THE PRODUCTION OF INTRACELLULAR ACIDITY BY NEUTRAL AND ALKALINE SOLUTIONS CONTAINING CARBON DIOXIDE

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It has recently been pointed out by the author (1) that the apparently contradictory views as to the mode of action of CO₂ on the mammalian respiratory center expressed on the one hand by Winterstein (2), Hasselbalch and Lundgaard (3), (1) and others, and on the other by Laqueur and Verzar (5), Hooker, Wilson and Connet (6) and Scott (7) are not necessarily in conflict. Carbonic acid may conceivably, like any other acid, act primarily through its hydrogen ions, as the first group of workers have held, and yet differ so greatly from other acids in certain peculiarities connected with its powers of penetrating living cells as to have practically as specific an action as that postulated by the second group.

One of the most interesting of these peculiarities is the relatively small importance of the hydrogen ion concentration of a solution containing carbon dioxide in determining the degree of intracellular acidity produced in a cell exposed to it. The author has already called attention to the fact that a neutral or even a slightly alkaline CO₂-bicarbonate mixture is practically as toxic to tadpoles as a pure CO₂ solution of the same concentration, and that such a solution has a sour taste. He has interpreted these results as being due to the fact that the H₂CO₃, whose dissociation is held in check by the bicarbonate, can freely enter cells (perhaps as CO₂), while the bicarbonate cannot, the carbonic acid dissociating when within the cells to the extent permitted by the new conditions of equilibrium prevailing there, which may easily result in a hydrogen ion concentration higher than that of the surrounding medium.

While the observations already recorded have probably been correctly interpreted, it has seemed desirable to supplement them with a case where the rise in intracellular acidity under the conditions in ques-
tion is actually visible to the eye. Such a case might be furnished by a cell containing a sufficiently sensitive natural indicator whose color changes would give visible evidence of any increase in the hydrogen ion concentration within the cell. After a rather lengthy search, material of this character has been found in the colored flowers of *Symphyotum peregrinum*, a cultivated plant belonging to the family Boraginaceae. These flowers, like those of many other members of the family, are pink in the bud, later becoming blue; the former color is associated with a higher, the latter with a lower hydrogen ion concentration. The exact pH of the turning point has not been determined, but is within the range of carbonic acid solutions.

The use of colored flowers to study cell penetration by acids, though Haas (8) obtained good results with this method, has not been very much favored in the past because of the fact that such cells are generally cuticularized and are not readily wetted by aqueous solutions. In the present case, however, it was found that if the end of the tubular corolla were snipped off with a pair of scissors and the whole flower were then slipped over the tapering end of a glass rod which could be dropped into a test-tube containing the solution to be studied, the change in color appeared quickly and regularly and could easily be followed, especially along the “ribs” corresponding to the attachment of the stamens, and at and above the depressions which are found at the points of attachment of the “corolla scales.” To observe the change in color, a hand lens was used, though even with the naked eye the results were easily visible. For example, the difference between a flower exposed to a saturated solution of CO₂ in distilled water was usually apparent in five minutes at a distance of five or six feet. The change in color with weak solutions of acids always began on the “ribs” and above the depressions mentioned, and gradually spread from them to other parts of the flower.

As a considerable number of similar experiments with this material all gave essentially the same results, it will be sufficient to describe a typical one. Four flowers from the same plant, as nearly alike in color as possible, were selected. The first was exposed to a saturated solution of CO₂ in distilled water (pH approximately 3.8); the second to pure distilled water of pH between 5.0 and 6.0 (the slight acidity being due to CO₂ absorbed from the air); the third to an M/2 solution of NaHCO₃ saturated with CO₂ at atmospheric pressure and having a pH of ca. 7.4, and the fourth to an M/2 solution of NaHCO₃.
considerably more alkaline than pH 8.0. The course of the experiment may be indicated most clearly in tabular form as shown in table 1.

It will be observed that the increase in acidity of the cells was not proportional to the pH of the external medium, since those exposed to

