THE EFFECT OF pH ON THE METABOLISM OF RABBIT BONE MARROW

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In 1924 Warburg and coworkers (1) reported that variation in both the pH and the bicarbonate concentration of the medium affected the anaerobic glycolysis of Flexner-Jobling rat carcinoma in vitro. When the bicarbonate level was held constant, an increase in CO₂ tension was accompanied by a fall in the rate of anaerobic glycolysis. At a constant pH, increasing the bicarbonate concentration from 3.1 to 15.5 millimoles per liter was associated with a progressive increase in the rate of anaerobic glycolysis; further increase in bicarbonate concentration produced no significant effect. Craig and Beecher (2) demonstrated that lowering the pH from 7.48 to 7.18 in a bicarbonate-containing medium depressed aerobic glycolysis of rat retina without significant alteration in oxygen consumption. At a constant pH, aerobic glycolysis was increased with increasing CO₂ tensions from 1 to 5 volumes per cent. Similar effects have been reported for the aerobic glycolysis of cat cortex and medulla (3). Warren (4) reported that the presence of bicarbonate resulted in a 20 to 40 per cent increase in the oxygen consumption of rabbit bone marrow.

The work of Summerson, Gilder, and Lee⁴ on the effects of pH and bicarbonate on the in vitro metabolism of mouse lymphosarcoma prompted parallel studies on normal rabbit bone marrow, which according to Warren (5) exhibits metabolic characteristics approaching those of tumor tissues. An attempt was made to answer the following questions. What are the metabolic characteristics of normal bone marrow at various ranges of pH? Is the metabolism at a given pH influenced by changes in bicarbonate concentration? Can the respiratory metabolism be accounted for in terms of glucose utilization? What fraction of the total aerobic acid production is represented by lactic acid? Can differences be found between the metabolism of bone marrow cells and the cells of lymphosarcoma?

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EXPERIMENTAL

Methods

The marrow was obtained from the femora and tibiae of male New Zealand white rabbits. All animals had been fed ad libitum. They were killed by a blow on the head, bled, and the pencils of marrow from the cracked bones delivered into Ringer's solution at room temperature. The marrow, free of the larger blood vessels, was pressed with the aid of a small pestle through an ordinary kitchen sieve into a few ml. of Ringer's solution in a Petri dish. The resulting tissue suspension was made up to a volume of approximately 16 ml. with Ringer's solution and agitated gently and continuously by a magnetic stirrer to insure uniformity during the subsequent sampling. For the manometric studies, exactly 0.5 ml. of this tissue suspension was pipetted into each manometric vessel. Two 0.5 ml. samples of each suspension were taken for the determination of total cell nitrogen.

Examination of stained smears of the marrow, made before and after the preparation of the suspension as described above, gave no evidence of excessive cellular destruction. The percentage of myeloid and of erythroid cells per 500 nucleated cells was determined for each animal. The marrows varied from 45 to 65 per cent erythroid in composition. There was no obvious correlation between metabolic patterns and cellular variation within this range. No attempts were made to obtain marrows showing wide variation in cellular composition.

The Summerson constant volume differential manometer (6) was used for the manometric measurements. This manometer permits the simultaneous measurement on a single sample of tissue of oxygen consumption, respiratory CO₂ production, and the production of CO₂ due to the total aerobic acid formation. One may also determine precisely the bicarbonate concentration of the medium and the CO₂ tension of the gas phase at both the beginning and the end of an experiment.¹ From these data, by using the Henderson-Hasselbalch equation, pH may be determined at the beginning and end of an experiment, and by interpolation for any time during the experimental period. These calculated values agree within 0.03 unit of pH with those measured in a Beckman pH meter.² We have chosen to report as most representative the pH at the mid-time of the experimental run; i.e., the pH of the medium 60 minutes after the beginning of the experiment.

