Respiration and Glycolysis of Normal Human Lymphocytes

By CARL J. HEDESKOV AND VIGGO ESMANN

THE DETERMINATION of the respiratory and glycolytic activity of normal human lymphocytes from blood has been made possible by the recent development of methods for the separation of these cells from blood in sufficient numbers. Available reports show consistent results with respect to the rate of respiration, but threefold variations in glucose uptake and lactic acid production.

Previous experiments on normal human polymorphonuclear (PMN) leukocytes have shown that the metabolic activity of these cells is profoundly inhibited by increasing the concentration of cells employed in the experiments; that is, the activity is the object of a "crowding effect." The present experiments have been undertaken to provide values for oxygen uptake, glucose utilization, and lactic acid production from a sufficient large series of experiments with lymphocytes isolated from normal human blood. Evidence is presented that in incubation experiments with lymphocytes a pronounced crowding effect appears, as well as small but significant Pasteur and Crabtree effects.

MATERIAL AND METHODS

The lymphocytes were obtained from 80 hospitalized patients and healthy members of the staff ranging in age from 18-70 years. The patients suffered from various diseases, primarily duodenal ulcers, functional muscular disorders and psychoneuroses, none of these known to be associated with an abnormal lymphocyte metabolism.

Isolation: Venous blood, 250 ml., was mixed with 190 ml. of a saline solution of dextran (Pharmacia Ltd., TDR 205-II-B-I) and heparin to a final concentration of 1 per cent and 0.05 mg./ml., respectively. The red blood cells (RBC) were allowed to sediment in 50 ml. glass tubes at 37 C. for 45 minutes. The supernatant plasma was then centrifuged at 150 g for 10 minutes, and the residue containing PMN leukocytes, lymphocytes, and the remaining RBC and platelets was resuspended in 10 ml. of the patient’s own plasma previously freed from cells.

The separation of PMN leukocytes and lymphocytes was conducted according to the method of Rabinowitz. The cell suspension was added to a glass column, 25 cm. in length by 1.3 cm. in diameter, which was packed with glass beads (d = 0.2 mm.) to a height of 22 cm. The columns were left at 37 C. After 30 minutes the lymphocytes (and remaining RBC) were eluated at 37 C. with approximately 35 ml. of plasma at a rate of 1.5 ml. per minute. The cells were concentrated by centrifugation at 600 g for 2 minutes and suspended in the appropriate amounts of incubation medium. The PMN leukocytes adhering to the glass beads were discarded.

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The concentration of lymphocytes was determined in duplicate. The coefficient of variation of all countings averaged 6 per cent. The differential counts averaged 98.3 ± 0.4 (s.c.m.) per cent lymphocytes. The yield was 30–35 per cent of the total lymphocytes of the blood specimen.

**Platelet and RBC Contamination:** Most platelets were eliminated by the slow centrifugation of the supernatant plasma after dextran sedimentation, whereas all RBC remaining in the supernatant would ultimately be recovered together with the lymphocytes. In 7 experiments the ratio of platelets to lymphocytes averaged 0.8 ± 0.1, whereas the ratio of RBC to lymphocytes in 11 experiments was 9.5 ± 0.5. The RBC contamination is fourfold that observed by Rabinowitz.1

**Incubation:** The final suspension of lymphocytes was made in a buffer containing NaCl 96 mM., KCl 4.7 mM., MgSO4 1.2 mM., CaCl2 2.5 mM., NaH2PO4 0.2 mM., Na2HPO4 1.0 mM., Tris 45 mM., glucose 16.7 mM., and gelatine 1 per cent. The pH of the suspension was 7.38, but decreased during 4 hours of incubation according to the amount of lactic acid produced, usually 0.2 pH.

Depending on the cell concentration, 1 or 3 ml. aliquots of the lymphocyte suspension was incubated in conventional Warburg vessels for 4 hours after 15 minutes of equilibration with 100 per cent oxygen. The center-well held 0.3 ml. of 5 n NaOH and filter paper. The incubation was terminated by adding perchloric acid from the sidebulb to a final concentration of 3.0 per cent.

In some experiments anaerobic conditions were produced by flushing the reaction flasks with nitrogen previously passed through an alkaline solution of pyrogallol.