<table>
<thead>
<tr>
<th>TIME</th>
<th>(1) DISTILLED WATER + CO₂</th>
<th>(2) DISTILLED WATER</th>
<th>(3) 2/3 NaHCO₃ + CO₂</th>
<th>(4) 2/3 Na₂CO₃</th>
</tr>
</thead>
<tbody>
<tr>
<td>11:20* Blue</td>
<td>Blue</td>
<td>Blue</td>
<td>Blue</td>
<td>Blue</td>
</tr>
<tr>
<td>11:21 Pink color appearing on lower “ribs” and above depressions</td>
<td>No change</td>
<td>Pink color appearing on lower “ribs” and above depressions</td>
<td>No change</td>
<td>No change</td>
</tr>
<tr>
<td>11:23 Upper “ribs” becoming pink</td>
<td>No change</td>
<td>Upper “ribs” becoming pink</td>
<td>No change</td>
<td>No change</td>
</tr>
<tr>
<td>11:25 Pink spreading laterally from lower “ribs”</td>
<td>No change</td>
<td>Pink spreading laterally from lower “ribs”</td>
<td>No change</td>
<td>No change</td>
</tr>
<tr>
<td>11:27 Lower portion of flower mostly violet with pink “ribs”</td>
<td>No change</td>
<td>Lower portion of flower mostly violet with pink “ribs”</td>
<td>No change</td>
<td>No change</td>
</tr>
<tr>
<td>11:31 Upper portion of flower shows considerable pink and violet outside of “ribs”</td>
<td>No change</td>
<td>Upper portion of flower shows less violet than (1) but “ribs” are, if anything, pinker</td>
<td>No change</td>
<td>No change</td>
</tr>
<tr>
<td>11:38 About the same</td>
<td>No change</td>
<td>About the same</td>
<td>No change</td>
<td>No change</td>
</tr>
<tr>
<td>11:45 Same</td>
<td>No change</td>
<td>Same</td>
<td>Becoming slightly bluer than (2)</td>
<td>Beginning to turn greenish</td>
</tr>
<tr>
<td>1:05 Same</td>
<td>No change</td>
<td>Slightly bluer than before but still pinker than (2)</td>
<td>Slightly bluer than before but still pinker than (2)</td>
<td>Beginning to turn greenish</td>
</tr>
<tr>
<td>2:30 Same</td>
<td>No change</td>
<td>About the same as (2)</td>
<td>Decidedly greenish</td>
<td>Decidedly greenish</td>
</tr>
</tbody>
</table>

* Started.

the slightly alkaline CO₂-bicarbonate mixture became distinctly acid, while those in the distilled water with a hydrogen ion concentration perhaps one hundred times as great did not. There was also comparatively little difference (up to the time when the bicarbonate finally
began to penetrate) between the two solutions saturated with CO₂, though the concentration of hydrogen ions in the one was approximately four thousand times that in the other. Evidently, therefore, the neutrality or slight alkalinity of a solution does not rule out the possibility of decided effects of hydrogen ions where CO₂ is one of the substances concerned, and the stimulation of the mammalian respiratory center in Scott’s (7) experiments by blood of pH 7.6 would not necessarily be in conflict with the orthodox view of the role of hydrogen ions in this process.

It may perhaps be of interest to show that the effects of CO₂ on living cells here described may be imitated by a simple model whose construction depends on the fact that CO₂ is freely soluble in xylene (as well as in other lipid solvents and lipoids) while NaHCO₃ is not. The “cell” is made as follows. A small “shell vial,” 10 by 35 mm., is filled to within 4 mm. of the top with a solution of phenolsulphonphthalein made very slightly alkaline by the addition of a trace of NaHCO₃. It is next filled level full with xylene (from which the CO₂, if necessary, has been removed by allowing it to stand in contact with an aqueous Ba(OH)₂ solution, and the escape of the xylene is then prevented by the following simple expedient. A test tube is dipped into a fairly thick colloidin solution and the film which closes its mouth on removal is immediately pressed over the opening of the vial to which it adheres as a thin transparent covering, freely permeable to salts, acids, etc., but preventing the loss of the xylene when the “cell” is immersed in water. The vials as thus prepared are kept until needed in an upright position in water. In some cases cottonseed oil was used in place of xylene with essentially similar results, though it is less convenient to work with, and results are obtained more slowly with it than with xylene.

A typical experiment with “artificial cells” was the following. The solutions used were the same as those studied with the Symphytum flowers, namely, distilled water saturated with CO₂, distilled water, M/2 NaHCO₃ saturated with CO₂, and M/2 NaHCO₃. With the distilled water and NaHCO₃, there was no change in the color of the indicator; with the two solutions containing CO₂, though, as before, the actual hydrogen ion concentration of the one was perhaps four thousand times as great as that of the other, there was an approximately equal rate of change in color of the red indicator solution to a bright yellow. In both cases, the change began to be visible within two
minutes and was complete in about an hour and a half. The line of
demarcation between the red and the yellow portions of the solution
was very sharp, especially at first.

The result of one alkaline solution turning acid when placed in
another alkaline solution is a rather striking one. In this case it evi-
dently depends on the relative solubilities of CO₂ (and perhaps H₂CO₃),
on the one hand, and of NaHCO₃ on the other, in lipoids and lipoid
solvents. In living cells there is frequently a close correlation between
the lipoid solubility of substances and their powers of penetration.
Without at all postulating a lipoid membrane in Overton's original
sense, it is nevertheless possible that the relative solubilities of the
substances concerned in the lipoid and aqueous phases encountered in
the structure of a typical cell may have much to do with the physio-
logical behavior of CO₂-bicarbonate mixtures.

