THE RELATIVE IMPORTANCE OF pH AND CARBON
DIOXIDE TENSION IN DETERMINING THE CESSA-
TION OF CILIARY MOVEMENT IN
ACIDIFIED SEA WATER.

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The activities of all living cells are so profoundly influenced by the
hydrogen ion concentration of their surroundings that for a number
of years physiologists have concerned themselves very extensively
with determinations of the pH of physiological fluids without
sufficient recognition of the fact that two solutions of exactly the same
pH may have totally different effects upon the internal reaction of
cells exposed to them. It has been shown by Jacobs (1-3), in the
case of systems containing CO₂ and bicarbonate or free ammonia and
ammonium salts, that external and internal reactions frequently do not
run parallel. Intracellular acidity may be produced by alkaline
solutions, or intracellular alkalinity by acid ones. The experiments
of Smith and Clowes (4, 5) also show that while the reaction of the
surrounding sea water is a factor of much importance in determining
the rate of cleavage of As terias and Arbacia eggs, the effects produced
at the same pH are very different according to whether the buffer
system used contains much or little free CO₂. Very recently, similar
results have been obtained with rabbit intestine by Fraser (6) and
with human leucocytes by Pearse (7).

In the present series of experiments an attempt has been made to
determine whether the same principles apply to cilia, which because
of their small volume and relatively enormous surface might be ex-
pected to show less contrast between external and internal effects
than is the case with most other protoplasmic structures. The
general result of these experiments is to indicate that, while the pH
of the surrounding medium is a factor of much importance in determining cessation of ciliary movement, the actual character of the buffer system concerned—in particular its CO₂ tension—is a factor of equal or even greater importance.

The material used was obtained from the gills of the mussel, *Mytilus edulis*. The behavior of the cilia of the gill filaments was followed in

![Diagram](https://i.imgur.com/3J12.png)

**Fig. 1.** Curves showing the effect of various CO₂ tensions upon the length of time required for cessation of ciliary beat in *Mytilus* gill. The abscissa represents the pH; the ordinate represents time in minutes up to 40 minutes, above which, at the top of the figure, are indicated 24 hour values.

I. Low CO₂ tension. (Sea water acidified and aerated.)
II. Moderate CO₂ tension. (Sea water acidified, but not aerated.)
III. High CO₂ tension. (Sea water and NaHCO₃, acidified, but not aerated.)

sea water of low, medium, and high CO₂ tension and of varying pH values. The method employed in preparing the solutions was essentially that of Smith and Clowes (5). The experiments were carried out in small, glass-stoppered bottles of 35 to 40 cc. capacity which were filled and kept closed to prevent the escape of CO₂. The desired ranges of pH and CO₂ tensions were obtained by using (I) sea water
which, after the addition of sufficient $n/2 \text{HCl}$, was aerated in an automatic shaker for 1 hour to reduce the CO$_2$ tension to that of the atmosphere; (II) sea water to which HCl was added without subsequent aeration; and (III) a 2.5 per cent solution of $n/2 \text{NaHCO}_3$ in sea water to which HCl was also added without subsequent aeration. Solutions II and III were prepared in the bottles used for the experiments in order that the loss of CO$_2$ might be minimized. Since the indicators employed (brom-phenol blue, methyl red, brom-cresol purple, and brom-thymol blue) were shown by control experiments to be non-toxic in the slight concentrations required for adjusting the reactions of the solutions, they were added directly to the latter.

The procedure employed in the experiments was the following. Three or four small pieces of *Mytilus* gill were introduced into a bottle nearly filled with the solution to be used and containing only sufficient indicator to make the necessary pH adjustment; HCl was then quickly added, the bottle stoppered, and the contents mixed by gentle shaking. The pH was determined by comparison with a set of standard tubes. The pH values here given must, for strict accuracy, be corrected for the salt error due to the use of sea water, but for comparative purposes they are sufficiently exact. After various lengths of time the bottles were opened and the pieces of gill were removed for immediate microscopic examination. Because it often appeared that the cilia in different portions of the gill were affected to different extents by the acid medium, the time determined was that required for the cessation of the beat of the smaller terminal cilia, which were very easily seen in profile. When a bottle to which HCl had been added was opened, its contents were discarded after the first examination, since the CO$_2$ content of the solution could then no longer be controlled.

In the accompanying graph the various experiments made have been grouped together to show not merely the average values obtained but the range of variation encountered. In the case of single experiments made on material from the same gill the data when plotted gave fairly smooth curves similar in form to those which have, for the sake of clearness, been drawn to represent the general trend of the observations. It will be seen from inspection of Curve I that the pH as such is evidently a factor of importance, since in this case the CO$_2$
tension was not allowed to vary but was at all times that of the room air. That the pH of the solution is not the only, or indeed the most important factor, however, is shown by a comparison of this curve with the other two. It will be observed that in the first curve (where the CO₂ tension is low) that at a pH of 5.6, for example, the beat continued for over 24 hours; it ceased in the second (where the CO₂ tension is medium) at the same pH in about 30 minutes or less; and in the third (where the CO₂ tension is high) it ceased instantly. With each of the given CO₂ tensions a zone exists which may be considered as a critical region for the cessation of the ciliary beat. The upper limit of this zone is represented by the lowest pH at which the beat continues for 24 hours; the lower limit is the pH which stops the cilia at once. The values for this region for each CO₂ tension would then be approximately as follows:

- Low CO₂ tension: pH 3.7-5.2
- Moderate CO₂ tension: pH 4.4-6.4
- High CO₂ tension: pH 5.9-6.9

Evidently, therefore, in the case of *Mytilus* cilia, as in the experiments of Smith and Clowes on echinoderm eggs, the CO₂ tension has an effect of its own.

Whether carbon dioxide produces its characteristic effects on cilia by increasing the intracellular acidity as has been supposed by Jacobs (2) to be the case in some of his experiments, or acts in some other more specific manner, must for the present be left undetermined. It is at any rate clear that in work done on this and similar material where the pH of the sea water is changed by the addition of acid the results will be of little value if care is not taken to control at the same time the carbon dioxide tension of the medium.

**SUMMARY.**

The length of time that cilia from the gills of *Mytilus* continue to beat in acidified sea water depends to some extent on the pH of the solution but to a greater extent on its carbon dioxide tension.

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BIBLIOGRAPHY.