Aerobic Glycolysis in Homogenates of Normal and Tumor Tissues*

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Previous investigations from this laboratory have revealed that the over-all oxidative capacity of tumor tissues, in general, falls in the range of the less active normal tissues and at a level much lower than normal liver, kidney, heart, or brain (12), while the enzymes of the glycolytic system occur in quantities of the same order of magnitude in all these tissues (5, 6). Since the conclusions regarding oxidative capacity have been questioned (19), further studies with systems not previously employed are here reported.

In earlier studies on the malic dehydrogenase system (10, 16), information was obtained concerning the DPN1-cytochrome c reductase content of various tissues. This was done by adding a crude preparation of malic dehydrogenase that contained no cytochrome reductase to a homogenate, with the result that the rate-limiting reaction was the reduction of cytochrome c and that the measurement of oxygen uptake could be taken as an assay of the DPN-cytochrome c reductase when DPN and cytochrome c were added in excess. The data showed that in liver homogenates the cytochrome c reductase was present in excess, and it was concluded that, in the absence of added malic dehydrogenase, the rate of oxygen uptake was a measure of the latter enzyme in the homogenate. However, in primary rat hepatomas there was such a narrow margin between the two enzymes that it could not be assumed that either enzyme was limiting in the unsupplemented homogenate (10, p. 317). More recent work by Weinhause et al. (21, 22) has indicated that the malic dehydrogenase component is not low in tumor tissue, and this observation suggests that the cytochrome reductase was limiting even in the measurements carried out without a malic dehydrogenase supplement. These studies yielded Q02 values of 103.5 and 15.1, respectively, for liver and hepatoma (10).

Reaction mixtures for the study of glycolysis in tumor homogenates have been perfected by LePage (5) and have shown that the glycolytic enzymes are present in tumor homogenates at a level that should deliver sufficient reduced DPN to saturate the DPN-cytochrome c reductase system in tumor tissue at the levels reported earlier (10). Thus, the rate of oxygen uptake in a properly supplemented tumor homogenate carrying out aerobic glycolysis might be used as a measure of the DPN-cytochrome c reductase or of the over-all DPN-oxidizing capacity of the tissue. The present report deals with the properties and limitations of such a system.

MATERIALS AND METHODS

Normal tissues were taken from 200-250-gm. male albino rats. Flexner-Jobling carcinomas and Walker 256 carcinosarcomas were taken 10 days after subcutaneous transplantation into 180-150-gm. female rats of the same strain, while Jensen sarcomas were excised 6 days after transplantation. Mouse Ehrlich ascites tumors were harvested 5 days after transplantation. The Novikoff hepatoma was recently obtained from Dr. A. B. Novikoff of the University of Vermont. It was transplanted intraperitoneally and grew rapidly to a weight of 10–15 gm. of non-necrotic tissue in 5-9 days. It was originally obtained by transplanting a primary liver tumor that arose after feeding 4-dimethylaminoazobenzene.

Normal and tumor tissues were rapidly excised from decapitated rats and placed in small beakers containing isotonic sucrose (0.25 m) and standing in cracked ice. The tissues were homogenized with a suitable volume of cold isotonic sucrose in a pestle-homogenizer. The ice-cold homogenates were pipetted into Warburg vessels standing in cracked ice and already containing all other constituents of the system. The vessels were then attached to the manometers without delay with air as the gas phase. At the end of the incubation period, 0.1 ml. of 50 per cent perchloric acid was pipetted into each flask to precipitate the proteins. After centrifuging, the supernatant fluid was analyzed for inorganic phosphate by the method of Fiske and Subbarow and for lactic acid by the method of Barker and Summerson, both as described by LePage (18).

HDP was obtained as the barium salt from Schwartz Laboratories, Inc., New York, while adenosine ATP was obtained as the barium salt from the Pabst Laboratories, Milwaukee. In each case, the barium was precipitated with sulfuric acid and sodium hydroxide, respectively. The DPN used in these experiments was prepared by the method of LePage (18) and assayed as 85 per cent DPN.

