphosphate \( (0.0053 \text{ M}) \), phosphate buffer, pH 7.4 \( (0.01 \text{ M}) \),

Fig. 1. Methods of varying \([s]\) in the determination of G-3-PDH kinetics. \( A \), plot of reciprocal of observed \( AD \) in G-3-PDH assay with fructose 1,6-diphosphate as substrate-precursor versus reciprocal of duration of preincubation period. Details in the text. \( B \), time-course of aldolase reaction with hydrazine added (●) and omitted (○). \( C \), the G-3-PDH assay with fructose 1,6-diphosphate as substrate-precursor. Time scale of preincubation period is compressed. All cuvettes contained potassium arsenate \( (0.006 \text{ M}) \), KF \( (0.04 \text{ M}) \), and Tris buffer, pH 8.5 \( (0.03 \text{ M}) \), all but those for Curve 3 contained cysteine \( (0.0004 \text{ M}) \), and all but those for Curve 4 contained fructose 1,6-diphosphate \( (0.0005 \text{ M}) \). Curve 1, DPN \( (0.0001 \text{ M}) \) added before homogenate, no preincubation; Curve 2, homogenate added at zero time and preincubated for 30 minutes; reaction initiated with DPN; Curve 3, cysteine omitted from preincubation. DPN promoted no reaction until cysteine added at a; Curve 4, fructose 1,6-diphosphate omitted throughout; Curve 5, hydrazine \( (0.63 \text{ M}) \) added to preincubation mixture. \( D \), Lineweaver-Burk plot of \( K_a \) for leucocyte G-3-PDH. \( s = \text{G-3-P (○); substrate-precursor = HDP (●).} \)
MgCl₂ (0.0053 M), ATP (0.0011 M), DPN (0.007 M), and cytochrome c (1.4 × 10⁻⁵ M), details concerning which have been published (2). Lactic acid was measured by the method of Barker and Summerson (20). Lactic acid production proceeded linearly for 60 minutes in most experiments. Lactic acid production per minute was calculated from the 20 minute rate.

**TABLE I**

Comparisons of Normal, Myelocytic Leukemia, and Lymphocytic Leukemia Leucocyte Aerobic Glycolytic Rate, Glycolytic Enzyme Activity, and Pyridine Nucleotides

The figures represent activity levels relative to mean activity in normal cells (100) ± standard error of the mean, except those for pyridine nucleotides which are given in micromoles. Figures in bold-faced type differ significantly from normal (P < 0.001).* Number of cases studied in parentheses.

<table>
<thead>
<tr>
<th>Type of leucocyte</th>
<th>Aerobic glycolytic rate</th>
<th>G-3-PDH</th>
<th>LDH</th>
<th>Aldolase</th>
<th>Isomerase</th>
<th>Pyridine nucleotides</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>100 ± 12</td>
<td>100 ± 12</td>
<td>100 ± 12</td>
<td>100 ± 6</td>
<td>100 ± 11</td>
<td>1.39 ± 0.14</td>
</tr>
<tr>
<td>(13)</td>
<td>(9)</td>
<td>(13)</td>
<td>(18)</td>
<td>(13)</td>
<td>(10)</td>
<td></td>
</tr>
<tr>
<td>Myelocytic leukemia</td>
<td>42 ± 5</td>
<td>59 ± 6</td>
<td>41 ± 3</td>
<td>107 ± 5</td>
<td>106 ± 8</td>
<td>1.26 ± 0.13</td>
</tr>
<tr>
<td>(12)</td>
<td>(8)</td>
<td>(14)</td>
<td>(12)</td>
<td>(5)</td>
<td>(11)</td>
<td></td>
</tr>
<tr>
<td>Lymphocytic leukemia</td>
<td>16 ± 2</td>
<td>19 ± 2</td>
<td>24 ± 3</td>
<td>35 ± 1</td>
<td>32 ± 3</td>
<td>1.27 ± 0.13</td>
</tr>
<tr>
<td>(10)</td>
<td>(6)</td>
<td>(10)</td>
<td>(12)</td>
<td>(4)</td>
<td>(8)</td>
<td></td>
</tr>
</tbody>
</table>

