The Effect of Phytohemagglutinin upon Glucose Catabolism in Lymphocytes

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With the technical assistance of Julia Woransberg and Jackie Spangler

The nature and the function of the metabolic pathways responsible for the utilization of glucose by human lymphocytes are yet to be thoroughly investigated. In 1938 Warburg et al. demonstrated that with regard to utilization of glucose by leukocytes, anaerobic catabolism is far more important than aerobic catabolism. Kits reported that in splenic and thymic lymphocyte preparations, the pentose phosphate (PP) pathway is operative as evidenced by experimental findings using 14C specifically labeled glucose as substrates. Beck has estimated, on the basis of findings in enzyme studies with white blood cell homogenates, that approximately 3 per cent of the glucose utilized was by way of the PP pathway. MacHaffie and Wang reported that radiorespirometric experiments with 14C specifically labeled glucose as substrate demonstrated that glucose is utilized by intact lymphocytes predominantly by way of the glycolytic pathway, leading to the formation of lactate and pyruvate. Their results also revealed that the PP pathway is playing a rather minor role responsible for the utilization of not greater than 2 per cent of the catabolized glucose (Fig. 1).

More recently, the effect of phytohemagglutinin upon mitosis of lymphocyte cells has been reported by Nowell. However, it is not known whether the phytohemagglutinin exerts any effect upon the catabolic mechanism for glucose utilization by lymphocytes.

In the present work, radiorespirometry has been employed to gain information on the utilization of glucose by intact lymphocytes, under physiologic conditions, in the presence and in the absence of phytohemagglutinin. Studies were directed to the detection of the effect, if any, of phytohemagglutinin upon glucose catabolism in these cells.

Materials and Methods

Lymphocyte Cells. For each series of experiments, 200 ml of whole blood was collected aseptically from healthy male donors and to it was added 2.0 ml of a 0.1 per cent heparin solution (U.S.P. Grade, Sigma Chemical Company, St. Louis, Missouri) in normal physiologic saline and 45 ml of sterilized fibrinogen solution (Bovine, Fibrinogen I. Nutritional Bio...
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Fig. 1.—EMF = Emden-Meyerhoff-Parnass Anaerobic Glycolytic Pathway. PP = Pentose Phosphate Pathway. PC = Pentose Cycle Pathway. GA = Glucuronic Acid Pathway. TCA = Krebs Cycle Pathway.

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Chemicals. Cleveland, Ohio). The erythrocytes and neutrophiles were allowed to settle while the mixture was placed in an incubator which was maintained at 37.5 C. for 4 to 5 hours. The supernatant was carefully drained off aseptically into individual incubation flasks. The suspension was found to be composed of approximately 90 per cent of lymphocytes and 10 per cent of other blood cells.

Rate of Glucose Utilization. The rate of glucose utilization by lymphocytes was determined by the use of the "glucostat" (glucose oxidase) method in contrast to the studies of previous investigators based on the method of Somogyi-Nelson. The lymphocyte suspension prepared in the foregoing described manner was incubated at 37.5 C. in an atmosphere of air enriched with CO₂ to the extent of 5 per cent. Samples of the suspension
were withdrawn periodically, under aseptic conditions, for glucose analysis. Total incubation period was 72 hours. Lymphocytes isolated from the blood samples of six male donors were used in this study.

Radiorespirometry. For the radiorespirometric experiment, 5 ml aliquots of the lymphocyte suspension, prepared in the previously described manner and containing approximately 2 to 3 \( \times 10^7 \) cells, were transferred aseptically into a respiration flask equipped with a side arm. The side arm, sealed with a sterile serum cap, was loaded with 1 ml of a sterilized solution containing a prescribed amount of the \( ^{14}\text{C} \) specifically labeled glucose, buffered with phosphates at pH 7.4. In experiments involving the use of phytohemagglutinin, 0.1 ml of the mitogen in saline was added either into the flask or into the side arm. The respiration chamber was flushed with a stream of sterile air, enriched with \( \text{CO}_2 \) to the extent of 5 per cent. The inlet and outlet aeration tubings of the respiration flask were closed off and the lymphocyte suspensions in the flask were incubated for a prescribed period of time at 37.5 to deplete glucose in the incubation medium. This was followed by the tip-in of the contents in the side arm to the cell suspension, and incubation was allowed to continue for an additional 48 hours.

