THE EFFECT OF pH ON THE RESPIRATION OF BRAIN TISSUE;
THE pH OF TISSUE SLICES*

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With slices of various guinea pig tissues suspended in bicarbonate-free
horse serum adjusted to various pH values, Canzanelli et al. (1) found that
maximum rates of oxygen uptake occurred in all cases in media with pH
values well above 7.4. With brain slices, the curve relating respiration rate
to the pH of the medium rose with increasing pH, rapidly up to pH 6, less
rapidly from 6 to 8, and then rapidly again to give a marked maximum at
pH 9 to 9.5. It finally fell sharply with further increase in pH. Can-
zanelli et al. summarized previous work on the effects of pH on tissue res-
piration. Much of it was unsatisfactory with regard to pH control and
measurement and for other reasons. The surprisingly high optimum found
by Canzanelli et al. suggested that the question should be studied further.

As a result of the studies here reported we conclude that the true optimum
pH for the respiration of brain tissue is in the usual physiological range and
that the actual pH within slices is usually appreciably different from that
of the medium in which they are immersed.

Methods

Slices of rat brain cortex were prepared in a cool humid chamber (2, 3)
by means of a Stadie and Riggs (4) microtome designed to cut slices about
0.43 mm. thick. For respiration measurements the blade was not
moistened and the slices were weighed on a torsion balance.

Isotonic suspensions (5) of whole brain were prepared in warm calcium-
free Ringer’s solution, or in bicarbonate-free horse or sheep serum,1 con-
taining 0.01 M glucose.

Oxygen uptake was measured by standard procedure in the Barcroft,
apparatus at 38°. The vessels contained oxygen for slices, air for suspen-
sions. In some experiments samples of medium or suspension were ad-
justed to various pH levels and 3 ml. were placed in the vessels. More
usually each vessel received 2 ml. of suspension or slices and 2 ml. of me-
dium, and 1 ml. of medium containing the amounts of phosphate buffer,

* An abstract of this paper has appeared (Federation Proc., 6, 249 (1947)).

1 The serum was not usually inactivated, since in several experiments the respira-
tion rates of slices in inactivated (i.e., heated to 58°) and untreated serum were the
same.

51
NaOH, or HCl, needed to produce the required pH in the mixture was tipped in from a side bulb shortly before zero time. The final concentration of phosphate, when used, was 0.03 M. All mixtures were isotonic. Duplicate vessels were set up. One was removed from the bath at zero time, the other at the end of the experimental period, and the pH of the medium in each was determined immediately with an ordinary glass electrode.

For the measurement of the pH of slices themselves, a small cylindrical stand made of plastic, 25 mm. in diameter, was covered with wide mesh gauze. The slice was laid on this and held in place by a second layer of gauze. These layers were held down by elastic bands around the stand. The stand, with slice and coverings, was lowered into a 50 ml. beaker containing the medium and the latter was continuously aerated with the appropriate gas. The beaker was placed in a larger vessel containing water maintained at about 37°C. The pH was measured by means of a micro glass electrode (glass membrane-saturated Ag acetate in 0.5 per cent acetic acid-silver) and reference electrode (cotton wick, normal saline, chlorided silver wire) similar to those described by Nims (6). These were connected to an ordinary Beckman pH meter, the connection to the glass electrode being shielded, and calibrated against buffers of known pH. The electrodes were bound to a flexible bronze strip which was fastened to the arm of a dissecting microscope stand. By means of the ratchet of this stand they could be very gently lowered into contact with the slice or raised to measure the pH of the medium.

Results

In Fig. 1, a, the results of experiments with rat brain cortex slices suspended in bicarbonate-free horse serum are shown. These results confirm those obtained by Canzanelli et al. with guinea pig brain in that maximum respiration rates are observed around pH 9. A large number of experiments with slices in Ringer-phosphate-glucose solution indicated that maximum respiration rate occurred at a relatively high pH in this medium, as in serum. However, the pH fell so rapidly in the high range that it was impossible to determine the effect of pH with accuracy.

It has been shown (5) that brain suspensions prepared in isotonic medium respire at a rate comparable with that of slices and present a number of advantages in the study of brain tissue metabolism. The pH of these suspensions can be readily adjusted after homogenization and does not change so greatly as does the medium with slices. Aerobic glycolysis is less active than that of slices (7) and respiratory CO₂ production is lower in the alkaline range, where CO₂ strongly affects pH. Rather dilute suspensions, 30 to 50 mg. of tissue per ml. of final volume, were used to minimize the pH changes.
In Fig. 1, b, c, and d, the relation of the respiration rate of whole brain suspensions in various media to pH is shown. The optimum pH seems to be about 7 to 7.5.

![Graphs](image_url)

**Fig. 1.** Effects of pH on the respiration rate of slices of cortex and suspensions of whole brain. Similarly marked points were obtained in experiments on samples of the same tissue. The pH shown was the mean of the values for the beginning and the end of the 30 minute experimental period.

