The Effect of Certain Glycolytic Inhibitors on Aerobic Lactic Acid Production by Human Leukocytes in Vitro

By Gordon R. McKinney and Samuel P. Martin

No mechanism is known for the reaction whereby normal polymorphonuclear leukocytes have the capacity to form relatively large quantities of lactic acid in the presence of oxygen. Since leukocytes from healthy individuals contain an active glyoxalase system, methylglyoxal may serve as a possible precursor of this lactic acid. Levene and Meyer stated that the conversion of methylglyoxal into a mixture of DL- and D-lactic acids furnished evidence for the concept that methylglyoxal was an intermediate in the formation of D-lactic acid from D-hexoses. Also several reports exist in the literature describing the isolation of derivatives of methylglyoxal upon the incubation of blood cells with either glucose or hexosediphosphate, indicating that these cells may form this compound as an intermediate in their breakdown of carbohydrate to lactic acid. Barrensee isolated the semicarbazone of methylglyoxal from dog blood. By isolating its 2,4-dinitrophenylhydrazone, Schneider and Widmann demonstrated that both human erythrocytes and leukocytes formed methylglyoxal. These latter investigators observed that 70-75 per cent of the glucose passes through the methylglyoxal stage. Thus it seems possible that mature granulocytes may possess a pathway for lactic acid formation which bypasses at least a portion of the Myerhof-Emden scheme of glycolysis. Data with inhibitors described in this report indicate that such an alternate scheme might exist in normal, mature leukocytes.

Experimental

Leukocytes employed in these experiments were obtained and processed by the method previously described. When broken cells were desired, the preparation of intact cells was ground with sea sand in a ground glass homogenizer (Tissue Grinder, Ten Broeck, Scientific Glass Apparatus Co.). Activity in broken cell preparations was quantitated on the basis of cell count in the sample of intact cells before grinding. After incubation in Warburg vessels for four hours at 37 C., the lactic acid was determined in an aliquot of cell suspension by the method of Barker and Summerson. The colorimetric analyses were read in a Coleman Junior Spectrophotometer, Model 6A. Lactic acid production was found to be linear with time. Oxamic acid, D-l-glyceraldehyde, and sorbose-1-phosphate were products of Bios Laboratories, Inc. The solution of D-l-glyceraldehyde was placed in a water bath at 85 C. for 5 minutes to avoid dimerization, before addition to the cell preparation. The
EFFECT OF GLYCOLYTIC INHIBITORS ON AEROBIC LACTIC ACID

Table 1.—Effect of Various Chemicals on Aerobic Lactic Acid Production by Human Leukocytes in vitro

<table>
<thead>
<tr>
<th>Additions</th>
<th>(final concentrations)</th>
<th>Intact Cells</th>
<th>Broken Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>dl-Glyceraldehyde</td>
<td>(0.02M)</td>
<td>+51.1</td>
<td>+48.9</td>
</tr>
<tr>
<td>dl-Glyceraldehyde</td>
<td>(0.002M)</td>
<td>+28.1</td>
<td>+30.4</td>
</tr>
<tr>
<td>dl-Glyceraldehyde (0.002M) + Glucose</td>
<td>(0.0056M)</td>
<td>+20.9</td>
<td>+8.5</td>
</tr>
<tr>
<td>Sorbose-1-phosphate (0.001M) + Glucose</td>
<td>(0.0056M)</td>
<td>+19.4</td>
<td>-3.1</td>
</tr>
<tr>
<td>Oxamic acid</td>
<td>(0.1M)</td>
<td>-20.1</td>
<td>-23.7</td>
</tr>
<tr>
<td>Oxamic acid (0.01M)</td>
<td>(0.01M)</td>
<td>-23.7</td>
<td>-22.9</td>
</tr>
<tr>
<td>Oxamic acid (0.01M) + Glucose</td>
<td>(0.0056M)</td>
<td>-29.4</td>
<td>-22.8</td>
</tr>
<tr>
<td>Oxamic acid (0.01M) + Pyruvate</td>
<td>(0.0056M)</td>
<td>+0.2</td>
<td>+12.5</td>
</tr>
<tr>
<td>Sodium iodoacetate</td>
<td>(0.001M)</td>
<td>-98</td>
<td>-92</td>
</tr>
<tr>
<td>Sodium iodoacetate (0.0001M)</td>
<td></td>
<td>-68.4</td>
<td>-73.1</td>
</tr>
<tr>
<td>Sodium fluoride</td>
<td>(0.01M)</td>
<td>-14.2</td>
<td>-19.1</td>
</tr>
<tr>
<td>Sodium fluoride</td>
<td>(0.001M)</td>
<td>+20.2</td>
<td>+16.9</td>
</tr>
</tbody>
</table>

