THE EFFECT OF CARBON DIOXIDE TENSION ON TISSUE METABOLISM (RETINA)

BY FRANCIS N. CRAIG AND HENRY K. BEECHER*

(From the Anesthesia Laboratory of the Harvard Medical School at the Massachusetts General Hospital, Boston)

(Received for publication, February 27, 1943)

I

During the study of the effect of oxygen tension on the metabolism of retina (Craig and Beecher 1943), our attention was drawn to the observation of Laser (1936, 1937) that the metabolism of this tissue in medium containing bicarbonate is twice as great as it is in medium containing phosphate buffer. The purpose of the experiments described here was to investigate whether this quantitative difference might be related to the concentration of the carbon dioxide-bicarbonate system. Warburg, Posener, and Negelein (1924) had already demonstrated the sensitivity of anaerobic glycolysis in a tumor to the concentration of the carbon dioxide-bicarbonate buffer system.

II

The material and its preparation, the media, except for changes as noted, and the methods, were described in the preceding paper. The study of carbon dioxide tension presented two complications. In order to keep the pH constant it was necessary to vary bicarbonate in proportion to carbon dioxide. In order to maintain the osmotic pressure when bicarbonate was changed, it was necessary to vary chloride inversely with bicarbonate. To minimize the alteration in the chloride concentration, one series was run at a lower pH.

III

From the results on rat retina in Table I, the following are apparent: The chloride and pH changes did not alter the effect on metabolism of changes in carbon dioxide-bicarbonate concentration. Lowering the pH from 7.48 to 7.18 (as calculated from the Henderson-Hasselbalch equation) depressed glycolysis but had no significant effect on oxygen uptake. In both series the oxygen uptake was maximal at 5 per cent carbon dioxide, being significantly less at carbon dioxide tensions of 1 per cent and 20 per cent. In both series there was an increase in lactic acid output between 1 per cent and 5 per cent carbon dioxide but no further change beyond 5 per cent. The effects of carbon dioxide tension at pH 7.48 are shown in Fig. 1.

* Aided by a grant from the Milton Fund of Harvard University.

473
TABLE I

Effect of Carbon Dioxide Tension in Bicarbonate Medium on the Oxygen Uptake and Lactic Acid Output of Rat Retina

Results for the 1st hour and standard error of the mean. In recording the gas mixtures the vapor tension of water was not corrected for. This amounts to 7 volumes per cent at 38°.

<table>
<thead>
<tr>
<th>CO₂ vol. per cent</th>
<th>O₂ vol. per cent</th>
<th>NaHCO₃ M X 1000</th>
<th>NaCl M X 1000</th>
<th>No. of observations</th>
<th>QO₂</th>
<th>Q₀₂</th>
</tr>
</thead>
</table>
| Calculated pH 7.48
| 1  | 99  | 5  | 157 | 16  | 16 ± 2.0 | 26 ± 1.2 |
| 5  | 95  | 24 | 138 | 10  | 31 ± 2.3 | 46 ± 2.0 |
| 10 | 90  | 48 | 114 | 4   | 29 ± 3.9 | 46 ± 1.9 |
| 20 | 80  | 96 | 66  | 5   | 19 ± 4.2 | 48 ± 3.3 |
| Calculated pH 7.18
| 1  | 99  | 2.5 | 165 | 4   | 15 ± 2.7 | 17 ± 1.8 |
| 5  | 95  | 12 | 155 | 4   | 25 ± 2.4 | 31 ± 5.7 |
| 20 | 80  | 48 | 119 | 4   | 15 ± 4.2 | 30 ± 5.1 |

Fig. 1. Barred circles refer to $Q_0^2$ of retina (Table I), open circles to $Q_{O_2}$ of retina (Table I), and closed circles to $Q_0^{NS}$ of sarcoma (Warburg, Posner, and Negelein, 1924).
From the data in Table II it will be seen that the rates of oxygen uptake and of lactic acid output in phosphate medium are of the same order as those in bicarbonate medium with 1 per cent carbon dioxide.

Recent indications that carbon dioxide takes part in the Krebs cycle in minced pigeon liver suggested the test of whether the effect of carbon dioxide tension when increased from 1 per cent to 5 per cent, could be duplicated by the addition of succinate. The results obtained when neutralized sodium succinate was made available to the retina in high concentration (0.02 M) in addition to glucose, are recorded in Table II.

In phosphate medium succinate had no significant effect on oxygen uptake, respiratory quotient, or lactic acid output. In its inability to bring about the rapid oxidation of succinate in phosphate medium, rat retina differed from many normal tissues and resembled certain tumors (Craig, Bassett and Salter, 1941). The slight variation in carbon dioxide tension resulting from the presence or absence of NaOH in the inset did not influence the lactic acid output in the paired vessels used for the determinations of r. q. with phosphate medium. This point has been discussed by Laser (1942).

