The Carbohydrate Metabolism of the Brown-Pearce Carcinoma of the Rabbit in Normal and Hypoglycemic Serum*

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INTRODUCTION

The observation of Warburg, Wind, and Negelein (15) that the tumor tissue metabolism of tumor-bearing animals kept in insulin convulsions for several hours was not affected stimulated many other investigators to inquire into the relationship of substrate concentration to oxygen consumption of tumors (1, 4—7, 13). All these investigators were in agreement in showing a higher consumption of oxygen in the absence of glucose than in its presence. The largest difference (50 per cent) was reported by Burk et al. (4) for chicken tumors.

The present study was concerned with two principal aspects of the metabolism of carbohydrate in the Brown-Pearce epithelioma of the rabbit; namely, the relationship of serum sugar level and the ratio of lactic acid production to oxidation in tumor slices.

Only primary growths were used. They were removed from the testis at 14—28 days after inoculation. Fifteen to 30 mg. of the slices, prepared with a straight razor and kept in Ringer phosphate solution, buffered to pH 7.55 until ready for study, were suspended in 2 ml. of rabbit's serum during a determination.

Sera were prepared from rabbits which had been on a fast for 14-24 hours. Fifteen to 30 ml. of blood, an amount which depended on the number of flasks employed, were taken from the starved animal for the preparation of normal serum. The rabbit then received subcutaneous injections of 12 units regular insulin, and, when the pre-convulsive sugar level was reached, as manifested by a characteristic behavior, or when frank convulsions occurred, the animal was bled for preparation of the hypoglycemic serum.

Barcroft-Brody manometers with flasks designed by Marsh (9) were used for the determination of respiration. Lactic acid values were determined colorimetrically with the Klett-Summerson photoelectric colorimeter, according to the procedure of Barker and Summerson (2). Glucose determinations were made on zinc filtrates of the sera by the Somogyi (11) method.

RESULTS

Experiments performed after the manner of Warburg, Wind, and Negelein (15), with Ringer-phosphate medium free of CO₂ and glucose, showed that hypoglycemia in the tumor-bearing animal did not affect the rate of oxidation. In a comparison of sera from normal animals with sera from the same animals in insulin shock (of a degree which would certainly produce a difference in glucose level), an average increase of 17.2 per cent (eleven animals) was found in the Q₀₂ values from the tumor slices in the latter media over those in the former.

In six experiments on fortifying shock sera with 66—138 mg. per cent of glucose, the stimulating effect of shock sera on oxygen consumption was completely nullified. In six other experiments the addition of 50 and 100 mg. per cent of glucose to normal sera had no effect on Q₀₂ values.

The trend of the data assembled from the experiments just described is summarized with approximate accuracy in Chart 1.

First, it was necessary to prove that the tumors metabolizing at a higher rate were affected in the same proportion by a change in the serum glucose as those metabolizing at a low rate. From the group of eleven experiments mentioned above, the average ratio of the higher range Q₀₂ values in hypoglycemic sera to those in normal sera was 1.16. A similar average ratio of lower range Q₀₂ values to those in normal sera was 1.19. The difference is within the limits of experimental error.

Since it was shown that the metabolic rate of a tumor is not a factor in determining its response to glucose for two widely differing serum glucose levels, it was assumed that the same condition holds for intermediate serum glucose levels. That a given
tumor had a high or a low energy turnover was in every instance determined by measuring its $Q_{O_2}$ in normal serum as a base line; as shown above, its $Q_{O_2}$ would be high or low in the same proportion at any other glucose level within the range of the indicated by the squares were determined directly. Triangles indicate the glucose levels were estimated, based on average values for normal and for shock sera of 140 mg. per cent and 36 mg. per cent, respectively.

Although the wide spread of points at the higher glucose levels prevents accurate localization of the curve in this region, that it should follow a course nearly paralleling the glucose axis is indicated by the results which showed that glucose added to normal serum did not affect $Q_{O_2}$ values.

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**Aerobic glycolysis.**—Warburg, Wind, and Negelein (15) perfused tumors with arterial blood and observed that the tumor cells abstracted 57 per cent of the sugar from it, whereas in normal tissues the sugar lost from the blood ranged between 2 and 18 per cent. As long as adequate oxygen was supplied to normal tissues, none of the sugar was utilized for production of lactic acid, but tumors produced from 46 to 49 mg. of this acid for each

**Chart 1**

The formula used in adjusting $Q_{O_2}$ values is shown below:

$$B_{cx} = B_x \left[ \frac{(1/N)\Sigma (A_1 + A_2 + \ldots)}{A_x} \right] .$$

$A_x = Q_{O_2}$ of any tumor in normal serum.

$N = \text{number of determinations}.$

$B_x = Q_{O_2}$ found for same tumor in serum altered, up or down, with respect to glucose.