In Fig. 2 the results of experiments are illustrated in which suspensions were adjusted to various pH values, kept at room temperature for various times, and then readjusted to pH 7.4 before the respiration rate was determined. It may be seen that the effects of pH rapidly become irreversible, particularly in the alkaline range.
Fig. 2. Effects of keeping suspensions at room temperature in Ringer-phosphate medium at various pH values on the subsequent respiration rate at pH 7.4.

**Table I**

**pH of Rat Tissue Slices in Various Media**

<table>
<thead>
<tr>
<th>Medium*</th>
<th>Aeration gas</th>
<th>pH recorded from medium</th>
<th>pH recorded from slice</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Brain cortex</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Horse serum t</td>
<td>100% O₂</td>
<td>8.8</td>
<td>6.7</td>
</tr>
<tr>
<td>&quot;</td>
<td>100% &quot;</td>
<td>6.0</td>
<td>5.9</td>
</tr>
<tr>
<td>Ringer-0.03 M phosphate</td>
<td>100% &quot;</td>
<td>8.8</td>
<td>6.5</td>
</tr>
<tr>
<td>Same</td>
<td>100% &quot;</td>
<td>8.4</td>
<td>6.6</td>
</tr>
<tr>
<td>&quot;</td>
<td>100% &quot;</td>
<td>8.4</td>
<td>7.3‡</td>
</tr>
<tr>
<td>&quot;</td>
<td>100% &quot;</td>
<td>8.0</td>
<td>6.5</td>
</tr>
<tr>
<td>&quot;</td>
<td>100% &quot;</td>
<td>7.4</td>
<td>6.6</td>
</tr>
<tr>
<td>&quot;</td>
<td>100% &quot;</td>
<td>5.8</td>
<td>5.7</td>
</tr>
<tr>
<td>&quot;</td>
<td>100% &quot;</td>
<td>4.6</td>
<td>5.0</td>
</tr>
<tr>
<td>Ringer-0.02 M bicarbonate</td>
<td>100% &quot;</td>
<td>8.7</td>
<td>7.0</td>
</tr>
<tr>
<td>Same</td>
<td>95% O₂-5% CO₂</td>
<td>7.4</td>
<td>6.7</td>
</tr>
<tr>
<td>Ringer-0.001 M bicarbonate</td>
<td>95% &quot;</td>
<td>5% &quot;</td>
<td>6.0</td>
</tr>
<tr>
<td>Ringer, no bicarbonate</td>
<td>95% &quot;</td>
<td>5% &quot;</td>
<td>4.8</td>
</tr>
<tr>
<td><strong>Liver</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ringer-0.03 M phosphate</td>
<td>100% O₂</td>
<td>8.5</td>
<td>7.3‡</td>
</tr>
<tr>
<td><strong>Kidney</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ringer-0.03 M phosphate</td>
<td>100% O₂</td>
<td>8.4</td>
<td>7.3‡</td>
</tr>
</tbody>
</table>

* The media contained glucose, except where noted.
† Similar results were obtained in sheep serum.
‡ No glucose in the medium.

While the respiration rate of suspensions is only 40 to 50 per cent of maximum at pH 9, the respiration rate with slices is at its maximum in medium of this pH. This suggests that the pH of the tissue in a slice is not the same as that of the medium in which it is suspended, but is actually
lower and nearer the optimum. That this is true was indicated by the experiments with the micro glass electrode. When electrodes which recorded a pH of nearly 9 in the medium were gently lowered into contact with the slice, the recorded pH immediately fell and, after about 5 minutes, became constant at about 6.6. On raising the electrodes, the original pH of the medium was again recorded. A steady pH value was not recorded immediately when the electrode touched the tissue, presumably because time is required for the fluid within the concavity of the electrode to come into equilibrium with that of the tissue. In glucose-containing media of pH above 7, the pH recorded from the slice was 6.5 to 6.7 and seemed usually to be independent of the pH of the medium. In media of lower pH, the pH of the slice fell lower. However, in media of pH below 5, the pH of the slice was found to be appreciably higher than that of the medium.

Similar results (see Table I) were obtained when the medium was serum, Ringer-phosphate, or Ringer-bicarbonate. (Since the volumes of the media were large, their pH did not change greatly.) With liver and kidney cortex

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**Fig. 3.** Changes in pH recorded from brain slices under various conditions. Ringer-0.03 M phosphate medium. Substrates added to the medium and aeration gas changed at times shown by the arrows. Glucose 0.01 M, sodium l-lactate 0.02 M. Solid lines, glass electrode on the slice; broken lines, glass electrode in the medium above the slice.
slices similar differences between the pH of the slice and the medium were observed.