Data calculated per 10⁹ cells

<table>
<thead>
<tr>
<th>Type of leucocyte</th>
<th>Aerobic glycolytic rate</th>
<th>G-3-PDH</th>
<th>LDH</th>
<th>Aldolase</th>
<th>Isomerase</th>
<th>Pyridine nucleotides</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>100 ± 12</td>
<td>100 ± 12</td>
<td>100 ± 12</td>
<td>100 ± 6</td>
<td>100 ± 11</td>
<td>0.77 ± 0.08</td>
</tr>
<tr>
<td>(13)</td>
<td>(9)</td>
<td>(13)</td>
<td>(18)</td>
<td>(13)</td>
<td>(10)</td>
<td></td>
</tr>
<tr>
<td>Myelocytic leukemia</td>
<td>45 ± 5</td>
<td>67 ± 7</td>
<td>43 ± 3</td>
<td>115 ± 5</td>
<td>113 ± 8</td>
<td>0.75 ± 0.08</td>
</tr>
<tr>
<td>(10)</td>
<td>(6)</td>
<td>(14)</td>
<td>(12)</td>
<td>(5)</td>
<td>(11)</td>
<td></td>
</tr>
<tr>
<td>Lymphocytic leukemia</td>
<td>40 ± 5</td>
<td>46 ± 5</td>
<td>59 ± 7</td>
<td>86 ± 3</td>
<td>80 ± 6</td>
<td>1.75 ± 0.18</td>
</tr>
<tr>
<td>(10)</td>
<td>(6)</td>
<td>(10)</td>
<td>(12)</td>
<td>(4)</td>
<td>(8)</td>
<td></td>
</tr>
</tbody>
</table>

* Calculation of standard error of the mean, s.e. = \( \sqrt{\sum d^2/(n(n - 1))} \). P derived by Fisher's "t" test.

**EXPERIMENTAL**

Enzyme and Pyridine Nucleotide Levels—Table I presents the mean levels of glycolytic activity, enzyme activity, and pyridine nucleotide concentration found in a survey of normal, myelocytic leukemia, and lymphocytic leukemia leucocyte homogenates. Because measurements of glycolysis and enzyme activity were performed under arbitrary conditions, the data are meaningful chiefly as they permit comparison of the three tissues. The results, therefore, are presented on the basis that activity in normal cells equals 100, except for pyridine nucleotide levels which are
given in micromoles. Data are shown both as calculated per cell and per mg. of N to dispel the possible influence of cell size on activity ratios. It is seen that the observed levels of dehydrogenase activity closely paralleled glycolytic activity, whereas aldolase and triosephosphate isomerase activity were, if anything, higher in myelocytic leukemia leucocytes, while glycolysis was lower than normal. These conclusions are not substantially altered when data are calculated per mg. of N, although the patterns in the two leucemic tissues are brought closer together by this calculation. Pyridine nucleotides were about the same per cell but higher per mg. of N in lymphocytic leukemia leucocytes.

Several comments are in order concerning the assay methods. Because, in the G-3-PDH assay, substrate was produced from fructose 1,6 diphosphate by endogenous tissue aldolase, DHA-P accumulated along with the G-3-P, presumably exceeding it in concentration owing to triosephosphate isomerase activity. Tissue α-GPDH, if present, would reduce DHA-P and oxidize DPNH simultaneously with G-3-PDH-catalyzed DPN reduction, thus lowering the observed ΔD (21). To assess the extent of possible interference from this source, the tissue α-GPDH reaction was measured in both directions in systems containing DHA-P plus DPNH and α-GP plus DPN. Activities were compared with ΔD values obtained in the G-3-PDH assay with G-3-P as substrate and identical quantities of homogenate. As shown in Table II, α-GPDH activity was low in the tissues studied.

Since aldolase activity was demonstrably low in lymphocytic leukemia cells, G-3-P (5 × 10⁻⁴ M) was used as substrate in surveying lymphocytic leukemia leucocyte G-3-PDH activity to permit comparison of the three tissues at essentially equal substrate concentrations.