**Analytical:** The oxygen consumption (ΔO2) was determined by the direct method of Warburg. The glucose uptake (ΔGluc.) and lactic acid production (ΔLA) were determined from the concentrations of the respective compounds in the deproteinized supernatant before and after incubation. The initial values were obtained from a separate flask after the equilibration period. The results are expressed in micromoles per 1010 lymphocytes per hour. The reference to the units of cell and time will be omitted from the text in the later presentation.

Glucose and lactic acid concentrations were determined by enzymatic methods (Boehringer, Biochimica test combinations).

The accuracy of the experiments were estimated by calculating the average coefficient of variation from duplicate determinations. In 10 experiments values of 2 per cent and 7.2 per cent were found for ΔO2 and ΔLA, respectively. In 5 experiments the corresponding value for ΔGluc. was 7.5 per cent.

**Cell Damage:** The extent of cell damage to PMN leukocytes isolated by dextran sedimentation has previously been determined.6 The following experiments attempt to make similar observations for isolated lymphocytes, as the isolation procedure has been varied from that of PMN leukocytes on two points, besides the introduction of the separation of the lymphocytes from the PMN leukocytes by means of the glass bead column. The total time taken for the preparation of the lymphocyte suspensions was 180–200 minutes, which is 3–4 times more than needed to prepare a suspension of PMN leukocytes. Secondly, a greater force of centrifugation is necessary to sediment the lymphocytes.

Visible or microscopic agglutination of the lymphocytes was never seen.

It was found that ΔO2 was linear with time for at least 4 hours. The effect of the separation of the lymphocytes on the glass bead column was evaluated in experiments with lymphocytes from a patient with chronic lymphatic leukemia having 98 per cent lymphocytes in the peripheral blood. The ΔO2 was found 10 per cent lower for lymphocytes passed through the column than for lymphocytes separated directly from the supernatant plasma after dextran sedimentation.

Cell damage was also investigated by calculating the proportion of lymphocytes which were colored by trypan blue after 30 minutes of incubation at 37 C. with a 1 per cent saline solution of the dye. Immediately after completion of the isolation procedure, less than 1 per cent of the cells took up trypan blue. After 4 hours of incubation 5–6 per cent of the cells were colored.

Finally, leakage of enzymes to the incubation medium was investigated. The extracellular
enzyme activity was expressed in terms of total activity obtained after complete disruption of the cells. The lymphocytes were sonicated at 4°C for 30 seconds (MSE ultrasonic disintegrator, 20,000 kc.). Cellular debris was removed by centrifugation at 1000 g for 10 minutes. Hexokinase activity was measured in a system containing Tris (pH 7.50) 91 mM., human crystalline albumin 0.14 mg/ml., glucose 2 mM., ATP 3 mM., MgCl₂ 3 mM., NADP 0.5 mM., glucose-6-P dehydrogenase 1.1 U./ml., and enzyme corresponding to 1–10 × 10⁶ lymphocytes per ml. in a final volume of 3 ml. Lactic acid dehydrogenase was measured in 3 ml. of a mixture of phosphate buffer (pH 7.50) 50 mM., sodium pyruvate 0.3 mM., NADH 8 mM., and enzyme corresponding to 1–10 × 10⁶ lymphocytes per ml. The activity of hexokinase and lactic acid dehydrogenase averaged 20 and 685 μmoles per 10¹⁰ lymphocytes per minute, respectively. In four experiments the extracellular activity of hexokinase after 4 hours of incubation averaged 1.9 per cent of the total activity. The corresponding value in three determinations of extracellular lactic acid dehydrogenase activity was 2.7 per cent. The extracellular enzyme activity before incubation was approximately half of these values.

Chemicals: NADP, NADH, and glucose-6-P dehydrogenase were obtained from Boehringer and Söhne. GmbH, Mannheim, Germany. ATP and Tris were obtained from Sigma Chemical Company, and the source of human crystalline albumin was Statens Seruminstitut, Copenhagen, Denmark.

Preparation of Glassware: Glass beads were treated with concentrated nitric acid followed by repeated distilled water rinses. After drying, the glass beads were siliconized with Silicote (Bie and Berntsen). The glass beads were discarded after each run.