Upon completion of the incubation period, 0.5 ml of the cell suspension was transferred out by way of the serum cap of the side arm, using a sterilized plastic syringe. One-half of the sample so collected was placed onto a nutrient agar plate to ascertain the sterility of the incubation mixture. Two-tenths ml of the sample was used for the determination of glucose content by means of the glucostat method. The remaining portion of the sample was examined microscopically with regard to cell morphology and viability and tested for pH which was maintained between pH 7.2 and 7.4. To the main portion of the cell suspension remaining in the respiration chamber was added, by way of the serum cap of the side arm, 2 ml 2 N HCl and the air in the respiration chamber, containing respiratory \( ^{14}\text{CO}_2 \), was flushed by way of the inlet and outlet tubings with a stream of air at 60 ml/min into a \( \text{CO}_2 \) trap containing ethanolamine-ethanol (2:1 v/v) for a period of 20 minutes. The radioactivity in the ethanolamine solution was then measured by means of liquid scintillation counting, according to the method of Wang.

The cell suspension was neutralized with an aqueous NaOH solution and the cells were separated from the incubation medium by means of centrifugation. The cells were washed three times with a phosphate buffered solution, dissolved in N.C.S. solubilizer (Nuclear-Chicago Corporation, Des Plaines, Ill.), and an aliquot of the cell solution was mixed with ethanolamine-ethanol-toluene solution for radioactivity measurement by means of liquid scintillation counting. An aliquot of the clear incubation medium was mixed thoroughly with a thixotropic gel preparation and the radioactivity of the sample so prepared was also counted by means of liquid scintillation counting.

RESULTS

The rate of glucose utilization by human lymphocyte cells isolated from blood samples of six male donors was found to range from 0.102 to 0.268 \( \mu\text{M/hr.} \times 10^7 \) cells. The mean rate of utilization being calculated as 0.17 \( \pm 0.07 \) \( \mu\text{M/hr.} \times 10^7 \) cells. The comparison of the findings in the present work and that reported in the literature is given in Table 1.

<table>
<thead>
<tr>
<th>Investigators</th>
<th>Rate of Glucose Utilization (( \mu\text{M/hr.} \times 10^7 ) cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vanotti et al.</td>
<td>0.348 ( \pm 0.067 )</td>
</tr>
<tr>
<td>Kalant &amp; Sucher</td>
<td>0.35 ( \pm 0.06 )</td>
</tr>
<tr>
<td>Kiss &amp; Schuler</td>
<td>0.45 ( \pm 0.06 )</td>
</tr>
<tr>
<td>Lohr</td>
<td>0.10 ( \pm 0.017 )</td>
</tr>
<tr>
<td>Dumm</td>
<td>0.35 ( \pm 0.03 )</td>
</tr>
<tr>
<td>MacHaffie &amp; Wang</td>
<td>0.17 ( \pm 0.07 )</td>
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</tbody>
</table>
Table 2.—Utilization of \(^{14}\)C Specifically Labeled Glucose Substrate by Lymphocytes in the Presence or Absence of Phytohemagglutinin*

<table>
<thead>
<tr>
<th>Substrates</th>
<th>Substrate Radioactivity Inventory (^{14})C of Administered Glucose</th>
<th>Respiratory (^{14})CO₂</th>
<th>Cells</th>
<th>Incubation Medium</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Control PHA</td>
<td>With PHA</td>
<td>Control PHA</td>
</tr>
<tr>
<td>Glucose</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C-1 0.05</td>
<td></td>
<td>2.0</td>
<td>4.0</td>
<td>0.2</td>
</tr>
<tr>
<td>C-2 0.05</td>
<td></td>
<td>2.0</td>
<td>3.0</td>
<td>0.3</td>
</tr>
<tr>
<td>C-3(4) 0.05</td>
<td></td>
<td>1.0</td>
<td>2.0</td>
<td>0.1</td>
</tr>
<tr>
<td>C-6 0.05</td>
<td></td>
<td>0.5</td>
<td>0.5</td>
<td>0.4</td>
</tr>
</tbody>
</table>