The demonstration that in brain measurable DPNase is related to particle size following homogenization (22) suggested a possible source of error in the pyridine nucleotide method. This was investigated with four degrees of particle size: intact cells, McShan-Erway glass homogenizer, Nelco blender, and French pressure cell homogenate with and without added nicotinamide. Particle size was roughly gaged by microscopic inspection and by measuring the per cent of total homogenate N in the supernatant fraction after centrifugation of the whole homogenate for 10 minutes at 600 X g. Table III presents the effect of added nicotinamide and particle size on measurable DPNase activity and fluorometrically meas-

3 A large series of determinations of normal, myelocytic leukemia, and lymphocytic leukemia leucocyte homogenate total nitrogen gave mean values (± standard error) of 180.2 ± 11.4, 168.6 ± 11.6, and 72.2 ± 1.8 mg. of N per 10¹⁰ cells, respectively, reflecting the morphologically obvious size difference between granulocytes and lymphocytes.
ured pyridine nucleotide. Unless nicotinamide was added, the demonstrable pyridine nucleotide level was critically dependent on the degree of

**Table II**

**Relative Activities of $\alpha$-GPDH and G-3-PDH in Leucocyte Homogenates As Determined in Systems Containing Pure Substrates**

The figures represent observed $\Delta$D$_{260}$ per 30 seconds per $10^7$ cells. $\alpha$-GPDH was measured spectrophotometrically in two systems: (a) $s = \alpha$-GP and $c = DPN$ and (b) $s = DHA-P$ and $c = DPNH$. The cuvettes contained (final concentration in parentheses) (a) $\alpha$-GP (0.006 M), DPN (0.003 M), phosphate buffer, pH 7.4 (0.01 M), “aged” leucocyte homogenate ($5 \times 10^7$ cells), and water to 3.0 ml. and (b) DHA-P (0.006 M), DPNH (0.008 M), and buffer, water, and homogenate as above. Because $\alpha$-GPDH reactions were initiated with homogenate, separate tubes containing no added substrate or coenzyme were run to control endogenous optical activity. G-3-PDH was assayed as described in the text, with G-3-P as substrate.

<table>
<thead>
<tr>
<th>Type of leucocyte</th>
<th>G-3-PDH</th>
<th>$\alpha$-GPDH</th>
<th>Endogenous</th>
<th>Calculated $\alpha$-GPDH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>+0.145</td>
<td>-0.058</td>
<td>-0.020</td>
<td>-0.024</td>
</tr>
<tr>
<td>Myelocytic leucemia</td>
<td>+0.105</td>
<td>-0.028</td>
<td>-0.013</td>
<td>-0.016</td>
</tr>
<tr>
<td>Lymphocytic leucemia</td>
<td>+0.033</td>
<td>-0.012</td>
<td>-0.008</td>
<td>-0.006</td>
</tr>
</tbody>
</table>

**Table III**

**Effect of Degree of Homogenization and Addition of Nicotinamide on Measurable DPNase Activity, Fluorometrically Determined Pyridine Nucleotides, and Supernatant Nitrogen in Myelocytic Leucemia Leucocytes**

All figures are percentage values. For pyridine nucleotides and DPNase, the maximal levels found in pressure cell homogenates are designated 100 per cent. When used in pyridine nucleotide assays, nicotinamide, 0.1 M, was added to the cell suspension before homogenization. For nitrogen, the figures represent actual per cent of total homogenate nitrogen in supernatant fraction after 10 minutes centrifugation of whole homogenate at 600 × $g$.

<table>
<thead>
<tr>
<th>Preparation</th>
<th>Supernatant nitrogen</th>
<th>DPNase</th>
<th>Pyridine nucleotides</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>− Nicotinamide</td>
</tr>
<tr>
<td>Intact cells</td>
<td>9.6</td>
<td>22.5</td>
<td>22.4</td>
</tr>
<tr>
<td>All-glass homogenate*</td>
<td>67.0</td>
<td>37.2</td>
<td>75.2</td>
</tr>
<tr>
<td>Neleco blender homogenate</td>
<td>79.0</td>
<td>63.0</td>
<td>29.9</td>
</tr>
<tr>
<td>French pressure cell homogenate</td>
<td>88.2</td>
<td>100.0</td>
<td>10.0</td>
</tr>
</tbody>
</table>

* Prepared by grinding for 4 minutes in all-glass McShan-Erway homogenizer.
homogenates, with nicotinamide present, closely approached maximal values and this technique was used for routine assays.