SUPPL. 3. 1 Supplied by the Holtzman-Rolfsmeyer Company of Madison.
RESULTS

Reaction Mixture for the Anaerobic Glycolysis of HDP

In order for glycolysis to proceed from HDP to lactic acid, oxidized DPN must be available to mediate the oxidation of glyceraldehyde-3-phosphate to 1,3-diphosphoglycerate. The two main pathways of hydrogen transport for the re-oxidation of reduced DPN are presumably (a) by DPN-cytochrome c reductase and the cytochrome system to oxygen and (b) by coupling with the enzymatic reduction of pyruvate to lactate. LePage has shown (5) that the glycolysis of HDP under anaerobic conditions and in the absence of fluoride is stimulated only 27 per cent by the addition of pyruvate; this proves that sufficient oxidized DPN is present, even under anaerobic conditions, to initiate the glycolysis of HDP. Once pyruvic acid has been formed, DPNH can be reoxidized, and glycolysis can continue unhindered.

To demonstrate the full oxidative capacity of the DPN-cytochrome c reductase system at the 3-phosphoglyceraldehyde step, a complete break of the coupling with the pyruvate system would be necessary. This can best be achieved in the absence of pyruvate. While complete suppression of pyruvate formation is not possible, the quantity of pyruvate is a minimum at the start of the reaction period, and a fluoride block at the phosphoglyceric acid level inhibits pyruvate formation. Under these conditions, the oxygen uptake of the glycolytic system should be a measure of DPN-cytochrome c reductase, since the glycolytic enzymes have been demonstrated to be very active in homogenates of normal and neoplastic tissues (6).

A system for the aerobic glycolysis of HDP has already been described by Potter (11), while a system for the anaerobic glycolysis of HDP has been described by LePage (5, 6). In the present series of experiments an attempt has been made to adjust the concentrations of the various reactants used previously to secure optimum conditions for oxygen uptake. Unless otherwise specified, a homogenate of rat heart in isotonic potassium chloride was used as source of enzymes in the following experiments.

Substrate.—Table 1 shows experiments on which the choice of substrate concentrations used in the assay medium were based. The minimum substrate concentration required to give close to maximal oxygen uptake was chosen.

Bicarbonate.—Table 1 shows the effect of potassium bicarbonate, which was added to neutralize lactic acid produced in the process of glycolysis. Carbon dioxide liberated from the bicarbonate was absorbed by alkali placed for that purpose in the center-well of the Warburg flasks. This procedure has been discussed elsewhere (11). Table 1 shows that a fivefold variation in bicarbonate did not greatly affect the optimum result.

Pardée center-well mixture.—The effect of the Pardée center-well mixture (8) was tested in the system. This mixture is capable of maintaining a constant pressure of carbon dioxide within the flask, when used in conjunction with an appropriate level of bicarbonate in the medium. However, experiments with a carbon dioxide pressure of \( \frac{3}{4} \) per cent, 1 per cent, 3 per cent, and 5 per cent did not constitute an improvement over the usual center-well addition of alkali.

pH of buffer.—Addition of phosphate buffer to provide substrate for phosphorylation reactions was held to a minimum in order to facilitate accurate measurements of changes in inorganic phosphate during the course of glycolysis. No appreciable effect was obtained by varying the pH of the phosphate addition from pH 7.4 to pH 8.2.

ATP.—ATP was added as a source of high energy phosphate bonds at a level previously used (5). Doubling this level of ATP had no effect on oxygen uptake.

Fluoride.—Fluoride inhibits ATP-ase activity (11) and also inhibits glycolysis at the phosphoglyceric acid stage. Fluoride was added at a previously determined level (11), which produced considerable stimulation of oxygen uptake (6), as experiments reported in a later section show. Trebling this level of fluoride caused inhibition of oxygen uptake.

Cytochrome c.—Cytochrome c has been shown to stimulate oxygen uptake in this system and was added at a level close to that previously recommended (11); doubling this level had no effect on

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**TABLE 1**

Effect of Substrate (HDP) and Potassium Bicarbonate on Oxygen Uptake in Heart Homogenates

Each compound was studied at varying concentrations, with all other components held constant at the values given in Table 3. The concentrations selected for assay are indicated by italics. A 10 per cent homogenate of rat heart in isotonic saline was used as source of enzyme and \( Q_{o,} \) values are based on oxygen uptake readings during the first 10 minutes of the experiment.