All other glassware was first treated with carbon-tetrachloride followed by a mixture of equal amounts of concentrated sulfuric and nitric acid. Next, silicone was removed by 10 per cent sodium hydroxide. Finally, the glassware was rinsed with dilute hydrochloride acid and distilled water. All glassware was freshly siliconized before use.

RESULTS

Influence of Lymphocyte Concentration: Figure 1 shows the results of ΔO₂ from 43 experiments with lymphocytes incubated for 4 hours. It is seen that ΔO₂ was highly dependent on the concentration of lymphocytes employed in the individual experiments. At concentrations below 15 × 10⁶ lymphocytes per ml. there is a steep increase in ΔO₂, and values between 150–750 μmoles might be observed. At higher lymphocyte concentrations the influence of the cell concentration on ΔO₂ is not so critical, and above 40 × 10⁶ lymphocytes per ml. values between 100–150 μmoles are usually observed. Readings for ΔO₂ were obtained at intervals during the 4-hour incubation. The same crowding effect appears after ½, 1, and 2 hours of incubation as after 4 hours.

The influence of the cell concentration on ΔLA was not so easily demonstrated as for ΔO₂. In 47 experiments the average ΔLA was 134 ± 7 μmoles. When these results were plotted against the lymphocyte concentrations employed in each experiment, no significant correlation could be obtained. When the results of incubations carried out with lymphocyte concentrations below and above 40 × 10⁶ cells per ml. were compared, the average for 21 and 26 experiments were 145 ± 11 and 124 ± 9 μmoles, respectively. This difference is not statistically significant. Table 1, however, demonstrates that a crowding effect does exist for ΔLA. This table shows the results of experiments with lymphocytes from ten subjects. The cells from each person were incubated at two different concentrations. It is seen that within a large range of cell concentrations 7 of 10 experiments showed a crowding effect for ΔLA. In two experiments there was no difference in ΔLA, and in one experiment ΔLA was
Fig. 1.—Correlation between the lymphocyte concentration and oxygen uptake $(\Delta O_2)$ in 43 experiments with normal human lymphocytes. $\Delta O_2$ is expressed in $\mu$moles per $10^{10}$ lymphocytes per hour. When the results are expressed in a loglog system, a straight line appears: $\log \Delta O_2 = -0.369 \log L + 2.759$ with $s_{\log \Delta O_2, \log L} = 0.085$, where $L$ is the lymphocyte concentration in millions per ml. The regression coefficient and the correlation coefficient $R = 0.91$ are both statistically significantly different from zero ($p < 0.001$). The logarithmic expression has been converted to the more readable form $\Delta O_2 = 574 \times L^{-0.369}$. The dashed lines represent the conversion of $s_{\log \Delta O_2, \log L}$ to the nonlogarithmic system.

Higher at the high lymphocyte concentration. For comparison, the results of $\Delta O_2$ from the same experiments are also given.

The $\Delta$Gluc. of lymphocytes was found low. It was not possible to obtain reliable measurements in experiments with a lymphocyte concentration below $20 \times 10^6$ per ml. In 28 experiments with higher lymphocyte concentrations $\Delta$Gluc. was $66 \pm 5 \mu$mol. Neither in the overall material nor in individual
Table 1.—Crowding Effect of Normal Human Lymphocytes