$B_{cx} = \text{corrected } Q_{O_2}$ in altered serum.

$$\frac{1}{N} \Sigma (A_1 + A_2 + \ldots) = 5.01 \text{ (mean of 11 exps.)} .$$

Chart 1 shows a graph of corrected $Q_{O_2}$ values plotted against the glucose level of the serum in which the respiration was studied. Sugar levels in-
100 ml. of blood. However, when the sugar concentration is reduced by insulin shock, the formation of lactic acid is also reduced, as may be seen in Table 1.

It is evident that, whereas the oxygen consumption of the Brown-Pearce epithelioma is stimulated in hypoglycemic sera, its aerobic glycolysis is halved.

Within the past few years, observations have indicated a close relationship between the rate of glycolysis (whether aerobic or anaerobic) and the pH of the medium. Schrek (10), studying the survival of many kinds of tissue cells from different species when exposed to a glucose-containing salt solution kept at 45° C., found that all kinds, whether exposed to oxygen or not, produced acid, and that the increase in acid production with time paralleled the decrease in pH. Summerson, Gilder, and Lee (12) reported that the aerobic production of lactic acid by lymphosarcoma cells proved to be a linear function of the pH. (For example, 1.05 μl lactic acid/10⁶ cells/hr at pH 7.6; 0.78 μl at 7.4, and 0.3 μl at 6.9.) The only kind of acid produced by lymphosarcoma, they maintained, is lactic, and as glucose disappears an approximately equivalent amount of lactic acid appears. LePage (8), using homogenates of rat tissues, has demonstrated that the rates of glycolysis (anaerobic) of normal tissues are equal to or greater than that of rat carcinoma (Flexner-Jobling).

These observations appear to accentuate the importance of knowing the relationship of glycolysis to respiration.

The Warburg ratio.—In his book on tumor metabolism Warburg (14) evolved a formula based on theoretical considerations for estimating by the manometric method the ratio of the total sugar decomposed (glycolyzed) to the quantity of sugar oxidized. In this paper this ratio is designated as Warburg's ratio. Since 1 mole of lactic acid will release 1 mole of CO₂ from a bicarbonate medium, and since 1 mole of lactic acid is equivalent to ½ mole of glucose, the Q₀₂ value of a tissue expressed in moles divided by 2 will give the moles of glucose glycolyzed. Assuming that all the oxygen consumed by the tissue goes to burn glucose (probably an unwarranted assumption), the Q₀₂ value expressed in moles divided by 6 will give the moles of glucose oxidized. The ratio of the Q₀₂ value to the Q₀₆ value multiplied by 8 will then be equivalent to the ratio of the quantity of glucose glycolyzed to the quantity of glucose oxidized.

In studying the Flexner-Jobling rat carcinoma, Warburg found a value of 12 for his ratio, which he interpreted as meaning that for every 13 molecules of glucose attacked 12 were split into lactic acid. On this basis he states that the metabolism of the carcinoma tissue in oxygen is predominately one of glycolysis.

In summarizing certain of the results recorded in the literature, Burk (5) lists values for Warburg's ratio (aerobic glycolysis to equivalent respiration) ranging from 5.1 for human skin carcinoma to 17.7 for carcinoma of the penis, with intermediate values of 10 and 12 for the Flexner-Jobling rat carcinoma and the Rous chicken sarcoma, respectively. Inasmuch as these values are based on manometric measurements, it seemed to us important to determine Warburg's ratio for the Brown-Pearce epithelioma more directly by chemical methods.

Aerobic glycolytic studies were made in normal serum under approximately 700 mm. partial pressure of oxygen, and the lactic acid produced and the glucose consumed were ascertained as described in the section on methods. Lactic acid and glucose levels in the normal serum were determined; then levels of these substances were ascertained as described in the section on methods. The ratios determined for lactic acid production to glucose consumption are shown in Table 2.

The consistency of the results may be attributed to the following features of the experiment:

1. The time variable was eliminated.
2. Errors in sampling were reduced, as the lactic acid and sugar determinations were made on the same zinc filtrate.
3. The necessity for tissue controls was abolished.

### TABLE 1

<table>
<thead>
<tr>
<th>Exp. no.</th>
<th>Lactic acid production in normal serum (μg.)</th>
<th>Glucose level (mg.)</th>
<th>Lactic acid production in shock serum (μg.)</th>
<th>Glucose level (mg.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(per cent)</td>
<td></td>
<td>(per cent)</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0.028</td>
<td>140</td>
<td>0.028</td>
<td>25</td>
</tr>
<tr>
<td>2</td>
<td>0.036</td>
<td>155</td>
<td>0.010</td>
<td>26</td>
</tr>
<tr>
<td>3</td>
<td>0.025</td>
<td>144</td>
<td>0.018</td>
<td>26</td>
</tr>
<tr>
<td>4</td>
<td>0.048</td>
<td>182</td>
<td>0.017</td>
<td>44</td>
</tr>
<tr>
<td>5</td>
<td>0.031</td>
<td>182</td>
<td>0.014</td>
<td>46</td>
</tr>
<tr>
<td>Av.</td>
<td>0.038</td>
<td></td>
<td>0.016</td>
<td></td>
</tr>
</tbody>
</table>

* Over a period of approximately 8 hours, 45 minutes at room temperature and 75 minutes at 37.5° C.
4. The ratio obtained did not depend upon recovery and weighing of tissues.