With brain slices under aerobic conditions the pH of the slice was appreciably lower with glucose present in the medium than with no substrate or lactate in the medium. With glucose the pH remained constant but with lactate or with no substrate the pH often rose slowly. On changing from aerobic to anaerobic conditions, with glucose present, the pH sometimes showed no immediate change, but after a variable time it rose; the latter trend could be reversed by return to aerobic conditions. In the absence of glucose under anaerobic conditions the pH usually rose, and the rise could be slowed down or arrested, but not reversed, by the addition, after a short time, of glucose. (Dickens and Greville (8) found that brain slices lost metabolic activity very rapidly in the absence of both oxygen and glucose.) It seems evident that the pH of a slice is at least partially dependent upon metabolic activity. Typical records of the above-mentioned observations are shown in Fig. 3.

DISCUSSION

The difference in pH between the slice and the medium might be due to the following factors: (a) difference between the carbon dioxide concentrations in the medium and in the inner layers of the slice; (b) difference between the lactic acid concentrations in the medium and in the slice, (c) buffering effect of tissue proteins and other substances; and (d) other factors connected with tissue and cell structure, permeability, and metabolism.

Warburg (9) calculated that a respiring slice of a tissue of thickness approaching the limit for adequate oxygenation of its inner layers would have, at equilibrium, a carbon dioxide tension in its inner layers about 3 per cent of an atmosphere higher than in the surrounding medium. A saline solution buffered with 0.02 M bicarbonate has at 38° a pH of about 9.6 in equilibrium with air and 7.35 with 5 per cent carbon dioxide. An increase of the CO₂ tension by 0.03 atmosphere would lower the pH to 7.5 and 7.1 respectively. If phosphate-buffered solutions, 0.03 M, having initial pH values of 8.0 and 7.4 in air, are equilibrated with 3 per cent CO₂, it may be calculated that their pH should drop to 7.2 and 7.0 respectively. (The pH of a medium at or below pH 6 would of course scarcely be affected by CO₂.) Thus the concentration of CO₂ within the slice could theoretically account for a considerable pH difference between the innermost layers of a slice and the medium in the alkaline range under aerobic conditions.

Some determinations were made of the lactic acid concentration in brain slices and in the suspending Ringer-phosphate-glucose medium (pH 7.5) after a period of aeration with oxygen. The slices were rapidly drained and ground up with weighed amounts of sand and 5 per cent trichloroacetic
acid and the mixture reweighed. A sample of the medium was also treated with trichloroacetic acid. Lactic acid was determined on the filtrates by the method of Barker and Summerson (10). The concentrations of lactic acid in the slice and in the medium were found to be about 8 and 3 micromoles per ml. respectively. The lactic acid concentration within the slice is thus definitely higher than in the surrounding medium and probably contributes to the pH difference, but the extent of this effect cannot readily be assessed. Perhaps the differences in CO₂ and lactic acid content of the tissue could together account for most of the pH differential of slices respiring in glucose-containing media.

Under anaerobic conditions in the presence of glucose, the pH of the slice was well below that of the medium in spite of the fact that respiratory CO₂ production would not be contributing to the pH difference. The differential might be accounted for in this case by a large accumulation of lactic acid within the slice.

In the presence of oxygen but in the absence of glucose, aerobic glycolysis would not occur but rather residual lactate within the slice would be consumed. With added lactate there would be definite consumption of lactate with liberation of base. It is thus to be expected that the pH of the slice would be higher in these conditions than in the presence of glucose and oxygen.

It seems probable to us that the buffering effect of the tissue materials must also be concerned. This is probably the main factor which keeps the pH of slices higher than that of the medium when the latter is below 5. Doubtless other factors are also concerned.

The pH of the interstitial fluids within the slice may vary with the distance from the surface and it is uncertain to what portion of the slice the pH recorded by the present method refers. Since the tips of the electrodes were a little less than 1 mm. in diameter and the slice thickness nearly 0.5 mm., it might be suggested that the recorded pH refers to a point nearly 0.5 mm. from the free surface. Since the deepest level in a slice of the usual thickness is half this distance, it is possible that the difference in pH between the medium and the innermost layers of a thin slice is somewhat less than that shown.

Since similar differences in hydrogen ion concentration between the medium and rat brain, kidney, and liver slices have been observed, the phenomenon is presumably general. In most studies on the metabolism of tissue slices in media at about pH 7.4, the actual pH of the interstitial fluids in the slice has evidently been lower than what is commonly considered to be the normal physiological level. But it seems possible that the difference between the pH recorded by an electrode placed on a slice and the pH of the surrounding medium may represent a true physiological
situation and not merely a result of the lack of capillary circulation. This is rendered more probable by in vivo observations of Elliott and Jasper (11), who found that the pH recorded from an electrode placed against the pial surface of a lightly anesthetized cat was independent of the pH of fluid irrigating the brain and surrounding the electrode, except its tip, and was always appreciably lower than that of the venous blood. The pH so recorded varied from point to point, depending, apparently, upon the proximity of larger blood vessels, and values as low as 7.0 were obtained.

**SUMMARY**

The true optimum pH for brain tissue respiration is about 7 to 7.5. The pH of slices of brain and other tissues is lower than that of the suspending medium at all pH values above 6.

**BIBLIOGRAPHY**

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