<table>
<thead>
<tr>
<th>Exp.</th>
<th>Lymphocyte Concentration</th>
<th>Oxygen Uptake</th>
<th>Lactic Acid Production</th>
</tr>
</thead>
<tbody>
<tr>
<td>14</td>
<td>17.7</td>
<td>151</td>
<td>103</td>
</tr>
<tr>
<td></td>
<td>5.5</td>
<td>262</td>
<td>118</td>
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<tr>
<td>15</td>
<td>60.2</td>
<td>138</td>
<td>146</td>
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<tr>
<td></td>
<td>17.4</td>
<td>187</td>
<td>200</td>
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<tr>
<td>16</td>
<td>38.0</td>
<td>164</td>
<td>112</td>
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<tr>
<td></td>
<td>11.7</td>
<td>263</td>
<td>110</td>
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<tr>
<td>17</td>
<td>28.6</td>
<td>180</td>
<td>133</td>
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<td></td>
<td>9.1</td>
<td>240</td>
<td>141</td>
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<td>18</td>
<td>22.1</td>
<td>170</td>
<td>124</td>
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<tr>
<td></td>
<td>5.5</td>
<td>333</td>
<td>289</td>
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<td>19</td>
<td>15.5</td>
<td>216</td>
<td>140</td>
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<tr>
<td></td>
<td>5.0</td>
<td>298</td>
<td>210</td>
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<td>20</td>
<td>41.6</td>
<td>139</td>
<td>98</td>
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<tr>
<td></td>
<td>12.3</td>
<td>197</td>
<td>174</td>
</tr>
<tr>
<td>22</td>
<td>30.0</td>
<td>145</td>
<td>126</td>
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<tr>
<td></td>
<td>8.9</td>
<td>485</td>
<td>187</td>
</tr>
<tr>
<td>23</td>
<td>32.0</td>
<td>154</td>
<td>162</td>
</tr>
<tr>
<td></td>
<td>14.3</td>
<td>230</td>
<td>139</td>
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<tr>
<td>25</td>
<td>82.2</td>
<td>122</td>
<td>114</td>
</tr>
<tr>
<td></td>
<td>2.3</td>
<td>614</td>
<td>186</td>
</tr>
</tbody>
</table>

*× 10⁶ per ml.
†μmoles per 10¹⁰ lymphocytes per hour.

Experiments with cells from the same person could a crowding effect be demonstrated for ΔGluc.

Influence of RBC: The influence of the contaminating RBC on the experimental results obtained with the lymphocyte suspensions was evaluated. Table 2 shows the results of experiments conducted with pure suspensions of RBC at concentrations within the range contaminating the lymphocyte suspensions. It is seen that no glucose utilization could be detected. The ΔLA averaged 3.0 μmoles. With an average ratio of 9.5 RBC per lymphocyte, this means that an average value of ΔLA for pure lymphocytes might be obtained by subtracting 30 μmoles from the value observed with a lymphocyte suspension contaminated with RBC. The ΔO₂ of RBC apparently also exhibits a crowding effect (Table 2), and the necessary correction to apply on ΔO₂ for the lymphocyte suspensions contaminated with RBC would vary between 5-20 per cent for lymphocyte concentrations between 70–7 × 10⁶ cells per ml.

Table 2.—Metabolism of Pure Suspensions of Red Blood Cells

<table>
<thead>
<tr>
<th>Exp.</th>
<th>Red Blood Cell Concentration</th>
<th>Oxygen Uptake</th>
<th>Glucose Utilization</th>
<th>Lactic Acid Production</th>
</tr>
</thead>
<tbody>
<tr>
<td>40</td>
<td>76</td>
<td>5.3</td>
<td>not detectable</td>
<td>3.0</td>
</tr>
<tr>
<td>41</td>
<td>140</td>
<td>2.7</td>
<td>not detectable</td>
<td>2.8</td>
</tr>
<tr>
<td>41</td>
<td>253</td>
<td>1.7</td>
<td>not detectable</td>
<td>3.0</td>
</tr>
<tr>
<td>40</td>
<td>366</td>
<td>1.8</td>
<td>not detectable</td>
<td>3.4</td>
</tr>
<tr>
<td>42</td>
<td>500</td>
<td>0.9</td>
<td>not detectable</td>
<td>2.9</td>
</tr>
<tr>
<td>42</td>
<td>788</td>
<td>0.7</td>
<td>not detectable</td>
<td>2.9</td>
</tr>
</tbody>
</table>

*× 10⁶ per ml.
†μmoles per 10¹⁰ red blood cells per hour.
HEDESKOV AND ESMANN

Table 3.—Metabolism of Normal Human Lymphocytes and Polymorphonuclear Leukocytes

<table>
<thead>
<tr>
<th></th>
<th>Oxygen Uptake</th>
<th>Glucose Utilization</th>
<th>Lactic Acid Production</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>observed</td>
<td>corrected</td>
<td></td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>131 ± 4.5†</td>
<td>(n = 22)</td>
<td>124 ± 9.0</td>
</tr>
<tr>
<td></td>
<td>corrected for red blood cells‡</td>
<td>117</td>
<td>62</td>
</tr>
<tr>
<td>Polymorphonuclear</td>
<td>117</td>
<td>437</td>
<td>640</td>
</tr>
<tr>
<td>Leukocytes†</td>
<td>117</td>
<td>437</td>
<td>640</td>
</tr>
</tbody>
</table>

*µmoles per 10¹⁷ cells per hour.
†Mean ± s.e.m.
‡Corrected for an average contamination of 9.5 red cells per lymphocyte.
§Calculated for experiments at a cell concentration of 40 × 10⁶ per ml.

