EFFECTS OF AN ADRENOCORTICAL EXTRACT ON TISSUE GLYCOLYSIS IN VITRO*

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Several investigators have reported that a commercial adrenal extract, Lipo-Adrenal Cortex,1 possesses marked lymphocytolytic action in vitro not manifested by other preparations (1-3). In the hope of correlating this finding with a change in some specific metabolic function, we have investigated the influence of this preparation on tissue metabolism in vitro. The results obtained indicate that L. A. C. produces a marked increase in aerobic glycolysis, and that this effect cannot be attributed to cortical steroids oxygenated at carbon 11, which are known to be present. Correlations of this phenomenon with lymphocytolytic response to L. A. C. are reported elsewhere (4).

Methods

Tissue slices prepared in the conventional manner were suspended in Ringer-Krebs-phosphate buffer (5). Homogenates were made in the Potter-Elvehjem glass homogenizer.

Thymus lymphocytes were obtained by mincing the thymus gland from a young rat in cold Ringer-Krebs-phosphate buffer in an ice bath. The suspension was then diluted to the desired volume and filtered through several layers of gauze.

Hexokinase and glycolysis in extracts of fresh rat brain or brain acetone powders were determined according to Ochoa (6) and Muntz and Hurwitz (7), and in rat muscle extract according to Colowick, Cori, and Slein (8); adenosinetriphosphatase was studied by the method of DuBois and Potter (9), diphosphopyridine nucleotidase in brain homogenate according to McIlwain and Rodnight (10), and aerobic phosphorylation according to Potter (11). Respiration and glycolysis in fortified homogenates of thymus and brain were determined as described by Reiner (12).

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A preliminary report of this work has appeared (Federation Proc., 12, 247 (1953)).

1 For convenience, Lipo-Adrenal Cortex will be referred to in the text of this paper as L. A. C.
Dry weights of thymus lymphocyte suspensions in the Ringer-Krebs medium were obtained by drying a representative sample and correcting for the salts present in the medium. The initial experiments were performed on thymus slices. However, loss of cells in the course of an experiment makes the dry weights somewhat unreliable. This source of error was eliminated by working with suspensions of thymus lymphocytes.

After precipitation of proteins with \( \text{CCl}_2\text{COOH} \), lactic acid was determined by the method of Miller and Muntz (13) as modified by Barker and Summerson (14), and pyruvic acid according to Friedemann and Haugen (15). All aerobic glycolysis values are calculated from chemical estimation of lactic acid.

Solutions of cortisone and hydrocortisone in Ringer-Krebs-phosphate buffer were prepared by adding the crystalline material to the medium and autoclaving at 15 pounds pressure for 10 to 15 minutes. The calculated amount of phosphate buffer was added after cooling.\(^2\) Desoxycorticosterone was dissolved in a few drops of acetone; cottonseed oil was then added and the volatile solvent was removed by overnight incubation in a vacuum oven at room temperature.

Most experiments reported here were performed with a commercial adrenocortical extract, L. A. C., in cottonseed oil, which we obtained through the courtesy of Dr. H. F. Hailman of The Upjohn Company. In all cases, controls were set up containing the same volume of the cottonseed oil vehicle. We are indebted to Dr. Dalton Jenkins for samples of cortisone (free alcohol) (Merck), desoxycorticosterone, and crystalline zinc insulin (Lilly), and to Dr. Oscar Hechter for a sample of crystalline hydrocortisone (free alcohol).

Adenosinetriphosphate (ATP) (barium salt), diphosphopyridine nucleotide (DPN), cytochrome c, and the phosphorylated sugars were all commercial preparations, obtained from either Sigma Chemical Company or the Schwarz Laboratories, Inc.

**Results**

*Effect on Respiration and Glycolysis of Whole Cells*—The addition of L. A. C. to a suspension of normal lymphocytes from thymus produces an inhibition of the oxygen uptake and the anaerobic glycolysis. At the time of the 60 minute reading in a typical experiment, the oxygen uptake is inhibited 21 per cent by 0.05 ml. of L. A. C. in a total volume of 1.5 ml. and the anaerobic glycolysis 25 per cent.

On the other hand, aerobic glycolysis is stimulated to a marked degree by L. A. C. Table I shows that as little as 0.025 ml. of L. A. C. can in-

\(^2\) I am indebted to Dr. Oscar Hechter for suggesting this method as one which permits solubility without loss of activity.
crease the aerobic glycolysis of thymus lymphocytes 100 per cent without
inhibiting the respiration to a significant degree. Additional L. A. C. does

**Table I**

*Effect of L. A. C. on Respiration and Glycolysis of Rat Thymus Slices*

Ringer-Krebs-phosphate buffer, 0.2 m glucose; 1 hour at 37°, total volume 1.5
ml.