The total volume of the manometer vessels was approximately 18 ml. Each vessel contained exactly 1.0 ml. of Ringer-bicarbonate-glucose solution added to the 0.5 ml. of marrow suspension, with 0.2 ml. of 2.5 N HCl in the side bulb. The composition of the Ringer's solution was sodium chloride, potassium chloride, sodium bicarbonate, dextrose, and sodium lactate.

¹ Personal communication from Dr. W. H. Summerson.

² Personal communication from Dr. W. H. Summerson.
chloride 8.65 gm. per liter, potassium chloride 0.23 gm. per liter, calcium chloride 0.24 gm. per liter. The concentration of glucose was approximately 500 mg. per cent. According to the experimental pH desired, varying amounts of 0.15 M sodium bicarbonate were used in the preparation of the Ringer-bicarbonate-glucose solution. To bring this solution to the approximate pH desired, it was equilibrated for 20 minutes with the appropriate O$_2$-CO$_2$ gas mixture before being placed in the vessels. The gas mixtures varied from 80 per cent O$_2$-20 per cent CO$_2$ to 97.5 per cent O$_2$-2.5 per cent CO$_2$.

The experimental temperature was 37.5°C, and the shaking rate approximately 120 strokes per minute. Prior to an experiment the appropriate gas mixture necessary to establish approximately the desired pH was passed through the vessels for a 20 minute equilibration period with the vessels in the thermostat at 37.5°C. The exact pH was determined experimentally, as described above. An equal rate of gas flow was maintained through all the vessels during the preliminary gassing period. 5 minutes were then allowed for equilibration at atmospheric pressure and a final 5 minutes for temperature equilibration. At zero time, acid was tipped into the control vessels. All aerobic experiments were 120 minutes in duration. Upon the completion of the experiment, acid was tipped into the experimental vessels.

The method of determining anaerobic glycolysis was essentially that outlined by Umbreit (7). The gas mixture was composed of 95 per cent N$_2$-5 per cent CO$_2$. Variations in pH were obtained by varying the molarity of the bicarbonate in the medium. Anaerobiosis was established by passing this gas mixture through the vessels for a 30 minute equilibration period. Anaerobic experiments were of 60 minutes duration.

To the total contents of each vessel was added 0.5 ml. of 10 per cent sodium tungstate for the precipitation of protein, at a final volume of 25 ml. This was filtered and the filtrate analyzed for glucose and lactic acid. Glucose was determined by the method of Benedict (8) in the Klett-Summerson photoelectric colorimeter. Lactic acid was determined according to the method of Barker and Summerson (9). The analytical accuracy was controlled for each series of experiments by the ability to recover in a separate sample known additions of glucose and lactic acid. Recovery errors averaged less than 3 per cent. With the manometric procedure used here, glucose utilization or lactic acid formation is established by the difference between analytical results in control and experimental vessels.

In this paper oxygen consumption is symbolized by the conventional $Q_{O_2}$ and is defined as the number of c.mm. of O$_2$ consumed per mg. of cell protein per hour. $Q_{CO_2}$, $Q_{O_2}^+$, and $Q_{CO_2}^+$ are expressed in corresponding units, where $Q_{CO_2}$ represents respiratory CO$_2$, and $Q_{O_2}^+$ or $Q_{CO_2}^+$ represents aerobic or anaerobic acid production, in terms of CO$_2$ liberated from bicarbonate.
$Q_{\text{Gluc.}}$ and $Q_{\text{LA}}$ (glucose and lactic acid, respectively) are expressed in micrograms per mg. of cell protein per hour. Total cell nitrogen was determined by the Kjeldahl method and was converted into total cell protein by the customary factor of 6.25. If desired, the $Q$ values as given here may be converted into terms of "fat-free dry weight" (10) by multiplying by the factor 0.91.