In view of the existence of the crowding effect, it might be deceptive to tabulate average values for lymphocyte metabolism. Above a cell concentration of 40 × 10⁶ lymphocytes per ml., however, the influence of the cell concentration is not great, and reasonable figures might be given (Table 3). For comparison the similar values from earlier experiments with PMN leukocytes at a concentration of 40 × 10⁶ cells per ml. are included. It is seen that the respiratory activity of the two kinds of cells is equal, but that ∆Gluc. and ∆LA is 7 times lower in lymphocytes than in PMN leukocytes.

Influence of Glucose and Oxygen: In 7 paired experiments with high lymphocyte concentrations, the average ∆O₂ in experiments without and with 16.7 mM glucose was 137 ± 6 and 120 ± 7 µmoles, respectively, and a small but significant (p < 0.01) Crabtree effect was thus observed. Similarly, a small Pasteur effect could be demonstrated. The average ∆LA in 5 experiments was 147 ± 26 and 127 ± 26 µmoles during anaerobic and aerobic conditions, respectively. Treated as paired experiments this difference is statistically significant (p < 0.01). Control experiments with pure suspensions of RBC revealed that the observed Crabtree and Pasteur effects of lymphocyte suspensions could not be attributed to the contaminating RBC.

The influence of the glucose and oxygen concentration on the crowding effect was investigated by comparing a fivefold variation in lymphocyte concentration with fivefold variations in oxygen and glucose concentration. Columns b and c of Table 4 show the normal crowding effect on ∆O₂ when the lymphocyte concentration is increased fivefold. In column d the same ratio of lymphocyte concentration to oxygen concentration as in column b was obtained by lowering the oxygen concentration to 20 per cent. It is seen that in this way the same decrease of ∆O₂ as in column b was obtained at the low lymphocyte concentration. An attempt to abolish the crowding effect by increasing the glucose concentration fivefold (column a) was unsuccessful.

Influence of Incubation Time: Figure 2 shows the results of experiments in which the influence of the incubation time was evaluated. It is seen that ∆O₂
Table 4.—Effect of Varying Lymphocyte, Oxygen and Glucose Concentration

<table>
<thead>
<tr>
<th>Exp.</th>
<th>Lymph. $\times 10^6$/ml. (L)!*</th>
<th>Oxygen concentration</th>
<th>Glucose concentration</th>
<th>(\Delta \text{O}_2)</th>
<th>(\Delta \text{Gluc.})</th>
</tr>
</thead>
<tbody>
<tr>
<td>72</td>
<td>$5 \times L$ 1500 mg. % 100%</td>
<td>94</td>
<td>99</td>
<td>145</td>
<td>102</td>
</tr>
<tr>
<td>76</td>
<td>$5 \times L$ 300 mg. % 100%</td>
<td>62</td>
<td>59</td>
<td>70</td>
<td>51</td>
</tr>
<tr>
<td>78</td>
<td>$5 \times L$ 300 mg. % 100%</td>
<td>119</td>
<td>112</td>
<td>139</td>
<td>118</td>
</tr>
<tr>
<td>79</td>
<td>$5 \times L$ 300 mg. % 100%</td>
<td>103</td>
<td>104</td>
<td>141</td>
<td>114</td>
</tr>
</tbody>
</table>

*The lymphocyte concentration $5 \times L$ in columns a and b was five times the concentration (L) employed in the parallel experiments in column c and d. In individual experiments L varied between $12-20 \times 10^6$ lymphocytes per ml.

$\Delta \text{O}_2$ and $\Delta \text{Gluc.}$ were linear with time, whereas $\Delta \text{Gluc.}$ was much higher during the first 30 minutes of incubation than later. In the interval 30-240 minutes, $\Delta \text{Gluc.}$ was nearly linear.