*Experimental conditions: Lymphocyte concentration used in each of the experiments was approximately \(2.4 \times 10^7\) cells per 5 ml. of incubation medium. The cell suspension in the incubation flask was incubated without external glucose substrate for a prescribed period of time. At the end of this incubation period, samples were withdrawn from the respective incubation flasks aseptically for glucose analysis. When analysis indicated that the glucose in the medium has been completely exhausted, 0.05 mg. of \(^{14}\)C specifically labeled glucose substrate was tipped into the incubation flask from the side arm, and the incubation was allowed to continue for 45 hours.

In Table 2 are given the findings of radiorespirometric experiments designed to investigate the effect, if any, of phytohemagglutinin upon relative participation of glucose pathways in lymphocytes. Carbon-14 specifically labeled glucose samples were used as the test substrates. The results given in Table 2 represent the average value of four replica experiments. Deviation among replica experiments with a given substrate was less than 5 per cent.

Several conclusions can be drawn from these findings. First since in each of these series of experiments the administered glucose in the incubation medium was found to be completely utilized, the radioactivity found in the medium represents primarily that of lactic acid, pyruvic acid and amino acids. A crude examination of the nature of the radioactive compounds in the medium revealed that lactic acid represents approximately 50 per cent of the radioactivity in the medium, and the pyruvic acid represents approximately 10 per cent of the radioactivity in the medium. Of interest is the finding that approximately 30 per cent of the radioactivity in the medium resides in several amino acids, particularly valine, threonine, serine and alanine. The latter fact indicates that some of the metabolic intermediates such as pyruvate, derived from substrate glucose, have been involved in the biosynthesis of amino acids.

It is evident from the results given in Table 2 that whereas phytohemagglutinin did not alter the yield of respiratory \(^{14}\)CO₂ from C-6 of glucose, the mitogen did exert significant effect upon the yields of \(^{14}\)CO₂ from other labeled glucose carbon atoms. One finds that the conversion of C-2, C-3(4) and particularly C-1 of glucose to CO₂ was significantly increased. These findings can be interpreted to indicate that phytohemagglutinin enhanced the operation of the PP pathway and, to some extent, the operation of the pentose cycle (PC) pathway in lymphocytes. The conclusion is drawn from the kinetics of the formation of respiratory CO₂ from glucose carbon atoms via various pathways. As shown in Figure 1, the enhanced participation of the PP pathway in
the glucose catabolism would lead to an increased conversion of C-1 of glucose to respiratory CO₂. If the pentose phosphate formed via the PP pathway participated extensively in the PC pathway, one would expect increased yields in respiratory CO₂ from C-2 and C-3 and, to some extent, C-4 of glucose. The fact that an enhanced conversion of C-6 glucose to CO₂ is not observed in the PHA experiment leads one to believe that PHA does not exert noticeable effect upon the operation of the EMP pathway in lymphocytes.

**Discussion**

Phytohemagglutinin has been shown by Rigas and Johnson\(^1\) to be a mixture of a mucoprotein and a protein, both of which have mitogenic activity. The protein mitogen is poor in sulphur-containing amino acids, rich in aspartic acid, asparagine, serine, threonine and leucine with alanine as the only N terminal amino acid. This protein has a molecular weight of the order of 128,000.

The mitogenic activity of phytohemagglutinin has been reported in detail by Nowell et al.\(^5\) and Marshall and Roberts.\(^19\) These authors have shown that 48 hours after the addition of PHA to suspension cultures of lymphocytes, a high percentage of large dividing cells was observed. It was speculated that the small lymphocyte transforms into a large blast-like cell capable of mitosis.