<table>
<thead>
<tr>
<th>Volume of C. O.* or L. A. C. added</th>
<th>Respiration, QO₂</th>
<th>Aerobic glycolysis, Q₀₂⁴</th>
<th>No. of experiments</th>
</tr>
</thead>
<tbody>
<tr>
<td>ml.</td>
<td>C. O.</td>
<td>L. A. C.</td>
<td>C. O.</td>
</tr>
<tr>
<td>0.025</td>
<td>10.0</td>
<td>9.5</td>
<td>1.8</td>
</tr>
<tr>
<td>0.05</td>
<td>11.6</td>
<td>10.1</td>
<td>1.5</td>
</tr>
<tr>
<td>0.1</td>
<td>9.9</td>
<td>7.8</td>
<td>2.5</td>
</tr>
<tr>
<td>0.2</td>
<td>9.8</td>
<td>4.9</td>
<td>2.1</td>
</tr>
</tbody>
</table>

* Cottonseed oil control.
† Microliters of CO₂ equivalent to lactic acid per mg. of dry weight per hour.

not further stimulate aerobic glycolysis significantly. However, respira-
tion is inhibited at higher concentrations of L. A. C., indicating that these
may be separable phenomena.

Increased aerobic glycolysis after treatment with L. A. C. is not re-
stricted to thymus lymphocytes. Fig. 1 shows that aerobic lactic acid
formation by slices or whole cell preparations of many normal tissues is stimulated by L. A. C. at a concentration of 0.1 ml. in 3 ml. The most marked effects are observed with thymus, brain, and kidney; liver, diaphragm, lymph nodes, and spleen are stimulated to a smaller extent. The aerobic glycolysis of testis was actually inhibited in three of four experiments. The glycolysis of mouse sarcoma 180 (and of mouse sarcoma 37) is not affected.

Thymus cells from older rats were sometimes less sensitive to L. A. C. than cells from younger ones. This is not a consistent finding.

L. A. C. is standardized to contain the equivalent of 1 mg. of hydrocortisone per ml., according to the glycogen deposition test. The variabili-

**Table II**

*Aerobic Glycolysis and Respiration of Rat Thymus Cells; Effect of L. A. C., Cortisone, Hydrocortisone, and Desoxycorticosterone*

Ringer-Krebs-phosphate buffer, 0.2 M glucose; 1 hour at 37°.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Addition in 1.5 ml</th>
<th>$Q_o^2$</th>
<th>$Q_o^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cottonseed oil</td>
<td>0.025 ml.</td>
<td>1.5</td>
<td>5.8</td>
</tr>
<tr>
<td>L. A. C.</td>
<td>0.025 “</td>
<td>3.1</td>
<td>5.1</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>1.6</td>
<td>5.3</td>
</tr>
<tr>
<td>Hydrocortisone</td>
<td>400 γ</td>
<td>2.0</td>
<td>4.0</td>
</tr>
<tr>
<td>“</td>
<td>800 “</td>
<td>3.2</td>
<td>2.5</td>
</tr>
<tr>
<td>Cortisone</td>
<td>800 “</td>
<td>1.9</td>
<td>2.3</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>2.2</td>
<td></td>
</tr>
<tr>
<td>Desoxycorticosterone</td>
<td>1 mg.</td>
<td>1.9</td>
<td></td>
</tr>
</tbody>
</table>

The data in Table II which indicate that 0.025 ml. of L. A. C. in 1.5 ml., the equivalent of only 25 γ of hydrocortisone, increases thymus aerobic glycolysis from 1.5 to 3.1 with only a slight effect on respiration. To produce an equivalent stimulation of aerobic glycolysis, 800 γ of cortisone are required, but neither 800 γ of cortisone nor 1 mg. of hydrocortisosterone in 1.5 ml. has any significant effect on glycolysis. Both hydrocortisone and cortisone inhibit respiration at these concentrations.

**Effect on Enzymes and Homogenates**—Intact thymus lymphocytes, incubated in Ringer-Krebs bicarbonate buffer, liberate inorganic phosphate from ATP, and this is not significantly influenced by L. A. C. Lactic acid formation from glucose by an extract of rat brain acetone powder is in-
hibited approximately 20 per cent by 0.1 ml. of L. A. C. in a total volume of 1 ml. However, 0.1 ml. of L. A. C. has little or no effect on the activity of hexokinase from either rat brain acetone powder or muscle, on adenosinetriphosphate splitting by rat thymus or brain homogenate, on diphosphopyridine nucleotidase in guinea pig brain homogenates, nor on aerobic phosphorylation by rat kidney homogenate.