<table>
<thead>
<tr>
<th>Molarity</th>
<th>( Q_{o,} )</th>
<th>Molarity</th>
<th>( Q_{o,} )</th>
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<td>0.020</td>
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<tr>
<td>0.005</td>
<td>63</td>
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</table>

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808 Cancer Research
oxygen uptake. Further experiments on the effect of cytochrome c are reported in a later section.

Homogenizing media.—The effect of different homogenizing media on oxygen uptake was studied with liver from a rat starved for 24 hours as source of enzyme. The QO, values obtained with 10 per cent homogenates in water, isotonic potassium chloride, and isotonic sucrose were, respectively, 20.2, 20.0, and 20.5, showing essentially no difference between the different media.

Tonicity.—The effect of tonicity on oxygen uptake was studied with the same liver preparation. Table 2 shows that a slight inhibition was obtained with increasing tonicity. No addition was therefore made to raise the level of tonicity in the medium above the value of 0.63 that was due to its requisite constituents. In this table, isotonic sucrose (0.25 m) was given a value of 1.0. The nonsucrose additions were calculated to osmotic equivalents on the basis of their ionic strengths assuming 100 per cent ionization, and the final tonicity was adjusted with sucrose.

Creatine.—Although creatine has been shown to affect phosphate esterification in a similar system (11), no appreciable effect on oxygen uptake was obtained by the addition of 30 mg creatine/flask with either heart or kidney homogenates. Creatine was therefore omitted from the reaction mixture.

Arsenate.—Arsenate at a final concentration of 0.003 m slightly stimulated oxygen uptake, while at 0.01 m a small initial inhibition was obtained. However, the initial level of oxygen uptake declined less rapidly than in control flasks. These effects did not warrant the addition of arsenate to the reaction mixture.

DPN.—Chart 1 shows the effect of increasing DPN concentrations on the QO, (\(\mu\) M of O\(_2\) uptake/mg dry weight of tissue/hour) of one normal and three neoplastic tissues. On a 10-minute basis, near-maximal oxygen uptake was obtained at a level of 0.1 ml. of 0.4 per cent solution of DPN, or a final concentration of \(2.2 \times 10^{-4}\) m. On a 60-minute basis, this level of DPN was still satisfactory except in the case of Jensen sarcoma, where a higher level was more appropriate. In view of the depression of oxygen uptake readings with time (see below) which is probably due to the already mentioned production of pyruvate during the course of glycolysis, QO, values based on the first 10 minutes were thought to be the relevant values; to obtain these, a DPN concentration of \(2.2 \times 10^{-4}\) m was adequate (see footnote to Table 3).

Nicotinamide.—Chart 2 shows the effect of nicotinamide, which acts as a noncompetitive inhibitor of DPN-ase (24) to preserve DPN that is otherwise rapidly broken down in homogenates (7). On a 10-minute basis, 0.1 ml. of a 1.2 m solution of nicotinamide is optimal for all tissues tested, while on a 60-minute basis 0.1-0.2 ml. is optimal. The choice for the assay system was the lower level; it corresponds to a final concentration of 0.04 m.

Tissue concentration.—Chart 3,a shows that the oxygen uptake declines with time. With all tissues tested, this decline was smaller at low than at high tissue levels. Chart 3,b shows that a direct proportionality exists between oxygen uptake and tissue concentration.

The composition of the medium for the aerobic glycolysis of HDP is given in Table 3.

<table>
<thead>
<tr>
<th>TABLE 3</th>
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<td>COMPOSITION OF THE AEROBIC GLYCOLYSIS MEDIUM</td>
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<tr>
<td>Addition</td>
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<tr>
<td>Hexosediphosphate*</td>
</tr>
<tr>
<td>Adenosine triphosphate†</td>
</tr>
<tr>
<td>Diphosphopyridine nucleotide†</td>
</tr>
<tr>
<td>Nicotinamide</td>
</tr>
<tr>
<td>Cytochrome c</td>
</tr>
<tr>
<td>Magnesium chloride</td>
</tr>
<tr>
<td>Potassium fluoride</td>
</tr>
<tr>
<td>Potassium bicarbonate (fresh)</td>
</tr>
<tr>
<td>Potassium phosphate, pH 7.4</td>
</tr>
<tr>
<td>Tissue homogenate in 0.25 m sucrose</td>
</tr>
<tr>
<td>Water to make a final volume of 3.0 ml.</td>
</tr>
<tr>
<td>Potassium hydroxide (2.0 M): 0.2 ml. in center well</td>
</tr>
<tr>
<td>Gas mixture: air</td>
</tr>
</tbody>
</table>

* Potassium salt.
† Sodium salt.
† This concentration was chosen after initial experiments with heart homogenate as the source of enzyme. Later tests with several tissues, shown in Chart 1, indicated that for one tissue, Jensen sarcoma, a higher DPN concentration would be more appropriate to obtain near-maximal oxygen uptake over an extended period such as an hour. On this account, a DPN concentration of \(6 \times 10^{-4}\) m is recommended for future work.