In bicarbonate medium with 1 per cent carbon dioxide, the control data...
were lower than the data for respiration and glycolysis given in Table I because they represented the mean of a 2 hour period in which the rates were declining. Succinate raised the $Q_{O_2}$ to the level previously observed with 5 per cent carbon dioxide (Table I). It also raised $Q_A$, the sum of lactic acid production ($Q_L$) and respiratory carbon dioxide. Since an independent chemical determination showed that lactic acid production was not altered by succinate, the increment in $Q_A$ with succinate may be attributed to an increase in respiratory carbon dioxide output. Hence it is unlikely that the extra oxygen uptake due to succinate is the result of succinate dehydrogenation alone. Whether the substrate of the extra oxygen uptake was glucose or succinate remains to be determined.

IV

Sensitivity of lactic acid output by a tumor to carbon dioxide-bicarbonate concentration has been described by Warburg et al. (1924). The data are shown in Fig. 1. Laser (1936, 1937) noted that the respiration of retina and of sarcoma 189 was lower in phosphate than in bicarbonate medium. Our results in the previous paper (Craig and Beecher, 1943) indicated that in retina, lactic acid production as well as oxygen uptake, was lower in phosphate than in bicarbonate, and lower by the same fraction. Although at 99 to 100 per cent oxygen, metabolism was quantitatively almost the same in phosphate medium and in bicarbonate medium with 1 per cent carbon dioxide, the possibility of a specific effect of phosphate has not been eliminated. The sensitivity of respiration in 1 per cent carbon dioxide bicarbonate medium to oxygen tension remains to be studied. One specific difference between the buffers was observed, namely, the ability of succinate to increase the oxygen uptake in 1 per cent carbon dioxide-bicarbonate but not in phosphate. Greig, Munro, and Elliott (1939) found that 0.01 M succinate did not increase oxygen uptake by ox retina in the presence of 5 per cent carbon dioxide and the absence of glucose. In our experiment with succinate in bicarbonate, glucose was present and only 1 per cent carbon dioxide was used.

A striking phenomenon noted in the present study was the observation that the oxygen uptake of a nearly intact tissue in the presence of glucose could be doubled by raising the carbon dioxide tension from 1 per cent to 5 per cent. The observation supports the possibility that carbon dioxide may be important at an intermediate stage in metabolism for the synthesis of dicarboxylic acids. This possibility was first suggested in connection with recent developments in the study of the Krebs cycle by Krebs and Eggleston (1940), Evans and Slotin (1940), Wood et al. (1941), and Solomon et al. (1941). The subject has been reviewed by van Niel et al. (1942), and Evans (1942).

It is questionable whether the decrease in respiration of retina with 20 per cent CO$_2$ at pH 7.48 is related to the anesthetic effect of 20 per cent CO$_2$ in the
inspired air (Loewy, 1923, Leake and Waters, 1929, and Solomon, Kaufman, and D'Elseaux, 1931). Work in progress indicates that the metabolism of cerebral cortex and medulla oblongata is not depressed by 20 per cent CO₂ at pH 7.48.

Wallace and Hastings (1942) have presented data leading to the conclusion that in mammalian skeletal muscle the bicarbonate ion does not normally cross the cell wall. The question is raised whether the bicarbonate ion penetrates the cell wall of retina under the conditions of the present experiments as easily as carbon dioxide. The sensitivity of lactic acid production to pH, and the insensitivity to an increase of buffered CO₂ from 5 per cent to 20 per cent have been shown in Table I. If one assumed from the sensitivity of glycolysis to external pH that glycolysis is also sensitive to changes in pH within the cell, then the fact that glycolysis was not affected by the above mentioned increase in the carbon dioxide-bicarbonate system may mean that CO₂ and bicarbonate penetrate, if at all, at equal rates. If CO₂ entered the cell but not bicarbonate, then the cell would turn acid and glycolysis would be inhibited.

**SUMMARY**

The metabolism of rat retina was found to be sensitive to the concentration of the carbon dioxide-bicarbonate buffer system. Increasing the carbon dioxide from 1 per cent to 5 per cent at constant pH nearly doubled both respiration and glycolysis. Increasing the carbon dioxide at constant pH from 5 per cent to 20 per cent had no effect on glycolysis, but depressed the QO₂ from 31 to 19.

In a medium containing glucose and the 1 per cent carbon dioxide-bicarbonate buffer, the addition of succinate increased the QO₂ from 12 to 26, without affecting glycolysis. In a medium containing glucose and phosphate, succinate had no significant effect.

We are indebted to Dr. Fritz Lipmann for many helpful suggestions during the course of this study and to Miss Anna Murphy for her careful assistance with the chemical determinations.

**CITATIONS**