Comparison of Normal and Leukemic Enzymes—The observed differences in the activity levels of individual enzymes among the three tissues raises the following question: are the differences attributable to quantitative differences in the amount of enzyme activity per cell or to qualitative differences in normal and leukemic cell enzymes? In an attempt to answer this question, Michaelis constants were determined for the individual enzymes and for glycolysis (in the fortified system with glucose as substrate). The results as derived from Lineweaver-Burk plots are presented in Table IV.

<table>
<thead>
<tr>
<th>Type of leucocyte</th>
<th>Glycolysis</th>
<th>G-3-PDH</th>
<th>LDH</th>
<th>Aldolase</th>
<th>Isomerase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal.............</td>
<td>2.0 × 10⁻⁴</td>
<td>4.0 × 10⁻⁵</td>
<td>4.8 × 10⁻⁵</td>
<td>7 × 10⁻⁴</td>
<td>4.0 × 10⁻⁴</td>
</tr>
<tr>
<td>Myelocytic leukemia....</td>
<td>3.2 × 10⁻⁴</td>
<td>4.4 × 10⁻⁵</td>
<td>5.0 × 10⁻⁵</td>
<td>7 × 10⁻⁴</td>
<td>3.5 × 10⁻⁴</td>
</tr>
<tr>
<td>Lymphocytic leukemia....</td>
<td>2.2 × 10⁻⁴</td>
<td>3.8 × 10⁻⁵</td>
<td>5.0 × 10⁻⁵</td>
<td>8 × 10⁻⁴</td>
<td>3.8 × 10⁻⁴</td>
</tr>
</tbody>
</table>

\[ K_s = \frac{1}{V} \left( \frac{[S]}{[S]_{max}} \right) \]

\[ K_c = \frac{1}{V} \left( \frac{[S]}{[S]_{max}} \right) \]

For "two substrate" systems in which \([c] or [s]) is fixed, the concentration of fixed substrate is given. All values are in moles per liter.

* See the text.
(<10 per cent $V_{\text{max}}$); hence in the derivations $v$ can be related almost entirely to added $s$. It is interesting that the observed $K_s$ for glycolysis is somewhat higher than that reported for purified brain hexokinase (23).

However, in attempting to measure the DPN concentration at which glycolysis was $\frac{1}{2}V_{\text{max}}$, it was found that omission of added DPN depressed glycolysis in myelocytic leucemia and lymphocytic leucemia leucocytes to only 50 to 60 per cent of $V_{\text{max}}$, and in normal leucocytes to only 90 per cent of $V_{\text{max}}$. (3), as might be expected in whole homogenates in which reversible coupling of the DPN-DPNH system permits catalytic rather than stoichiometric coenzyme concentrations. DPN can be considered a "second substrate" only with pure enzymes or in homogenates when reverse reactions have been eliminated or controlled. If it is assumed that the pyridine nucleotides, as measured above, are dispersed throughout the cytoplasm only (neglecting for this calculation the effect of DPN binding), that cell pyridine nucleotide is 90 per cent DPN, that the cytoplasm represents 65 per cent of the cell volume, and that $10^9$ normal leucocytes occupy 6.0 ml., calculation shows that DPN in normal leucocyte cytoplasm is $3.2 \times 10^{-4}$ M or 13 times greater than the observed Michaelis constant for DPN ($K_s$) for LDH, the latter value having been determined in homogenate preparations substantially freed of endogenous DPN and, as noted below, in purified preparations of leucocyte LDH. The increase in glycolysis which follows addition of DPN may therefore reflect the extent of enzymic DPN destruction.

Fig. 2 shows typical Lineweaver-Burk plots of normal, myelocytic leucemia, and lymphocytic leucemia leucocyte LDH activity with varying DPNH levels at two fixed substrate concentrations. Although derived $K_s$ values were identical for the three tissues, calculated $V_{\text{max}}$ values were linear functions of cell LDH concentration, as shown in Fig. 2, D, where $V_{\text{max}}$ is plotted versus the mean of LDH levels observed in the above survey.