Tissue Differences in Oxygen Uptake

Table 4 shows the results obtained when the reaction mixture given in Table 3 was used to compare the oxygen uptake of different tissues. Column 1 of Table 4 shows a grading of DPN-cytochrome c reductase capacity from higher values for normal tissue to lower values for neoplastic tissues.

Effects of cytochrome c and BCB.—Column 3 of
Table 4 shows that omission of cytochrome c depresses the oxygen uptake of all tissues except the Ehrlich ascites tumor.

BCB acts in this and related systems as a direct link between diaphorase and oxygen (17), thereby providing a pathway for the oxidation of DPNH that bypasses DPN-cytochrome c reductase. Column 4 of Table 4 shows that, for the four normal tissues tested, BCB had no effect on oxygen uptake. In contrast, the oxygen uptakes of the four neoplastic tissues were raised to a mean value of 190 per cent of control. The result obtained with Jensen sarcoma stresses the desirability of working with several different types of neoplastic tissues, before drawing conclusions of a generalized nature.

The above results show that BCB strongly stimulates the oxygen uptake of three out of the four tumor tissues studied, while producing little or no effect with normal tissues. These results represent further evidence that the capacity of the...
DPN-cytochrome c reductase is limited in these tumors as compared to normal tissues.

Column 5 shows the effect of adding BCB in the absence of cytochrome c. It is seen that only two tissues, namely, Flexner-Jobling carcinoma and Walker 256 carcino-sarcoma, have their oxygen uptake stimulated by both cytochrome c (compare columns 1 and 3) and BCB (compare columns 5 and 8); and for both these tissues the presence of both cytochrome c and BCB is necessary for maximal oxygen uptake (compare column 4 with 1 or 5). This suggests that addition of BCB presents an alternative pathway for hydrogen transport but leaves intact, at least to some degree, the original pathway via cytochrome c.

**EFFECT OF INHIBITORS AND SUBSTRATES ON LACTIC ACID PRODUCTION AND PHOSPHATE RELEASE**

Tables 5 and 6 represent a survey of glycolytic reactions in a number of normal and neoplastic...
tissues. The medium shown in Table 3 was used, with certain omissions and additions. The values represent parallel measurements of oxygen uptake, net lactic acid production, and net phosphate release obtained during incubation in Warburg flasks for a period of 60 minutes at 38° C. To obtain net figures, the micromoles present in the reaction mixture at zero time (t₀) were subtracted from the values obtained after incubation.

Column 1 of Table 5 shows the results obtained with the medium precisely as in Table 3. Comparing column 1 with column 3, obtained with omission of fluoride, it is seen that fluoride stimulates oxygen uptake in all tissues except the Ehrlich ascites tumor. The effectiveness of fluoride in blocking lactic acid formation varies over a range of from 90 per cent for heart to 38 per cent for spleen. Fluoride strongly inhibits phosphate release in all tissues. No net phosphate uptake was possible even in the presence of fluoride, owing to the absence of phosphate acceptor.

The effect of antimycin A on oxygen uptake in the standard system has been described elsewhere (13, 15). In columns 5 and 6 of Table 5, fluoride has been omitted from the system, and the effect of blocking or weakening the cytochrome system by adding antimycin A or omitting cytochrome c has been studied. A comparison with column 3 shows that these procedures had little or no effect on lactic acid production. On the other hand, phosphate release was significantly reduced with heart, liver, and kidney but unaffected with spleen and the neoplastic tissues.

Fumarate addition.—All values given in Table 6

![Graph](chart5a.png)

**CHART 5a.**—Variation of oxygen uptake with time for the aerobic glycolysis of HDP. A 10 per cent kidney homogenate in isotonic sucrose was used as source of enzyme.