**Discussion**

The suspensions of lymphocytes used in these experiments were prepared essentially according to the principle of Johnson and Garvin\!* as modified by Rabinowitz.\!* Regarding the yield of lymphocytes and the almost complete elimination of PMN leukocytes, monocytes, and platelets, our results are in accordance with the work of Rabinowitz. The contamination with RBC was 3 times greater, which, however, essentially reflects the completeness of the dextran sedimentation. In our hands,\!* as well as in the original detailed investigation of Skoog and Beck,\!* the number of RBC per white cell in the supernatant plasma after dextran sedimentation is 2-3. Eliminating the PMN leukocytes and monocytes but retaining all the RBC would increase the number of RBC per white cell with a factor of approximately 3, which actually also was observed. In one minor respect our sedimentation procedure deviated from Rabinowitz’s, for we sedimented the RBC at 37 C. in order to accelerate the procedure. It is well known that the contamination with RBC decreases somewhat with decreasing temperature during the sedimentation, but this cannot explain the difference.

The contamination with RBC unfortunately proved to be of some significance for the magnitude of respiration and glycolysis of the lymphocyte suspensions. Attempts to eliminate the RBC by osmotic shock for 30 seconds as described by Fallon et al.\!* proved unsuccessful in our hands. It was therefore necessary to evaluate the influence of the contaminating RBC by control incubations with pure suspensions of red cells. The usually reported values\!* for $\Delta \text{Gluc.}$ of 1-1.5 $\mu$moles per $10^9$ RBC per hour were not detectable with concentrations of $0.7-7 \times 10^8$ RBC per ml contaminating our lymphocyte preparations, as the sensitivity of the method for glucose determination did not
Fig. 2.—Time course of respiration and glycolysis of lymphocyte suspensions. Lymphocyte suspensions (40 × 10 ml.) were incubated in Warburg flasks for 4 hours. After 15 minutes of equilibration with 100 per cent oxygen, glucose was tipped from the side bulb at zero time to a final concentration of 16.7 mM. Aliquots were drawn for glucose and lactic acid determinations at the indicated intervals. The results are expressed in μmoles per 10^10 lymphocytes ± s.e.m. on the basis of 8 (glucose uptake), 7 (oxygen consumption), and 4 (lactic acid production) experiments.

permit the reliable determination of concentration differences of less than 5 mg./100 ml. from an initial value of 300 mg./100 ml. glucose. ΔLA of RBC was 3.0 μmoles and showed no crowding effect, so that a constant correction for ΔLA might be used. RBC, however, exhibited a crowding effect with respect to ΔO_2 different from the crowding effect of the lymphocyte suspensions. The necessary correction was small at high lymphocyte concentrations, but in experiments with low cell concentrations the respiration of RBC amounted to 20 per cent of the respiration of the lymphocyte suspensions.

The different methods used to evaluate the viability of the isolated lymphocytes agreed well. Passage through the glass bead column lowered the respiration with 10 per cent in agreement with the value found by Rabinowitz. The respiration was, however, linear with time. The reason for the lower but seemingly undamaged respiration is not apparent. Cellular injury evaluated by the uptake of trypan blue or leakage of hexokinase and lactic acid dehydro-
genase to the extracellular fluid showed that 1 per cent of the lymphocytes were damaged by the isolation procedure and a further 1-4 per cent of the cells were injured during the incubation period of 4 hours. The extent of this damage corresponded to the damage reported earlier on PMN leukocytes isolated and incubated under similar conditions, and it must also here be concluded that the lymphocyte suspensions consisted predominantly of intact cells.

The most notable feature of the reported experiments is the demonstration of a pronounced crowding effect on $\Delta O_2$ of lymphocytes. This effect has previously been found with normal and leukemic PMN leukocytes and with leukocytes from peritoneal exudates of guinea pigs, not only on $\Delta O_2$ but also on $\Delta$ Gluc., $\Delta$ LA., and $\Delta$ Glycogen. In the present experiments the crowding effect was investigated over a very wide range of lymphocyte concentrations (0.5-128 x 10^6 cells per ml.), and a great regularity in the dependency of $\Delta O_2$ on the lymphocyte concentration was found. In addition, a crowding effect was also found to exist for $\Delta O_2$ of RBC, and thus all the main components of blood cells is now known to exhibit this peculiar phenomenon.