![Graph](https://via.placeholder.com/150)

**Fig. 1.** The effect of pH on the oxygen consumption of normal rabbit bone marrow cells. The average $Q_o_2$ at each pH range is represented by the position of the circle, the number within the circle representing the number of experiments performed. The spread of the vertical lines represents the spread of the individual determinations within each range of pH.

**Results**

87 aerobic experiments covering a pH range from 6.3 to 7.7 have been performed. To simplify the presentation of the data this range was subdivided into parts, each having a span of one-tenth of a pH unit, and on this basis the experimental results have been grouped. In the figures, the averages of the $Q$ values of each group are represented by circles. The numbers within the circles indicate the number of experiments performed. The range of variation from the arithmetical average of each group is represented by the span of the vertical lines.

The relation of oxygen consumption to the calculated pH of the medium at the mid-time of the experimental period is presented in Fig. 1. Oxygen
consumption was greatest in the pH range 6.9 to 7.3. For this range the average oxygen consumption was 3.6 c.mm. per mg. of cell protein per hour. The spread of $Q_{O_2}$ values from the average was large in each range of pH studied and the differences between the averages were small over the entire pH range.

From pH 6.3 to 7.3 the total aerobic acid production increased with increasing pH in a substantially linear fashion. This relation of $Q_{O_2}$ to pH is shown in Fig. 2. Aerobic glycolysis was greatest at pH 7.2, the average $Q_{O_2}$ being 4.3; it was smallest at pH 6.3, the average $Q_{O_2}$ being 1.8. Above pH 7.3 the glycolytic rate diminished with increasing pH to an average of 2.9 at pH 7.6.

The lactic acid production of aerobic glycolysis is related to pH in Fig. 3. The averages for the $Q_{LA}$ of each pH group fall into a pattern which approximates that shown in Fig. 2. At pH 6.3 $Q_{LA}$ averaged 7.6; at pH 7.1 it averaged 15.9. In pH ranges above 7.1, lactic acid production was diminished with increasing pH and $Q_{LA}$ averaged 8.9 at pH 7.6. The ratio between total acid and lactic acid production for each pH grouping is shown.
Fig. 3. The effect of pH on the production of lactic acid by rabbit marrow.

Fig. 4. The effect of pH on the ratio of total acid production to lactic acid formation in rabbit marrow.

In Fig. 4, $Q_G^{O_2}$ has been multiplied by 4 to convert it into terms equivalent to $Q_{LA}$. When total acid formation exceeds lactic acid produc-
Fig. 5. The effect of pH on the respiratory quotient in normal rabbit bone marrow cells.

Fig. 6. The effect of pH on the ratio of glucose consumption to lactic acid production in rabbit marrow.
Fig. 7. The ordinate of this graph represents the ratio of the oxygen equivalent of the glucose utilized in excess of lactic acid produced to the total oxygen consumption. This ratio is related to pH.

Fig. 8. The effect of pH on the anaerobic acid production of normal rabbit marrow.

The ratio is greater than 1. Such a ratio exists in nine of the fourteen groups. The average lactic acid production of all experiments was 88 percent of the average for total acid formation.
pH exerted no effect on r.q. This is shown in Fig. 5. For all experiments the r.q. averaged 0.95. Such a value directs attention to the glucose utilization by the marrow. At pH 7.1, $Q_{\text{Gluc.}}$ averaged 21.5. It was greatest at pH 6.9, averaging 22.2. At pH 6.3 and 7.6, $Q_{\text{Gluc.}}$ was low, averaging 13.5 and 13.6, respectively. As defined in this paper, $Q_{\text{Gluc.}}$ and $Q_{\text{LA}}$ may be compared directly. Their ratio is related to pH in Fig. 6. A value greater than 1 in Fig. 6 exists when glucose utilization exceeds lactic acid production. As may be seen in Fig. 6, all values are greater than 1. The glucose utilization in excess of lactic acid formation is theoretically available for oxidation. The ratio of the oxygen equivalent of this excess glucose to the $Q_{\text{O}_2}$ is related to pH in Fig. 7. Micrograms of glucose have been converted to equivalent microliters of oxygen by dividing the value in micrograms by 1.33. In Fig. 7, a ratio greater than unity indicates that more glucose is utilized than can be accounted for by both lactic acid production and oxygen consumption. In ten of the fourteen groups the ratio is greater than 1.