![Graph](chart5b.png)

**CHART 5b.**—Dependence of oxygen uptake on tissue concentration. The data were taken from Chart 5a.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Control</th>
<th>Endogenous</th>
<th>−Cytochrome c</th>
<th>+BCB (0.0058 m)</th>
<th>−Cytochrome c +BCB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart</td>
<td>56.5</td>
<td>14.6</td>
<td>40.9</td>
<td>56.8</td>
<td>42.9</td>
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<tr>
<td>Kidney</td>
<td>32.8</td>
<td>14.3</td>
<td>18.0</td>
<td>28.2</td>
<td>29.4</td>
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<tr>
<td>Liver</td>
<td>22.4</td>
<td>7.8</td>
<td>14.0</td>
<td>19.8</td>
<td>20.3</td>
</tr>
<tr>
<td>Spleen</td>
<td>15.3</td>
<td>4.6</td>
<td>6.9†</td>
<td>15.8</td>
<td>15.2</td>
</tr>
<tr>
<td>Flexner-Jobling carcinoma</td>
<td>13.0</td>
<td>3.2</td>
<td>4.6</td>
<td>25.9</td>
<td>21.6</td>
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<tr>
<td>Jensen sarcoma</td>
<td>12.0</td>
<td>7.6</td>
<td>7.2</td>
<td>10.4</td>
<td>7.6</td>
</tr>
<tr>
<td>Ehrlich ascites tumor</td>
<td>12.0</td>
<td>6.8</td>
<td>5.8</td>
<td>20.8</td>
<td>21.3</td>
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<tr>
<td>Walker 256 carcinoma-sarcoma</td>
<td>13.3†</td>
<td>5.0†</td>
<td>5.5†</td>
<td>22.3†</td>
<td>10.7†</td>
</tr>
</tbody>
</table>

* Qₒₒ values are based on oxygen uptake readings during the first 10 minutes of the experiment.
† These values were obtained in a single experiment.
TABLE 5

**EFFECT OF FLUORIDE (0.01 M), ANTIMYCIN A (5 \times 10^{-7} M), AND CYTOCHROME C (2 \times 10^{-8} M) ON THE AEROBIC GLYCOLYSIS OF HDP (50 \mu M)**

All measurements are for a 60-min. reaction period.

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<th>TISSUE</th>
<th>Wet wt., mg.</th>
<th>(Dry wt., mg.)</th>
<th>MEASUREMENT</th>
<th>+KF</th>
<th>Homo</th>
<th>+HDP</th>
<th>Homo</th>
<th>+Antimycin A</th>
<th>Homo</th>
<th>-Cyt. c</th>
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<tbody>
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<tr>
<td>Q_0</td>
<td>38.0</td>
<td>6.6</td>
<td>24.0</td>
<td>10.1</td>
<td>7.9</td>
<td>18.5</td>
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<td>Net lactate, ( \mu M )</td>
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<td>-0.9</td>
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<td>Net lactate, ( \mu M )</td>
<td>1.6</td>
<td>1.8</td>
<td>33.6</td>
<td>7.1</td>
<td>24.4</td>
<td>26.3</td>
<td></td>
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</tbody>
</table>

* Oxygen uptake is expressed as the Qo_2 (\mu l O_2/mg dry weight of tissue/hour).
† These values were obtained in a single experiment.

TABLE 6

**AEROBIC GLYCOLYSIS WITH HDP (5 \mu M), FUMARATE (5 mM), AND GLUCOSE (30 mM) AS SUBSTRATES WITHOUT ADDITION OF FLUORIDE TO THE MEDIUM**

All measurements are for a 60-min. reaction period.