In connection with the points already mentioned, it is of interest
to compare the penetrating powers of CO₂ with those of other acids,
particularly those which, from the results of previous workers, appear to
erate living cells with the greatest readiness. Such a comparison was
in progress at the time when a severe storm put a temporary end to the
available supply of Symphytum flowers. Reserving, therefore, for a
subsequent paper the details of the work already done and certain
points requiring further elucidation, it may be said briefly that of all
of the acids studied (carbonic, benzoic, salicylic, valeric, butyric, acetic,
sulphuric and hydrochloric), carbonic is by far the most effective when
pure solutions of equal pH are compared, in causing a visible change in
intracellular acidity. For example, with a solution saturated with
CO₂ at ordinary temperatures, a visible change in color usually begins
in one or two minutes, while with the acids next in the order of their
effectiveness (benzoic and valeric) fifteen to thirty minutes are required,
with butyric, acetic and salicylic acids following in the order named.
The mineral acids are only very slightly effective.

These results, as far as the acids other than carbonic (which appar-
ently has not yet been studied in this manner) are concerned, agree fairly
well with the findings of Haas (8) and of Collett (9) and also with the
observation of the author (1) that tadpoles are fatally injured in satu-
rated CO₂ solutions in two or three minutes, while in solutions of other
acids of the same pH, from one to several hours are required to produce
the same result.

If carbonic acid be compared with other acids of the same normality
instead of the same pH, it appears far down the list, as would be ex-
pected, not because it does not enter cells readily—for there is much evidence that it does—but because, on account of its weakness (i.e., its low degree of dissociation), it is necessary for so much more of it to enter the cell before a change in the color of the indicator can occur than in the case of the more strongly dissociated acids.

One further point may be noted. If a solution, e.g., N/10, of NaOH have various acids added to it (in as concentrated a form as possible to avoid dilution) until the point of neutrality, as shown by phenolsulphonephthalein is reached, there should at this point, in the case of fairly strong acids, be practically no free acid present, while in the case of a weak acid like H$_2$CO$_3$, there should be a considerable amount. This difference may easily be made visible in the case of carbonic and other lipoid-soluble acids, such as benzoic, salicylic and butyric, by using the "artificial cells" already described. In one such experiment where the solutions were all neutral and N/10 with respect to total base, the indicator solution within the vial began to change color in three minutes with carbonic acid, while with butyric, benzoic and salicylic acids (all of which are highly effective with the "artificial cells" in the free form on account of their solubility in xylene) no change had occurred in three hours. Had the water used been so free from CO$_2$ as to make possible the use of an indicator solution with a lower buffer value, the effect of the smaller amounts of the other acids could probably have been detected as well, but as the experiment was not intended to be accurately quantitative, but merely to show that a very considerable difference exists between carbonic and the other acids, it was not considered necessary to take this precaution.

As the final conclusion to be drawn from the various experiments described in this paper, which have been made partly on living plant cells and partly on a simple "artificial cell," it may be stated that the physiological behavior of CO$_2$ probably depends to a considerable extent on two of its chemical and physical peculiarities: $a$, the weakness of H$_2$CO$_3$ as an acid, permitting the existence of a relatively large amount of the free but undissociated acid (as well as dissolved CO$_2$) in the equilibrium that exists at neutrality or slight alkalinity; and $b$, the readiness with which the undissociated acid, or its anhydride, CO$_2$, enters living cells, perhaps in virtue of its lipoid solubility. The combination of these two peculiarities is responsible for certain of the remarkable, and in some respects, unique, physiological properties of carbon dioxide.
PERMEABILITY OF CELL WALL TO CARBON DIOXIDE

SUMMARY

1. It has been found that the flowers of Symphytum peregrinum contain a natural indicator sensitive to carbonic acid, which may be used to study cell penetration by CO₂.

2. Using this material, it appears that a condition of intracellular acidity can be produced by a slightly alkaline solution of CO₂ in M/2 NaHCO₃ almost as effectively as by a solution of CO₂ in distilled water, though the hydrogen ion concentration of the latter solution is approximately four thousand times as great as that of the former.

3. A similar result may be obtained by the use of an “artificial cell” whose construction is described.

4. From pure aqueous solutions of acids of the same pH, carbonic acid changes the color of Symphytum flowers more quickly than do any of the other acids studied.

5. The ability of neutral and slightly alkaline solutions containing CO₂ to produce intracellular acidity is probably due to at least two factors: a, the weakness of H₂CO₃ as an acid and b, the lipoid solubility of CO₂ or H₂CO₃ or both, and the lack of such solubility in the case of bicarbonates.

BIBLIOGRAPHY

(1) Jacobs: This Journal, 1920, li, 321.
(2) Winterstein: Pflüger's Arch., 1911, cxxviii, 167.
(7) Scott: This Journal, 1918, xlvi, 43.