The cause of the crowding effect is not known. With PMN leukocytes it has previously been shown that the crowding effect is not caused by variations of pH, lack of inorganic phosphate or glucose, nor is it due to an inhibitory action of accumulated lactic acid. Hartman was not able to demonstrate a crowding effect with PMN leukocytes early in the incubation, but in the present experiments with lymphocytes the same crowding effect was found after half an hour as after 4 hours of incubation, which seems to exclude that the crowding effect is caused by an accumulation of an inhibitor.

The experiments reported in Table 4 showed that a proportionate rise in lymphocyte concentration or decrease in oxygen concentration elicited the same fall in $\Delta O_2$. This might be coincidence, but it does seem to suggest that the crowding effect on $\Delta O_2$ might be caused by a reduced availability of oxygen in the crowded suspensions. It is known that the viscosity of PMN leukocytes suspended in plasma increases with increasing cell concentration within the range 40-600 x 10^6 per ml. It might be imagined that a similar relationship may be found for lymphocytes suspended in buffer. It is mentioned by Roughton that the rate of solution of gases depends on the viscosity of the liquid and that the rate of solution of gases in whole blood is only one-fifth of the rate in pure water. It might be possible that an increase in viscosity is responsible for a decreased oxygen concentration in the dense lymphocyte suspensions, even in the presence of a 100 per cent oxygen atmosphere. Direct measurements of the oxygen concentration in the cell suspensions by polarographic techniques would be suitable to test this hypothesis.

It was not possible to obtain a mathematical expression of the crowding effect on $\Delta$ LA, as has previously been found for PMN leukocytes. The presence of a crowding effect on $\Delta$ LA was, however, evident in experiments where lymphocytes from the same person were incubated at different concentrations. The failure of demonstrating a crowding effect on $\Delta$ Gluc, might have been caused by the difficulty of obtaining reliable measurements of the glucose
uptake at low lymphocyte concentrations, where the expected fall in glucose concentration would amount to 1 per cent or less of the initial concentration. On account of the crowding effect comparisons of our results with the results obtained in other laboratories are somewhat difficult, especially in case of experiments performed with low lymphocyte concentrations, where a small change in cell concentration causes a large increase in $\Delta O_2$. With suspensions of lymphocytes in buffer with added plasma, Rabinowitz\(^1\) found in 6 experiments $\Delta O_2$ 183 $\mu$moles at a cell concentration of $25 \times 10^6$ lymphocytes per ml. Rauch et al.\(^3\) isolated lymphocytes by 20 minutes of centrifugation at 320 g after loading the PMN leukocytes with iron particles. The lymphocytes suspended in Tris buffer at a concentration of $20-30 \times 10^6$ cells per ml. showed increasing glucose utilization with increasing glucose concentration. At the highest glucose concentration tested (9.5 mM.), these authors found $\Delta O_2$ 175 $\mu$moles, $\Delta$Gluc. 110 $\mu$moles, and $\Delta$LA 175 $\mu$moles in 6 experiments. By interpolation on Figure 1 it is seen that $\Delta O_2$ in the reported experiments agree very well with our results. The somewhat higher glycolytic activity found by Rauch et al. might very well be within the limits of our results, considering their few experiments and the large sample variation of $\Delta$LA and $\Delta$Gluc. Finally, Kiss and Schuler\(^4\) have reported glycolytic values for lymphocytes isolated from the blood of normal children by centrifugation in a modified Cushman tube at 800 g for 13 minutes. These authors found in five experiments with $10-20 \times 10^6$ lymphocytes suspended in plasma at pH 7.4 values for $\Delta$Gluc. and $\Delta$LA of 303 $\mu$moles and 610 $\mu$moles, respectively. These results are very different from ours, and might indicate either cell damage due to the prolonged centrifugation or possibly a real difference between lymphocytes of children and adults.