Data are reported as per cent change from control. Control value for intact cells was 0.236 micromoles lactic acid/10^7 cells/hr; for broken cells, 0.327 micromoles/10^7 cells/hr.

amount of color produced by the reaction of glyceraldehyde and the colorimetric reagents was determined by running a glyceraldehyde blank and subtracting that value from the samples containing both cells and glyceraldehyde. Values for lactic acid production were calculated on the basis of micromoles of acid formed by 10,000,000 white blood cells per hour, and are reported as the per cent change from control.

RESULTS

Under the conditions of these experiments the data in table 1 indicate that certain compounds, usually considered as glycolytic inhibitors, did not markedly affect lactic acid formation by normal, human leukocytes. In fact dl-glyceraldehyde actually increased the yield of lactic acid. Sorbose-1-phosphate was not effective, even in broken cells, in a concentration which Lardy et al. have reported to depress essentially all hexokinase activity. Likewise oxamic acid, a specific inhibitor of lactic dehydrogenase, was not markedly effective. But it was of interest that pyruvate did overcome the inhibition of oxamic acid. However, sodium iodoacetate was a potent inhibitor, and sodium fluoride depressed the acid yield in final concentrations above 0.01M. Usually much lower concentrations of fluoride (0.001-0.005M) actively inhibit glycolysis in muscle extracts. The absence of exogenous magnesium might possibly have made this system extremely sensitive to fluoride.

DISCUSSION

From these data one might assume that at least two enzyme systems, hexokinase and lactic dehydrogenase, of the overall glycolytic pathway are somewhat different in leukocytes than in muscle or yeast. These variations are of importance in view of reports in the literature describing the existence of glycogen in leukocytes obtained from citrated horse blood and its conversion of reducing and nonreducing intermediates by phosphorylase activity. Wagner and Yourke have isolated glucose-1-phosphate, fructose-6-phosphate, fructose-1,6-diphosphate, and phosphoglyceric acid from leukocytes. The presence of these
intermediates indicates the existence of phosphorylase, phosphoglucomutase, phosphohexoisomerase and triosephosphate dehydrogenase activity. In addition, Wagner and Yourke\(^8\) observed that hexokinase is present in the insoluble particles of leukocytes. Such evidence is indicative of the existence of a glycolytic pathway comparable to the classic Myerhof-Embden scheme.

The rise in lactic acid noted when cells were incubated with glyceraldehyde might indicate a possible conversion of the latter to the acid. Baker\(^9\) has said that glyceraldehyde stimulated glycolysis in liver and kidney. Needham and Lehmann\(^2\) described the conversion of \(d\)-glyceraldehyde to lactate via methylglyoxal. Embden et al.\(^21\) reported a similar observation with washed blood corpuscles of dogs. However, Levene and Meyer\(^22\) stated that a simple rearrangement of the glyceraldehyde molecule to yield lactic acid did not seem possible. Lardy et al.\(^11\) proved that \(l\)-glyceraldehyde was inhibitory only because it condensed with dihydroxyacetone phosphate to form sorbose-1-phosphate, which in turn was the actual inhibitor of hexokinase. Thus, the inability of glyceraldehyde and of sorbose-1-phosphate to inhibit leukocyte lactic acid production, even in the presence of glucose, may indicate that the hexokinase system as such is altered in mature leukocytes. Since Wagner and Yourke\(^8\) demonstrated the presence of hexokinase activity in the insoluble particles of leukocytes from horse blood, the data reported herein on human leukocytes might indicate a difference in the activity of that enzyme between species. Secondly, the possibility of variation in the hexokinase activity between leukocytes and other cells, yeast for example, is of interest. On the other hand, aerobic glycolysis is said to be more resistant to glyceraldehyde inhibition than anaerobic glycolysis.\(^19\)