The manometric results of eighteen experiments on the anaerobic glycolysis of normal rabbit marrow cells are presented in Fig. 8. The marrow of three animals was used. It is apparent that the $Q_{\text{O}_2}$ is affected in a linear fashion by changes in pH, averaging 9.3 at pH 7.4 and 1.3 at pH 6.2. Lactic acid production was also linearly affected by pH and accounted for the total anaerobic acid formation. Glucose utilization paralleled lactate production in approximately a 1:1 ratio throughout the pH range studied.

**DISCUSSSION**

We feel that it is important to stress precise knowledge of the bicarbonate concentration in the medium under experimental conditions. In order to draw conclusions regarding bicarbonate or pH effect on glycolysis or other aspects of cell metabolism, it is not sufficient merely to know the molarity of the bicarbonate in the medium prior to the equilibration period. This is illustrated by one typical set of experiments. Prior to the introduction of tissue, the Ringer-bicarbonate-glucose solutions contained 5.7, 11.2, and 22.4 mM of bicarbonate per liter, respectively. At the start of the experiment, after the preliminary equilibration, the bicarbonate concentrations for the same solutions were 3.5, 7.4, and 13.2 mM per liter. The breakdown of bicarbonate during the equilibration period is large, variable, and consequently not accurately predictable. Not only does this influence the calculation of pH but also, when one uses small quantities of bicarbonate in the medium, a rapidly glycolyzing tissue may decompose all of the bicarbonate either during equilibration or prior to the completion of the experimental period. Consequently, the interpretation of investigations reporting the effects on cell metabolism of either $pCO_2$ or of bicarbonate
may be subject to an unpredictable error when these quantities are not measured under conditions existing during the experimental period.

In the experiments here reported the average \( Q \) values for normal rabbit marrow in a bicarbonate-containing medium at pH approximating 7.4 were \( Q_{O_2}, 3.2; Q_{O^2}, 3.9; Q_{LA}, 11.8; \) and \( Q_{Gluco}, 13.4. \) These values for respiratory metabolism are very close to those previously reported in which the medium was Ringer-phosphate solution (5, 10, 11). Under experimental conditions different from those of the present study, Warren (4) reported that the respiration of rabbit bone marrow was approximately 40 per cent greater in Ringer-bicarbonate than in Ringer-phosphate solution. We are aware of no experiments comparable to those reported here on the effect of pH change on the in vitro respiration and glycolysis of bone marrow. Similar studies have been made with rat retina (2), cat cortex (3), and mouse lymphosarcoma (12).

| Table I |
| Partial Tabulation of Bicarbonate Concentration, pCO\(_2\), and Aerobic Glycolysis of Marrow in Range of pH 6.70 to 6.79 |

<table>
<thead>
<tr>
<th>(HCO(_3^)) (0 ) time</th>
<th>CO(_2) (0 ) time</th>
<th>( Q_{O^2} ) (0 ) time</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \text{mM per l.} )</td>
<td>( \text{vol. per cent} )</td>
<td>( \text{mM per l.} )</td>
</tr>
<tr>
<td>5.9</td>
<td>4.3</td>
<td>3.2</td>
</tr>
<tr>
<td>5.9</td>
<td>4.6</td>
<td>2.0</td>
</tr>
<tr>
<td>6.2</td>
<td>4.9</td>
<td>4.5</td>
</tr>
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<td>4.6</td>
<td>2.4</td>
</tr>
<tr>
<td>8.4</td>
<td>7.3</td>
<td>2.7</td>
</tr>
<tr>
<td>9.1</td>
<td>7.5</td>
<td>2.2</td>
</tr>
</tbody>
</table>