Because Michaelis constants were determined with whole homogenates, the possibility remains that variations in observed enzyme velocity produced by varying substrate or coenzyme concentration resulted indirectly from varying levels of activity in other enzyme systems. To resolve this question for LDH, highly purified enzyme preparations were isolated from normal, myelocytic leucemia, and lymphocytic leucemia leucocytes by a modification of the procedure of Gibson, Davisson, and Bachhawat (24). In a typical preparation (3.68 ± 0.11) $\times 10^9$, washed, intact leucocytes (approximately 23 ml. of packed wet cells) were subjected to homogenization for 5 minutes in the Nelo blender in ice-cold 0.154 M KCl solution. The material was centrifuged at 800 $\times g$ at 0° for 30 minutes. The supernatant fraction was rehomogenized and centrifuged twice more. The pooled supernatant solutions were adjusted to pH 6.5 with Na$_2$HPO$_4$,
0.1 M, and made up to 16 per cent ethanol at -5°. Following centrifuga-
tion at 800 X g at -5°, the supernatant solution was dialyzed against
0.5 M NaCl at 0°. Solid ammonium sulfate was added to a concentration
of 0.3 gm. per ml. The precipitate was dissolved in distilled water, dialyzed
against 0.03 M NaCl at 0°, and, after adjustment to pH 5.8 with acetic
acid, brought to 5 per cent ethanol at -3° and adjusted to 1/2 = 0.03

![Fig. 2. The kinetics of leucocyte LDH.](image)

with phosphate buffer, pH 5.8. The clear supernatant fraction obtained
on centrifugation was made up to 30 per cent ethanol at -3° and recen-
trifuged, and the precipitate was redissolved in phosphate buffer, 1/2 =
0.05, pH 7.7. Reprecipitation at -8° with 30 per cent ethanol was re-
peated several times and the final residue was dissolved in 1.0 M ammonium
sulfate made up in phosphate buffer, 0.1 M, pH 7.8. This product was
used for kinetic studies.

Table V shows recoveries and purifications obtained in the preparation
described.
As shown in Fig. 2, C, kinetic studies on LDH purified from normal, myelocytic leukemia, and lymphocytic leukemia leucocytes yielded identical $V_{\text{max}}$ values. $K_e$ and $K_s$ values were the same as those obtained on whole homogenates. Other leucocyte enzymes have not yet been similarly purified.

The measurement of $K_s$ for LDH (in whole homogenate and purified enzyme) is presented in Fig. 3. Enzyme activity was inhibited at high pyruvate concentrations, and intercepts were obtained by extrapolating the linear segment of the $1/v$ versus $1/s$ curves. The mechanism of the substrate inhibition and its possible significance for the calculated Michaelis constants were not investigated further.

**TABLE V**

Purification of Leucocyte Lactic Dehydrogenase

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Total nitrogen</th>
<th>Number of units</th>
<th>Per cent recovery</th>
<th>Specific activity $V_{\text{max}}$ units per mg.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Supernatant of homogenate</td>
<td>529</td>
<td>105</td>
<td>100</td>
<td>2.0</td>
</tr>
<tr>
<td>16% ethanol supernatant</td>
<td>235</td>
<td>75</td>
<td>71</td>
<td>3.2</td>
</tr>
<tr>
<td>Ammonium sulfate residue</td>
<td>73</td>
<td>75</td>
<td>71</td>
<td>10.3</td>
</tr>
<tr>
<td>1st 30% ethanol ppt.</td>
<td>18</td>
<td>37</td>
<td>35</td>
<td>20.6</td>
</tr>
<tr>
<td>2nd 30% &quot; &quot;</td>
<td>11</td>
<td>32</td>
<td>30</td>
<td>29.1</td>
</tr>
<tr>
<td>Final ammonium sulfate residue</td>
<td>7</td>
<td>30</td>
<td>29</td>
<td>43.0</td>
</tr>
</tbody>
</table>

* 1 unit equals $1 \times 10^{-4}$ M DPNH converted per minute per ml.

The pH-activity curves obtained for crude and pure LDH, G-3-PDH, and aldolase (Fig. 4) also show functional similarity between normal and leucemic enzymes. The reported specific effect of the buffer used (26) on aldolase was generally confirmed.