The results show that 63 per cent of the glucose metabolized appears as lactic acid. In other words, for every 100 mg. of glucose metabolized, 63 mg. are glycolyzed and 37 mg. oxidized or otherwise destroyed.

Assuming that all the glucose which is not glycolyzed is completely burned, calculating Warburg's ratio from Table 2, a value of 63/37 or 1.7 is obtained. This value is considerably lower than those listed by Burk, based on manometric measurements, for other malignant tissues, and corresponds more nearly to the values he lists for benign tumors. However, if the data obtained in this investigation are calculated by the method of Warburg, values for his ratio of the same order as those listed by Burk for malignant tissues will be obtained. Table 3 summarizes the results of experiments conducted in normal serum in which tissue controls were used so that Qa2 values could be determined. It is evident that the ratio of lactic acid produced to glucose consumed should be employed rather than manometric measurements as a more accurate index of the carbohydrate metabolism of a tumor.

SUMMARY

1. A study was made to determine the extent to which the metabolism of the Brown-Pearce tumor could be altered by lowering the circulating glucose level with insulin. The metabolism of tumor slices was investigated in normal sera from rabbits which had been on a fast and in sera prepared from animals in insulin shock. Oxygen consumption was measured manometrically and aerobic lactic acid production determined colorimetrically.

2. Six experiments were conducted in which the glucose level of the shock sera was restored approximately to normal or above, and six in which the effect of the addition of glucose to normal serum was ascertained. A graph showing the relationship between serum glucose and Qo2 values was determined.

3. The ratio of aerobic lactic acid production to glucose consumption in normal serum was ascertained. The ratio of aerobic glycolysis to equivalent respiration was calculated by data determined chemically and data determined manometrically.

4. The oxygen consumption of Brown-Pearce tumor slices is increased by 17.2 per cent and the aerobic lactic acid production decreased to 50 per cent in sera prepared from animals in insulin shock, as contrasted to determinations made in sera taken from the same animals on fast for 14–24 hours.

5. The stimulating effect of hypoglycemic sera on oxygen consumption is destroyed by the addition of glucose.

6. A value of 0.63 was obtained for the ratio of aerobic lactic acid production to glucose consumption in normal sera.

TABLE 2

<table>
<thead>
<tr>
<th>Exp. no.</th>
<th>Lactic acid/mg</th>
<th>Total lactic acid production (mg.)</th>
<th>Total glucose consumption (mg.)</th>
<th>Lactic acid/glucose Av.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.058</td>
<td>0.610</td>
<td>1.13</td>
<td>0.54</td>
</tr>
<tr>
<td>2</td>
<td>0.044</td>
<td>0.387</td>
<td>0.59</td>
<td>0.72</td>
</tr>
<tr>
<td>3</td>
<td>0.035</td>
<td>0.457</td>
<td>0.92</td>
<td>0.50</td>
</tr>
<tr>
<td>4</td>
<td>0.027</td>
<td>0.650</td>
<td>0.92</td>
<td>0.70</td>
</tr>
<tr>
<td>5</td>
<td>0.058</td>
<td>0.691</td>
<td>1.17</td>
<td>0.59</td>
</tr>
<tr>
<td>6</td>
<td>0.035</td>
<td>0.386</td>
<td>0.59</td>
<td>0.64</td>
</tr>
</tbody>
</table>

Total: 0.62

TABLE 3

DETERMINATION OF WARBURG'S RATIO IN NORMAL SERUM
(By Manometric Method)

<table>
<thead>
<tr>
<th>Exp. no.</th>
<th>Lactic acid/mg hr</th>
<th>Qo2</th>
<th>Qo2/ Qo2</th>
<th>Qo2/Qo2</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.024</td>
<td>5.95</td>
<td>3.38</td>
<td>5.3</td>
</tr>
<tr>
<td>2</td>
<td>0.017</td>
<td>4.22</td>
<td>2.89</td>
<td>4.4</td>
</tr>
<tr>
<td>3</td>
<td>0.035</td>
<td>5.68</td>
<td>3.61</td>
<td>7.2</td>
</tr>
<tr>
<td>4</td>
<td>0.020</td>
<td>19.40</td>
<td>4.72</td>
<td>7.9</td>
</tr>
</tbody>
</table>

Av. 6.2

7. A comparison between the metabolism of tumors taken from rabbits on fast with that from those in insulin shock was made in CO2-free Ringer's phosphate medium. No significant difference in Qo2 and R.Q. values was found.

8. In the light of determinations by chemical methods, it appears that the Warburg ratio ascertained manometrically does not accurately reflect the extent of tumor glycolysis. It is concluded that the ratio of lactic acid produced to glucose consumed should be employed as a more precise index of carbohydrate metabolism.
REFERENCES

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