<table>
<thead>
<tr>
<th>TISSUE</th>
<th>Wet wt., mg.</th>
<th>(Dry wt., mg.)</th>
<th>MEASUREMENT</th>
<th>+HDP</th>
<th>Homo</th>
<th>+Fumarate</th>
<th>Homo</th>
<th>+Glucose</th>
<th>Homo</th>
<th>+HDP</th>
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<tr>
<td>Heart 20</td>
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<td>Q_0</td>
<td>17.4</td>
<td>10.1</td>
<td>14.6†</td>
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<tr>
<td>Net lactate, ( \mu M )</td>
<td>8.7</td>
<td>0.9</td>
<td>-0.9†</td>
<td>15.2</td>
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<tr>
<td>Net phosphate, ( \mu M )</td>
<td>21.1</td>
<td>8.9</td>
<td>9.8†</td>
<td>20.3</td>
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<tr>
<td>Kidney 50</td>
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</tr>
<tr>
<td>Q_0</td>
<td>22.0</td>
<td>9.0</td>
<td>26.6†</td>
<td>40.0†</td>
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<td>Net lactate, ( \mu M )</td>
<td>9.8</td>
<td>-0.5</td>
<td>2.4†</td>
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<tr>
<td>Net phosphate, ( \mu M )</td>
<td>21.6†</td>
<td>8.7†</td>
<td>10.3†</td>
<td>19.7†</td>
<td>6.8†</td>
<td>19.7†</td>
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<td>Q_0</td>
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<td>19.1†</td>
<td>22.5†</td>
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<tr>
<td>Net lactate, ( \mu M )</td>
<td>7.4</td>
<td>-1.0</td>
<td>5.1†</td>
<td>12.0†</td>
<td>-0.9†</td>
<td>7.6</td>
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<tr>
<td>Spleen 60</td>
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<tr>
<td>Q_0</td>
<td>4.0</td>
<td>2.3</td>
<td>3.5†</td>
<td>4.3†</td>
<td>2.4†</td>
<td>4.0</td>
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<tr>
<td>Net lactate, ( \mu M )</td>
<td>8.4</td>
<td>1.2</td>
<td>4.0†</td>
<td>8.1†</td>
<td>2.0†</td>
<td>8.9</td>
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<tr>
<td>Flexner-Jobling carcinoma 60</td>
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<tr>
<td>Q_0</td>
<td>4.4</td>
<td>3.8</td>
<td>6.0†</td>
<td>4.3†</td>
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<tr>
<td>Net lactate, ( \mu M )</td>
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<td>0.8</td>
<td>8.3†</td>
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<tr>
<td>Net phosphate, ( \mu M )</td>
<td>21.2</td>
<td>7.2</td>
<td>4.7†</td>
<td>20.0</td>
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<td>Jensen sarcoma 60</td>
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<tr>
<td>Q_0</td>
<td>6.0</td>
<td>5.3</td>
<td>6.4</td>
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<td>5.5</td>
<td>6.2</td>
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<tr>
<td>Net lactate, ( \mu M )</td>
<td>15.0</td>
<td>5.8</td>
<td>6.7</td>
<td>12.7</td>
<td>9.1</td>
<td>16.0</td>
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<tr>
<td>Net phosphate, ( \mu M )</td>
<td>16.8</td>
<td>7.6</td>
<td>6.9</td>
<td>19.6</td>
<td>7.0</td>
<td>17.8</td>
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<td>Ehrlich ascites tumor 110</td>
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<tr>
<td>Q_0</td>
<td>8.0</td>
<td>5.3</td>
<td>11.3†</td>
<td>11.7†</td>
<td>6.4†</td>
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<tr>
<td>Net lactate, ( \mu M )</td>
<td>11.8</td>
<td>1.8</td>
<td>11.4†</td>
<td>15.0†</td>
<td>12.6†</td>
<td>16.1</td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

* Oxygen uptake is expressed as the Qo_2 (\mu l O_2/mg dry weight of tissue/hour).
† These values were obtained in a single experiment.
were obtained with omission of fluoride from the reaction medium. Column 3 of Table 6 shows that 5 \( \mu M \) of fumarate stimulated oxygen uptake to a degree that was generally only slightly higher than that obtained with the same level of HDP. These results indicate that the same factors may be limiting both in Krebs cycle oxidations and in the aerobic pathway for DPNH oxidation.

A possible interpretation of the above data is that the \( Q_{02} \) values observed in the glycolytic system were mainly due to the oxidation of glycolysis products via the Krebs cycle. This possibility appears to be eliminated by the data of column 4, which show that the addition of both fumarate and HDP stimulated oxygen uptake beyond that found with either substrate alone in all tissues studied, but especially in kidney. This stimulation would be unlikely if HDP acted simply to raise the level of Krebs cycle substrates.