The kinetic data permit comparisons of the three tissues in terms of the maximal velocity of the individual enzymes and glycolytic rate rather than in terms of the arbitrary activity levels presented in Table I. Here can be compared not only normal tissue with leucemic, but also, and of perhaps equal interest, absolute capacities of component glycolytic enzymes with tissue capacity for glycolysis itself. Table VI presents calculated $V_{\text{max}}$ values expressed as micromoles of triose produced per minute per $10^{10}$ cells.

Despite the apparent proportionality between dehydrogenase activity and glycolytic activity, the capacity for activity of the dehydrogenases $^4$

$^4$ The pyruvate concentration used by Meister (25) in his survey of normal and tumor tissue LDH ($3.3 \times 10^{-4}$ M) was inhibitory for leucocyte LDH.
far exceeds the actual activity which their rôle in glycolysis would seem to demand of them. Aldolase $V_{\text{max}}$ also exceeds that of glycolysis but by a considerably smaller margin. The similarity between the $V_{\text{max}}$ of LDH and G-3-PDH is also noteworthy.

**Table VI**

<table>
<thead>
<tr>
<th>Type of leucocyte</th>
<th>Glycolysis</th>
<th>Aldolase</th>
<th>Isomerase</th>
<th>G-3-PDH</th>
<th>LDH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>75</td>
<td>155</td>
<td>292</td>
<td>512</td>
<td>600</td>
</tr>
<tr>
<td>Myelocytic leucemia</td>
<td>33</td>
<td>167</td>
<td>310</td>
<td>320</td>
<td>300</td>
</tr>
<tr>
<td>Lymphocytic leucemia</td>
<td>11</td>
<td>53</td>
<td>93</td>
<td>96</td>
<td>180</td>
</tr>
</tbody>
</table>

**Fig. 3**

Fig. 3. The kinetics of leucocyte LDH (continued). Lineweaver-Burk plots of $1/v$ versus $1/s$ with $[c] = 6.25 \times 10^{-4} \text{ M}$. Symbols defined in Fig. 2.

**Fig. 4**

Fig. 4. pH-activity curves for leucocyte glycolysis, aldolase, LDH, and G-3-PDH. ○ indicates normal, ● myelocytic leucemia, ■ lymphocytic leucemia leucocyte homogenate, and ▲ LDH purified from myelocytic leucemia leucocytes. Abscissae, pH; ordinates, arbitrary velocity units. Details of incubations in the text. A, glycolytic activity. Phosphate buffer, pH 6.8 and 7.4 (0.01 M); Tris buffer, pH 8.0 (0.01 M), with supplemental orthophosphate added (0.01 M). B, aldolase. Activities at various pH levels with collidine, Tris, phosphate, and borate buffers. All buffers 0.01 M. Same aliquot of homogenate in each test. C, LDH activity. Acetate buffer, pH 4.4; phosphate buffer, pH 6.2 and 7.4; Tris buffer, pH 9.0; and borate buffer, pH 10.2. All buffers 0.033 M. D, G-3-PDH. Phosphate buffer, pH 6.8, 7.0, and 7.4; Tris buffer, pH 8.0, 8.6, and 9.0. All buffers 0.03 M.
The results permit construction of the families of curves presented in Fig. 5 where enzyme velocity and glycolysis velocity are plotted against the logarithm of substrate concentration. It should be noted that G-3-PDH and aldolase were measured at pH 8.6 rather than at pH 7.4. The pH-activity curves (Fig. 4) suggest that the comparisons of Fig. 5 would not be substantially altered had all assays been done at pH 7.4. As shown in Fig. 4, B, aldolase activity at pH 8.6 with Tris buffer was identical to that at pH 7.4 with collidine buffer. *In vivo* pH conditions are, of course, not known.