The results presented in Table 3 show that while the respiratory activity of normal PMN leukocytes and lymphocytes, expressed on a cellular basis, is the same, $\Delta$Gluc. and $\Delta$LA of lymphocytes are much smaller than for PMN leukocytes. It must, however, be concluded that lymphocytes have an appreciable aerobic glycolysis accounting for approximately 75 per cent of the glucose uptake. The metabolism of lymphocytes from patients with chronic lymphatic leukemia has usually been found low when compared with normal mixed white cells consisting predominantly of PMN leukocytes.\(^{10,11,17}\) The present results indicate that no great difference appears to exist between the glycolysis and respiration of normal lymphocytes and values reported on cells isolated from patients with chronic lymphatic leukemia.

The presence of a Pasteur effect has previously been found both in normal and leukemic lymphocytes\(^4,10,17\) and is also demonstrated in this series carried out with normal cells. The Crabtree effect has not previously been demonstrated with normal lymphocytes, while evidence for the presence of this effect in leukemic lymphocytes is equivocal.\(^{13,18}\)

After addition of glucose to the "starved" lymphocytes, a surplus of glucose apparently enters the cells rapidly. It is unlikely that the increased $\Delta$Gluc. during the first 30 minutes of incubation (Fig. 2) is caused by an accumulation of free intracellular glucose, as it must be assumed that any free intracellular glucose would be determined together with extracellular glucose after deplo-
teinization of the sample with perchloric acid. The implication is that since \( \Delta LA \) proceeds linear with time, the rate of glycolysis is controlled by some step other than the hexokinase reaction.

**Summary**

The metabolism of intact, normal, human lymphocytes in vitro was studied from a total of 80 subjects. Corrected for the metabolism of contaminating red blood cells, the glucose uptake, lactic acid production, and oxygen consumption were 62, 95, and 117 \( \mu \)moles per \( 10^{10} \) lymphocytes per hour, respectively, provided the cells were incubated at concentrations greater than \( 40 \times 10^6 \) lymphocytes per ml. At lower lymphocyte concentrations the oxygen consumption per lymphocyte rose steeply with decreasing cell concentration (crowding effect). A similar but weaker crowding effect was noted for the lactic acid production, but not for the utilization of glucose.

The oxygen uptake was lower with 20 per cent than with 100 per cent oxygen as gas phase. Small Pasteur and Crabtree effects were demonstrated. The oxygen consumption and lactic acid production proceeded linear with time, while the glucose utilization was higher during the first 30 minutes of incubation than later on.

It is concluded that lymphocytes have a low aerobic glycolysis accounting for 75 per cent of the glucose utilization. The respiration is severely inhibited at high cell concentrations and it is suggested that this is caused by an insufficient availability of oxygen to the cells.

**Summario in Interlingua**

Le metabolismo de intacte normal lymphocytos human esseva studiate in vitro pro un total de 80 subjectos. Post correction pro le metabolismo de contaminante erythrocytos, il esseva trovate que le acceptation de glucosa, le production de acido lactic, e le consumo de oxygeno esseva 62, 95, e 117 \( \mu \)mol per \( 10^{10} \) lymphocytos per hora, respectivemente, providite que le cellulas esseva incubate a concentrationes de plus que \( 40 \times 10^6 \) lymphocytos per ml. A plus basse concentrationes de lymphocytos, le consumo de oxygeno per lymphocyt montava marcatemente con le declino del concentration cellular (effecto del congerimento). Un simile sed minus marcate effecto esseva notate pro le production de acido lactic sed non pro le utilisation de glucosa.

Le acceptation de oxygeno esseva plus basse a 20 pro cento de oxygeno que a 100 pro cento. Micre effectos de Pasteur e de Crabtree esseva demonstrate. Le consumo de oxygeno e le production de acido lactic progredeva in linearitate con le tempore, durante que le utilisation de glucosa esseva plus alte durante le prime 30 minutas de incubation que plus tarde.

Ex concludite que lymphocytos ha un basse glycolyse aerobie, lo que es responsable pro 75 pro cento del utilisation de glucosa. Le respiration es severmente inhibitae a alte concentrationes cellular, e il es postulate que isto es causate per le insufficiente accessibilitate de oxygeno per le cellulas.

**REFERENCES**

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Respiration and Glycolysis of Normal Human Lymphocytes

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