It has also been observed that in fortified cell-free homogenates of both thymus and brain, unlike intact cells and slices, aerobic glycolysis is not increased by L. A. C. The experiments with homogenate were performed with glucose plus fructose diphosphate or with fructose diphosphate alone as substrate (12). The effect of L. A. C. on glycolysis of fructose diphosphate by intact thymus cells has therefore been studied.

Table III

Glycolysis of Phosphorylated Sugars by Intact Thymus Cells

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Concentration of substrate</th>
<th>( \frac{Q_{O_2}}{A} )</th>
<th>No. of experiments</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td></td>
<td>0.4</td>
<td>5</td>
</tr>
<tr>
<td>Glucose</td>
<td>0.02</td>
<td>2.0</td>
<td>10</td>
</tr>
<tr>
<td>Fructose-1,6-diphosphate</td>
<td>0.005</td>
<td>5.8</td>
<td>4</td>
</tr>
<tr>
<td>Fructose-6-phosphate</td>
<td>0.02</td>
<td>0.8</td>
<td>4</td>
</tr>
<tr>
<td>Glucose-1-phosphate</td>
<td>0.02</td>
<td>0.3</td>
<td>2</td>
</tr>
<tr>
<td>Fructose</td>
<td>0.02</td>
<td>0.8</td>
<td>2</td>
</tr>
</tbody>
</table>

Glycolysis of Fructose Diphosphate by Thymus Cells—Table III indicates that intact thymus cells can form lactic acid from fructose-1,6-diphosphate but not from fructose-6-phosphate, glucose-1-phosphate, or fructose. The effect of fructose-1,6-diphosphate is manifested at a concentration as low as 0.001 M. Furthermore, it is probably not attributable to an alteration in the cell membrane, for there is no change in the permeability of the cells to safranine.³

L. A. C. does not appear to stimulate lactic acid formation from fructose diphosphate. Thus, in Table IV, Experiment 1, the increment produced in glycolysis by L. A. C. with glucose as the substrate is 1.9, and only 0.4 with hexose diphosphate. Similarly, in Experiment 2, the increment with glucose is 1.7, and with hexose diphosphate 1.0 or 0.8, essentially the same as the increment from endogenous substrate.

Influence of Osmolarity of Medium—The glycolysis of whole cell preparations of certain tissues, especially brain cortex, is known to be extremely sensitive to variations in the ionic environment (16–20). It has seemed

³ Unpublished experiments, Z. Miller.
to us important to determine whether thymus lymphocytes show a similar response and to ascertain whether a relationship exists between this and the stimulation by L. A. C.

**Table IV**

*Effect of L. A. C. on Glycolysis of Glucose and Hexose Diphosphate by Intact Thymus Cells*

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Substrate</th>
<th>Concentration of substrate</th>
<th>$Q_A^{O_2}$</th>
<th>Increment due to L. A. C.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>C. O.</td>
<td>L. A. C.</td>
</tr>
<tr>
<td>1*</td>
<td>Glucose</td>
<td>0.02</td>
<td>2.2</td>
<td>4.1</td>
</tr>
<tr>
<td></td>
<td>Fructose-1,6-diphosphate</td>
<td>0.02</td>
<td>4.5</td>
<td>4.9</td>
</tr>
<tr>
<td>2</td>
<td>None</td>
<td>0.02</td>
<td>0.4</td>
<td>1.2</td>
</tr>
<tr>
<td></td>
<td>Glucose</td>
<td>0.02</td>
<td>1.6</td>
<td>3.3</td>
</tr>
<tr>
<td></td>
<td>Fructose-1,6-diphosphate</td>
<td>0.005</td>
<td>5.5</td>
<td>6.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.001</td>
<td>2.8</td>
<td>3.4</td>
</tr>
</tbody>
</table>

* Average of four experiments.

**Table V**

*Effect of NaCl Concentration on Thymus Cell Aerobic Glycolysis*

Ringer-Krebs-phosphate buffer, 0.2 M glucose, 1 hour at 37°.

<table>
<thead>
<tr>
<th>Concentration (m eq. per l)</th>
<th>Aerobic glycolysis, $Q_A^{O_2}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>154</td>
<td>3.6</td>
</tr>
<tr>
<td>310</td>
<td>6.9</td>
</tr>
<tr>
<td>234</td>
<td>5.1</td>
</tr>
<tr>
<td>194</td>
<td>3.9</td>
</tr>
<tr>
<td>154</td>
<td>2.5</td>
</tr>
<tr>
<td>80</td>
<td>3.1</td>
</tr>
<tr>
<td>56</td>
<td>3.9</td>
</tr>
</tbody>
</table>

Increasing K⁺ concentration of the medium 10-fold, decreasing Mg²⁺ to one-tenth or increasing it to 10 times its normal value, and omitting Ca²⁺ or increasing it 4-fold do not affect the aerobic glycolysis of thymus lymphocytes.