Fig. 5 suggests that, if glycolysis were proceeding at its maximal velocity, the velocities of the component enzymes would necessarily be the ordinate values of the points where their curves intersect the glycolytic $V_{\text{max}}$ asymptote. These points projected to the abscissa imply particular concentrations for the various enzyme substrates at which these velocities will prevail, low in the case of LDH substrate (pyruvate) and G-3-PDH substrate (G-3-P) and higher in the case of aldolase substrate (fructose 1,6-diphosphate). This deduction coincides with the known scarcity of pyruvate and triose phosphate in tissue extracts (27, 28). Fig. 5 also

---

**Fig. 5.** Graphs showing velocity of glycolytic enzymes (LDH, G-3-PDH, and aldolase) and of glycolysis versus logarithm of their substrate concentrations in normal, myelocytic leucemia, and lymphocytic leucemia leucocyte homogenates. Velocities expressed as micromoles of triose formed per minute per $10^{10}$ cells. The horizontal lines indicate calculated $V_{\text{max}}$ values for glycolysis and designated enzymes. The points where enzyme curves intersect the glycolytic $V_{\text{max}}$ line are projected to the abscissa to indicate substrate concentrations at which individual enzyme velocities would equal glycolytic $V_{\text{max}}$. 
shows that the somewhat higher than normal $V_{\text{max}}$ of aldolase in myelocytic leukemia leucocytes implies a lower than normal accumulation of fructose 1,6-diphosphate.

**DISCUSSION**

These conclusions may be considered in the light of Hinshelwood’s analysis (6) of steady state metabolism which shows that in a multienzyme sequence

$$k_\mu f_\mu(c_{\mu-1}) = k_{\mu-1}$$

where $k_\mu$ and $k_{\mu-1}$ = the velocity constant of the $\mu$th and $(\mu-1)$th enzyme of the series, respectively, $f_\mu$ = the degree of substrate saturation of the $\mu$th enzyme, and $c_{\mu-1}$ = the concentration of substrate provided by the previous enzyme of the series. The steady state could be attained in two cases: (1) with enzyme saturated with substrate, i.e. $f_\mu = 1$, $k_\mu$ would have to equal $k_{\mu-1}$ and (2) with the enzyme not saturated, its rate being responsive to change in $c_{\mu-1}$, then $c_{\mu-1}$ must adjust itself to such a value that the steady state is established. Were the rate-limiting enzyme for glycolysis shown in Fig. 5, its curve would presumably overlie the glycolysis curve, implying that, at maximal velocity, the enzyme would be substrate-saturated as in the first case of Hinshelwood, while, at submaximal velocities, it would be unsaturated as in the second case. Which of the two cases obtained would therefore depend upon substrate availability. In the absence of “branching” in the sequence, it would appear from the curves that, in steady state metabolism, the non-rate-limiting enzymes (with the possible exception of the first of the sequence) could not be substrate-saturated.

The possible significance of enzymic structural orientation remains undecided in the present case, particularly since the preparations used presumably lacked intact cell surfaces. It has been assumed for the present study that the glycolytic enzymes were uniformly dispersed throughout the cytoplasm (29) and hence the incubation medium. Preliminary experiments have shown that, when ordinary homogenates were centrifuged for 30 minutes at 18,000 × $g$ to eliminate mitochondria and nuclei, the glycolytic rate per unit volume of “remaining supernatant fraction” was about nine-tenths that of whole homogenate levels. Supernatant aldolase was slightly lower. The depressant effect upon glycolysis of omitting added DPN was sizably greater with “remaining supernatant fractions” than with whole homogenate, indicating that DPN loss had accompanied large granule sedimentation.

The assumption that the glycolytic enzymes are soluble in the medium will presumably be supported or refuted by studies now in progress on the
concentrations and turnover rates of glycolytic intermediates. As suggested above, these patterns should be predictable if homogeneous enzyme solutions are involved, diluted though they may be by homogenate technique.

The present data show interesting disparities (1) between normal and leucemic leucocytes and (2) between the relative levels of a multienzyme system and its component enzymes in analogous tissues. Despite their lower than normal glycolytic rate, myelocytic leucemia leucocytes had a normal or higher than normal aldolase content, possibly signifying an additional or different rôle in this tissue for aldolase, an enzyme noted for its lack of absolute substrate specificity. If the lower glycolytic activity in myelocytic leucemia cells were due merely to a smaller cytoplasmic volume, the disparity between aldolase and glycolysis would be relatively even greater.