Quaglino et al.\(^2\) reported that the activity of the dehydrogenase systems, associated with glycolysis, was high in both the blast cells and in the transforming lymphocytes. Hirschhorn et al.\(^21\) observed a sharp increase in the activity of acid phosphatase in lymphocytes stimulated by PHA, and the histochemical delineation of innumerable granules containing acid phosphatase in the cytoplasm at 48 to 72 hours.

The synthesis of RNA acid, in PHA-stimulated lymphocytes, was studied by Epstein and Stohlman,\(^22\) using tritium-labeled cytidine. DNA synthesis was also studied by examining incorporation of tritiated thymidine. During the first 24 hours RNA synthesis was evident, and at 72 hours 80 per cent of the cells, mostly the large blast-like cells, showed labeled RNA granules. No DNA synthesis occurred in the first 24 hours. Subsequently, DNA synthesis proceeded to a peak level at 72 hours when 55 per cent of all cells were labeled with thymidine-H\(^3\). These workers concluded that PHA induced RNA synthesis early in the culture of lymphocytes. This results in the transformation of the small lymphocytes to the large blast-like cells which are then capable of synthesizing DNA. The observations of Elves et al.,\(^23\) Hirschhorn et al.,\(^24\) and others of similar blastogenic transformation with the production of antibody-bearing gamma globulin by these transformed cells suggest that an antigenic stimulus triggers the transformation reaction. This is partially confirmed by Nowell's observation\(^25\) that prednisolone (a synthetic corticosteroid) blocks the transformation of lymphocytes induced by PHA.

From results of this work, it can be reasoned that the enhanced operation of the PP pathway may reflect the additional need of intermediates formed from glucose, such as pentoses, for biosynthetic functions during the induced mitosis. Thus one finds that, in the presence of phytohemagglutinin, glucose carbon atoms were incorporated into cellular constituents in noticeably greater
degree. The enhanced operation of the PC pathway may reflect an additional requirement of TPNH during the mitosis of lymphocytes, since it is known that the operation of the PC pathway is a major mechanism in the production of TPNH in biological systems, and that TPNH is required for the synthesis of DNA.

The alteration of glucose pathways resulting from change in cultural conditions have been reported by several authors. Thus, Cohen\textsuperscript{26} reported that under aerobic conditions, the rapid-growing cells of Escherichia coli metabolize glucose primarily by way of the PP pathway, whereas glucose metabolism of resting cells relies heavily on the glycolytic pathway. Heath and Koffler\textsuperscript{27} reported that the participation of the PP pathway in \emph{Penicillium chrysogenum} was increased with the rate of growth. Wang and Krackov\textsuperscript{28} observed that with \emph{Bacillus subtilis} cells grown in simple medium rely more on the PP pathway in comparison to the cells grown in complex medium.

**Summary**

The glucose metabolism in intact lymphocyte cells was found to be altered in the presence of phytohemagglutinin. The operation of the pentose phosphate pathway and, to some extent, the pentose cycle pathway in lymphocytes was found to be significantly enhanced. This finding is interpreted to reflect an additional need of biosynthetic intermediates and TPNH by the lymphocytes to accommodate mitotic activity induced by phytohemagglutinin.

**Summario in Interlingua**

Il esseva trovate que le metabolismo de glucosa in intae cellulas lymphocytic es alterate in le presentia de phytohemagglutinina. Le funzionate del circuito de phosphato de pentosa e, minus extensemente, del circuito del cyclo de pentosa se revelava, in le lymphocytos assi exponite, significativemente promovite. Iste constatation es interpretate como supporto del these que le lymphocytos, a fin que illos pote maestrar le activitate mitotic inducite per phytohemagglutinina, require le presentia de intermediarios biosynthetic e de reducite nucleotida tripfosphopyridinic.

**REFERENCES**

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