However, if the total osmolarity of the medium is markedly altered by varying the NaCl concentration, aerobic glycolysis is influenced to a considerable degree (Table V). Marked stimulation is also obtained with LiCl and NH₄Cl as well as with mannitol and sucrose, when these are
added to the Ringer-Krebs medium at a concentration of 140 or 280 milliosmoles per liter. KCl had little effect at the lower concentration, but was inhibitory at the higher. Urea was completely without effect at either concentration.

Influence of Insulin—The increased aerobic glycolysis of thymus cells induced by L. A. C. cannot be prevented by the simultaneous addition of 8 units of crystalline zinc insulin in vitro.

Heat Stability of Active Principle in L. A. C.—The factor or factors which stimulate aerobic glycolysis are resistant to high temperature. Heating L. A. C. at 100 degrees for 75 minutes did not affect its activity. Furthermore, the stimulation of glycolysis by L. A. C. cannot be attributed to contamination by inorganic substances, for the residue after ignition of an alcoholic extract of L. A. C. is completely without activity.

DISCUSSION

The experiments reported here indicate that an extract of the adrenal cortex possesses a substance or substances capable of stimulating in vitro the aerobic glycolysis of thymus lymphocytes and certain other tissues. This stimulation cannot be accounted for by cortisone, hydrocortisone, or desoxycorticosterone, for these substances are effective, if at all, only at concentrations considerably higher than are present in the extract. Fractionation studies also indicate that the glycolytic activity is found in relatively non-polar fractions which do not contain cortisone or hydrocortisone.4

The increased aerobic glycolysis can be produced by concentrations of L. A. C. which do not influence respiration significantly. Higher concentrations of L. A. C., as well as cortisone and hydrocortisone, do inhibit respiration. Kit and Barron (21) have also observed inhibition of rat thymus respiration by hydrocortisone. On the other hand, Roberts and White (22) found no change in the respiration of rat lymphoid tissue after treatment in vivo with an aqueous adrenocortical extract (A. C. E.) or adrenocorticotropic and found a slight inhibition following adrenalectomy. Neither procedure influenced the aerobic glycolysis of mesenteric lymph nodes. We have found that axillary and mesenteric lymph nodes are considerably less sensitive to L. A. C. in vitro than thymus lymphocytes.

Stimulation of glycolysis by L. A. C. appears to require an intact cell structure. No effect could be demonstrated with homogenates, and slight inhibition of cell-free, glycolyzing extracts has been observed. However, the failure of L. A. C. to influence the activity of enzyme systems studied in cell-free preparations does not preclude the possibility that these systems may be altered when L. A. C. is added to the whole cell. Thus, Sutherland

4 Unpublished experiments, O. Hechter and Z. Miller.
and Cori reported that, although the hyperglycemic factor from insulin has no effect on cell-free preparations of phosphorylase, it does stimulate phosphorylase in the liver slices (23).

Our experiments with thymus lymphocytes indicate that L. A. C. stimulates lactic acid formation from glucose but not from fructose diphosphate. This suggests that (1), in the thymus lymphocytes as in other tissues (24, 25), the rate of formation of fructose diphosphate is a limiting step and that (2) L. A. C. stimulates glycolysis by increasing the rate of formation of fructose diphosphate. This might result if the permeability to glucose is increased or if some enzyme which is involved in the formation of fructose diphosphate is stimulated.

The stimulation of glycolysis by L. A. C. as well as by solutions of high osmolarity may conceivably be referable to a relative dehydration of the cell, and a concomitant increase in K⁺ or NH₄⁺ concentration (26, 27). It is of interest to note that Muntz and Hurwitz (7) have observed that both K⁺ and NH₄⁺ increase the glycolysis of an extract of rat brain acetone powder. This effect has been attributed to stimulation of phosphofructokinase (28, 29).

**SUMMARY**

1. The addition of Lipo-Adrenal Cortex *in vitro* increases the aerobic glycolysis of thymus lymphocytes and of slices of brain, kidney, liver, and other tissues at concentrations which have no influence on respiration.

2. The effects observed cannot be attributed to cortisone, hydrocortisone, or desoxycorticosterone, and the activity is not affected by insulin.

3. An intact cell is required for this effect. The glycolysis of brain and thymus homogenates is not stimulated. Glycolysis by an extract of rat brain acetone powder is slightly inhibited. Hexokinase, ATPase, and DPNase in cell-free preparations are affected little, if at all.

4. Fructose diphosphate is glycolyzed more rapidly than glucose by intact thymus lymphocytes. This glycolysis is not stimulated by Lipo-Adrenal Cortex.

5. Glycolysis of thymus lymphocytes is also increased in a high osmolar environment.

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