No differences were demonstrated between the corresponding enzymes of normal and leucemic leucocytes with regard to Michaelis constants and pH-activity relationships, suggesting that the analogous enzymes may be functionally identical. Michaelis constants and pH optima found for leucocyte enzymes substantially agreed with those reported for enzymes from other biological sources (30). \( K \), for triosephosphate isomerase has not been previously reported.

Fig. 5 suggests that, in the steady equilibrium state, the glycolyzing leucocyte is using at least two of its enzymes well below their maximal capacity, even though their levels are proportional to the glycolytic rate. Of these two, G-3-PDH appears to be the key DPN-reducing enzyme and LDH the key DPNH-oxidizing enzyme. Unlike the situation reported to occur in yeast and muscle, \( \alpha \)-GPDH appears to have a minor rôle in the leucocyte "DPN cycle." Chance's analysis (31) of yeast suggests a DPN-DPNH shuttle chiefly between G-3-PDH and \( \alpha \)-GPDH. The low levels of \( \alpha \)-GPDH found in leucocytes suggest that the G-3-PDH-LDH shuttle is the more significant in this tissue, although the techniques of Chance would be helpful in clarifying the point. It is also interesting in view of the low \( \alpha \)-GPDH levels observed to note that triosephosphate isomerase levels and maximal capacities are lower in comparison to aldolase than Meyerhof and Beck (12) reported for other tissues.

The present data reveal no enzyme which can be considered rate-limiting. Experiments to be published later have shown no increase in glycolysis following addition of purified aldolase, isomerase, or LDH to leucocyte homogenate.

Further studies are now in progress on the kinetics of other glycolytic enzymes, particularly hexokinase, phosphofructokinase, and phosphohexose isomerase. Following characterization of the individual enzymes,
it should be possible to examine two, three, and four enzyme subsequences and their relationship to the total sequence. The previously reported (3) increase in glucose glycolysis which follows addition of fructose 1,6-diphosphate suggests replacement of a rate-limiting reaction in the glucose → fructose 1,6-diphosphate subsequence by one in the fructose 1,6-diphosphate → lactic acid subsequence, which may or may not be linked to the adenylic acid system.

**SUMMARY**

Previously reported differences in aerobic glycolytic rate between normal, chronic myelocytic leukemia, and chronic lymphocytic leukemia leucocytes have been investigated by comparing the activity levels, pH-activity curves, Michaelis constants, and maximal velocities of a number of individual enzymes of glycolysis in the three tissues. The results were compared with similar data on the glycolytic process itself in an attempt to discover the locus of the rate-limiting reaction of the glycolytic sequence.

It was observed that, although the $V_{\text{max}}$ per unit of tissue of any one enzyme studied was higher than the $V_{\text{max}}$ of glycolysis (i.e. the rate-limiting enzyme was not found), the activity levels of lactic dehydrogenase and glyceraldehyde-3-phosphate dehydrogenase were closely proportional to the glycolytic rate in the three tissues while greatly exceeding it in maximal velocity. Conversely, aldolase and triosephosphate isomerase activity was higher than normal in myelocytic leukemia leucocytes, although the glycolytic rate was lower than normal.

Michaelis constants for substrate and coenzyme and pH optima were essentially identical for the corresponding enzymes of the three tissues, although differing among different enzymes, suggesting functional similarity between normal and leucemic enzymes. α-Glycerophosphate dehydrogenase activity was low and it is suggested that the major DPN-DPNH shuttle in leucocytes occurs between glyceraldehyde-3-phosphate dehydrogenase and lactic dehydrogenase.

Kinetic studies suggest a basis for predicting the concentrations of glycolytic intermediates, if certain presuppositions are valid.

The author acknowledges the technical assistance of Julianne Hitt, Phyllis Talmage, Juanita Lamport, and Ione Crawford.

**BIBLIOGRAPHY**

A KINETIC ANALYSIS OF THE GLYCOLYTIC RATE AND CERTAIN GLYCOLYTIC ENZYMES IN NORMAL AND LEUCEMIC LEUCOCYTES

William S. Beck


Access the most updated version of this article at http://www.jbc.org/content/216/1/333.citation

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC’s e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/216/1/333.citation.full.html#ref-list-1