Oxamic acid is structurally related to pyruvic acid, and exhibits marked specificity as an inhibitor of lactic dehydrogenase.\(^22\) Since this analog depresses lactic acid production by only 20–25 per cent it may mean that only \(\frac{1}{5}\)–\(\frac{1}{4}\) of the total lactic acid may arise by the reduction of pyruvate. If lactic dehydrogenase activity does account for only this small portion of lactic acid, then this enzyme may be present in mature leukocytes in a smaller quantity than in other cells. A possible difference in lactic dehydrogenase activity, or content, in normal, human leukocytes may correlate with the active glyoxalase system observed in these cells.\(^2\) In this regard Beck\(^23\) has described the preparation of a highly purified lactic dehydrogenase from leukocytes. His data showed that the preparation from leukocytes essentially did not differ kinetically from this same enzyme obtained from other biological sources. Since oxamic acid did not affect the manometric determination of \(\text{CO}_2\) with methylglyoxal as substrate, it is feasible that the fraction of lactic acid production not depressed by oxamic acid may arise by way of this glyoxalase system. If, as these data indicate, only 20–25 per cent of the lactic acid does come from pyruvate, then 75–80 per cent must have another precursor. That figure is in agreement with the 70–75 per cent conversion of glucose to methylglyoxal reported by Schneider and Widmann\(^5\) and of hexosediphosphate to methylglyoxal described by Widmann.\(^6\)

The data reported in this paper tend to agree with earlier observations in the literature regarding the possible synthesis of methylglyoxal as an intermediate in lactic acid formation by leukocytes. Experiments in this laboratory have not yet demonstrated directly the formation and existence of methylglyoxal. How-
ever, preliminary results in analyzing for the appearance of methylglyoxal colorimetrically indicate its formation by human granulocytes under experimental conditions similar to those employed in this report. At least it seems that the coenzyme of glyoxalase, glutathione, may be an essential cofactor for leukocyte lactic acid production because experiments with dialyzed intact cells, poisoned with iodoacetate, have demonstrated its importance. After cells were incubated with 0.0025M sodium iodoacetate and then dialyzed to remove the sulfhydryl inhibitor, lactic acid formation could be restored by the addition of adenosine triphosphate, magnesium, glucose or fructose-1,6-diphosphate, and glutathione. It was of interest that triosephosphate dehydrogenase did not appear to be essential for normal lactic acid production, in contrast to the requirement of glutathione.

Whether the apparent differences in the hexokinase and lactic dehydrogenase systems between leukocytes and other cells indicated by the data reported herein, and the possible existence of methylglyoxal as an intermediate in the breakdown of carbohydrate to lactic acid, can account for the high rate of aerobic lactic acid formation by leukocytes cannot be stated. Neither do these data permit a precise statement regarding the mechanism of aerobic lactic acid production by mature leukocytes obtained from healthy individuals, but they do allude to the possibility of some variations in the pattern of carbohydrate metabolism between these and other cells.

**SUMMARY**

Oxamic acid depressed aerobic lactic acid formation by normal, human leukocytes by only 20-25 per cent. Glyceraldehyde increased lactic acid production. Sorbose-1-phosphate was without effect. Iodacetate inhibited markedly, but fluoride depressed only in high concentrations. The significance of these findings regarding the mechanism of aerobic lactic acid formation by mature leukocytes is discussed.

**SUMMARY IN INTERLINGUA**

Acido oxamico deprimeva le production aerobe de acido lactic per normal, leucocitos human per solmente 20 a 25 pro cento. Glyceraldehydo augmentava le production de acido lactic. Sorbose-1-phosphate eseva sin effecto. Iodacetato eseva un inhibitor de fortia marcate, sed fluorido habeva un effecto depressive solmente in alte concentrationes. Es discutite la signification de iste constatationes relative al mechanismo del formation aerobe de acido lactic per matur leucoctyos human.

**REFERENCES**


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