In each range of pH studied, the total aerobic acid production was greater than the lactic acid formation. No attempts were made to identify other than lactic acid. In ten of the fourteen groups into which the experiments were divided on the basis of pH, more glucose was utilized than could be accounted for by both lactic acid formation and oxygen consumption. If the analyses for glucose are dependable, this fact implies glycogenesis on the part of the marrow or the formation of intermediate carbohydrate metabolites. On the basis of incompleted studies, we doubt that significant glycogenesis occurs. No analyses were made for carbohydrate intermediaries. The work of Goldinger, Lipton, and Barron (11) indicates that in marrow glucose is metabolized by way of the Krebs’ tricarboxylic acid cycle and that there is a small synthesis of citric acid.

In view of the current interest in the utilization of carbonic acid in cellular metabolism (13), the question arises as to whether the bicarbonate concentration or the pCO\(_2\) influenced results per se. Various concentrations of
bicarbonate and carbon dioxide yielding the same pH were studied. Within any small range of pH, the glycolytic rates showed no recognizable trends which could be attributed to increasing molarity of bicarbonate. Table I presents a partial tabulation of experiments performed at pH 6.70 to 6.79.

Comparison of our data for normal rabbit marrow cells with those of Summerson, Gilder, and Lee (12) for mouse lymphosarcoma shows certain differences. Aerobic glycolysis of lymphosarcoma seems to be more sensitive to changes in pH than does that of marrow. This difference appears to be quantitative. In lymphosarcoma, aerobic glucose utilization can be entirely accounted for on the basis of lactic acid formation. Such is not the case with marrow. The R.Q. of marrow was found to be 0.95, whereas that of lymphosarcoma was lower, averaging 0.83. In general lymphosarcoma is a more actively metabolizing tissue than marrow. This is not the comparison of a normal tissue with its malignant counterpart. Rather it is a comparison of two distinct tissues, studied by identical experimental techniques, in each of which the pH of the medium markedly affected the rate of glycolysis but had slight effect on the respiratory rate. Therefore, we feel that any attempt to relate glycolysis and respiration in a quantitative way as a means of characterizing a tissue metabolically is open to question when the effect of pH has not been determined.

Grateful acknowledgment is made to Dr. W. H. Summerson for his interest and help in connection with the material presented here.

SUMMARY

1. The in vitro metabolism of rabbit bone marrow cells in Ringer-bicarbonate-glucose medium has been studied with the Summerson differential manometer. 87 experiments were performed under aerobic conditions, eighteen anaerobically. Analyses for glucose utilization and lactic acid production by the tissue have been compared with the manometric data. All metabolic rates have been correlated with the pH of the medium, as determined during the experimental period.

2. Aerobic metabolic rates were maximum at pH 7.2. Both aerobic and anaerobic glycolysis were depressed markedly and in linear fashion by depression of the pH of the medium from 7.2 to 6.3. The effect of change of pH on the oxygen consumption and R.Q. was small, the R.Q. averaging 0.95. Total aerobic acid formation was greater than aerobic lactic acid production. Aerobic glucose utilization was at least sufficient to account for both lactic acid production and oxygen consumption.

3. Since pH markedly affects the glycolytic rate but has small effect on oxygen consumption, any ratio relating glycolysis to respiration in rabbit bone marrow is variable. Such is also the case with mouse lymphosarcoma.
and probably other tissues as well. Hence, the significance of such a ratio in characterizing the metabolism of a tissue is questioned unless the influence of pH is taken into account.

4. The breakdown of bicarbonate in the medium during the preliminary equilibration period was large and variable. Consequently, the validity of experiments reporting the effects on cell metabolism of either bicarbonate or pCO₂ is also questioned when these quantities have not been measured under conditions existing during the experimental period.

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