It should be noted that considerable quantities of lactic acid were produced even with fumarate as substrate. The sum of lactic acid production with HDP (column 1) and fumarate (column 3), when corrected for endogenous (column 2), was larger than the experimentally observed production of lactic acid with both HDP and fumarate present (column 4). This suggests that fumarate sparks Krebs cycle oxidation of the pyruvate that results from glycolysis.

**Glucose addition.**—In comparing columns 2 and 5 of Table 6, it is seen that glucose slightly stimulated the \( Q_{02} \) of all tissues and similarly caused only slight changes in phosphate breakdown. Of particular interest is the finding that glucose produced no increase whatever in lactic acid production with heart, kidney, and liver and only an increase of doubtful significance (0.8 \( \mu M \)) with spleen; on the other hand, large amounts of lactic acid were formed with all the tumor tissues tested.

These data are placed in better perspective by column 6 of Table 6, which shows that, in the case of heart and kidney, the presence of both HDP and glucose results in a larger production of lactic acid than is obtained with either substrate alone (columns 1 and 5). This result is in agreement with previous findings in an anaerobic system (6) that a functioning glycolytic system tends to encourage lactic acid formation from glucose.

**DISCUSSION**

The present work confirms previous indications (10, 16) concerning the grading of DPN-cytochrome c reductase capacity from higher values in certain normal tissues to lower values in various tumor tissues, with some overlapping of data. Of particular interest is the comparison of the \( Q_{02} \) for the Novikoff hepatoma (Charts 1 and 2) with that of normal liver (Tables 5 and 6), which shows that the two tissues have almost the same \( Q_{02} \) in this system. This comparison is especially important because of the finding by Hogeboom and Schneider (9) that, in a study of this enzyme in mouse liver and the 98/15 mouse hepatoma, the latter had a significantly higher enzyme content. Lenta and Riehl (4) found almost the same activity in liver and 98/15 hepatoma but found that the hepatoma was much higher in activity than the other tumors tested.

The data are also in agreement with the studies by LePage (6) on anaerobic glycolytic systems, in which it was shown that brain and tumor tissues were able to phosphorylate glucose effectively while homogenates from normal tissues other than brain were unable to glycolyze glucose. The data for oxygen uptake in the system for aerobic glycolysis are of greatest interest in connection with the continuing discussion (cf. 19–23) as to whether tumors contain less oxidative capacity than normal tissues. If the present data are compared to those of earlier studies on the over-all malic acid system (10, 16), an interesting fact emerges—that the tumor tissues, regardless of the test system, never exceed an oxidative rate of 20–25 and are usually much lower than this, depending on the type of tumor tested. However, in the case of heart, liver, and kidney, the \( Q_{02} \) values in the malic system (10, 16) were 114, 103, and 81, as compared to values of 38, 18.5, and 23, respectively, in the present work. These data suggest either that the malic system was not limited by the DPN-cytochrome c reductase or that, in the aerobic glycolytic system, the triosephosphate dehydrogenase is unable to saturate the cytochrome reductase capacity in the three tissues mentioned. In the former case the complete system may have drawn in the succinic system as a result of the glutamate addition. What may be emphasized in any case is that the oxidative capacity of these three tissues can be shown to

---

*In evaluating these results of photometric measurements, it is relevant to consider the findings of Lehninger (9) that, when a particulate fraction of rat liver containing both mitochondria and nuclei was incubated in water for 5 minutes at 0° C. prior to the measurement of DPN-cytochrome c reductase activity, the enzyme activity was increased 4.4 times over the activity of untreated particles. This result was explained by the existence of a permeability or other structural barrier to the free diffusion of DPNH to the active centers of mitochondria. These data emphasize the fact that the physical state of the enzyme preparations used for the measurement of DPN-cytochrome c reductase activity is of great importance, and it cannot be assumed that the permeability of the mitochondria from tumors is the same as for those from liver.*
cytochrome c reductase in tumor tissues.

cresyl blue doubled the oxygen uptake of three out

values for normal tissues to low values for tumor

preted in terms of a limited capacity of the DPN-

of four tumor tissues, while no effect was observed

with four normal tissues. These data are inter

by tissue homogenates has been developed.

has a lower capacity than heart, liver, and kidney.

is emphasized that the oxidative system of tumor

hydrogen transport system is needed. However, it

and the triosephosphate systems it is clear that

with tissue slices. On the basis of these effects and

Elliott and Baker (1) who observed similar effects

tissues. These findings support the earlier work of

which increased the oxygen uptake in three of the

sues tested but had no effect on the normal

but had essentially no effect on lactic acid produc-

tion.

4. With the probable exception of brain, which

was not studied, a clear-cut difference in the capac-

ity to produce lactic acid from glucose was ob-

served between homogenates of normal and tumor

tissues. Brain homogenates carry out this reaction

anaerobically. The other normal tissues were un-

able to produce lactic acid from glucose in the ab-

sence of other substrates, while neoplastic tissues

produced considerable quantities of lactic acid

under identical conditions. However, addition of

HDP stimulated the production of lactic acid from

sugar in heart and kidney. These data confirm

previous findings by LePage for anaerobic gly-

olysis.

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be much greater than that of any of the tumor tis-

ues studied in this laboratory or by Weinhouse

and collaborators under any conditions (19–23).

Among the oxidative systems that have been

studied, the least ambiguous is the succinoxidase

system, and again the tumors exhibit the same
general level of oxidative capacity as in the other

systems, while heart, liver, and kidney have capac-

ities of 219, 88, and 195, respectively, in terms of

Qo, (12). Weinhouse has apparently accepted the

validity of these data but has interpreted the re-

sults as merely the reflection of the "low mito-

chondrial content of tumor tissues" (23). If it is

agreed that the mitochondrial content of tumor

tissues is low it cannot be argued that their oxida-

tive capacity is equal to that of tissues containing

vastly more mitochondria: the oxidative capacity

(or mitochondrial content) of tumor cells must be

judged relative to other cell components such as

the nucleus or the glycolytic enzymes, or even the

total cell mass. Any deficiencies in the tumor mito-

chondria will only accentuate the differences.

While the capacities of certain individual compo-

nents of the cytochrome system are still not estab-

lished, it seems clear that the capacities of these

components of tumors as a class are similar to

to those of the least active normal tissues and that

the latter contain only about 1/10 as much activi-

ty as the more active normal tissues (12).

The possibility of further differences is indicated

by the effects obtained with brilliant cresyl blue,

which increased the oxygen uptake in three of the

four tumors tested but had no effect on the normal

tissues. These findings support the earlier work of

Elliott and Baker (1) who observed similar effects

with tissue slices. On the basis of these effects and

the unexplained differences between the malic

and the triosephosphate systems it is clear that

further work on the individual components of the

hydrogen transport system is needed. However, it

is emphasized that the oxidative system of tumor

tissues as judged by three types of measurement

has a lower capacity than heart, liver, and kidney.

SUMMARY

1. A system for the aerobic glycolysis of HDP

by tissue homogenates has been developed.

2. A grading of oxidative capacity from high

values for normal tissues to low values for tumor

tissues has been observed in this system. Brilliant

cresyl blue doubled the oxygen uptake of three out

of four tumor tissues, while no effect was observed

with four normal tissues. These data are inter-

preted in terms of a limited capacity of the DPN-

cytochrome c reductase in tumor tissues.

3. Measurements of lactic acid production and

phosphate breakdown were used to study the ef-

fect of inhibitors and substrates on the aerobic

glycolysis of normal and tumor tissues. Lactic acid

production with HDP as substrate in the fluoride-
inhibited system was similar with normal and

tumor tissues. Quantitative data on the action of

fluoride in stimulating oxygen uptake and inhibit-

ing both lactic acid formation and phosphate

breakdown were obtained. Addition of antimycin

A or omission of cytochrome c reduced the oxygen

uptake and phosphate breakdown in some tissues

but had essentially no effect on lactic acid produc-

tion.

4. With the probable exception of brain, which

was not studied, a clear-cut difference in the capac-

ity to produce lactic acid from glucose was ob-

served between homogenates of normal and tumor

tissues. Brain homogenates carry out this reaction

anaerobically. The other normal tissues were un-

able to produce lactic acid from glucose in the ab-

sence of other substrates, while neoplastic tissues

produced considerable quantities of lactic acid

under identical conditions. However, addition of

HDP stimulated the production of lactic acid from

sugar in heart and kidney. These data confirm

previous findings by LePage for anaerobic gly-

colysis.


