# The Effects of Ions upon Ciliary Movement.

By

**J. Gray, M.A.,**

Fellow of King's College, Cambridge.

With 6 Text-figures.

THE experiments described in this paper constitute an attempt to determine the necessary external conditions which support ciliary action. Concerning the mechanism of the cell itself practically nothing is known, and comparatively little work has been done to determine the factors involved by the external medium. In 1902–1906 R. S. Lillie published a series of papers dealing with ciliary action; since then our knowledge of the physical chemistry of solutions of electrolytes has greatly increased, and it seemed desirable to re-open this line of investigation.

Almost from the beginning of the present series of experiments it became apparent that the hydrogen-ion was a factor of supreme importance. Although the work of Sorensen and Palitzch (**6**) has made the determination of the hydrogen-ion concentration of any particular sample of sea-water a comparatively simple matter, yet the conditions which determine this value are by no means clear. Sea-water contains calcium, magnesium, carbonates and phosphates, all of which influence the alkalinity, and at the same time form a complex chemical equilibrium with the carbon dioxide of the air. It will therefore be seen that if the equilibrium is upset by varying the amount of any of these constituents of sea-water the control of the hydrogen-ion concentration of the solution is

not an easy problem. It is quite clear that it is not permissible to compare the physiological effects of solutions unless their hydrogen-ion concentrations are adequately controlled—in other words, the qualitative effects of such ions as sodium, calcium or magnesium can only be studied when the hydrogen-ion factor has been excluded. The importance of this factor can hardly be exaggerated.

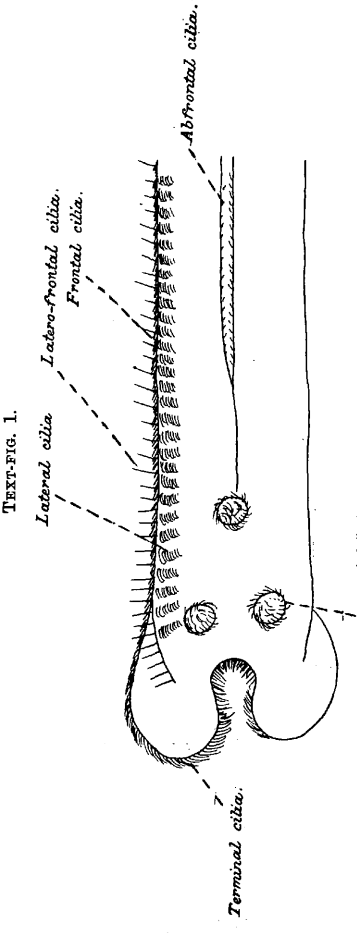
It is admitted that the results obtained neither indicate any specific property of the metallic ions contained in sea-water, nor are they of sufficiently critical value to exclude the existence of such properties. It would appear that such results will only be obtained when it is possible to find satisfactory experimental conditions for controlling the hydrogen-ion concentration of artificial salt-solutions for long periods of time. For example, a solution of isotonic calcium chloride rapidly changes its hydrogen-ion concentration owing to absorption of $CO_2$ from the air and subsequent precipitation of calcium carbonate. Such a solution, of course, forms an extreme case, but the importance of this point is made evident in the following pages.

The material chosen for this work was the gills of Mytilus edulis. It is simple to obtain, and the gill structure has been carefully observed; further, fragments of gills form a highly satisfactory subject for prolonged experiments. The structure of the gill of Mytilus has been fully described by Orton (**4**), but for the sake of convenience the following summary is given of these observations.

The main inhalent current of water is caused by the lateral cilia. These cilia are exceedingly numerous, and each cell of the epithelium bears several cilia (Text-fig. 4).

It is somewhat difficult to describe the direction of movement of the lateral cilia. Orton states that they "lash across the length of the filaments," but the most characteristic feature of their normal movement is a wave of pulsation which passes along the length of the whole line—the waves pass up on the oral side of the filament and down on the aboral side.

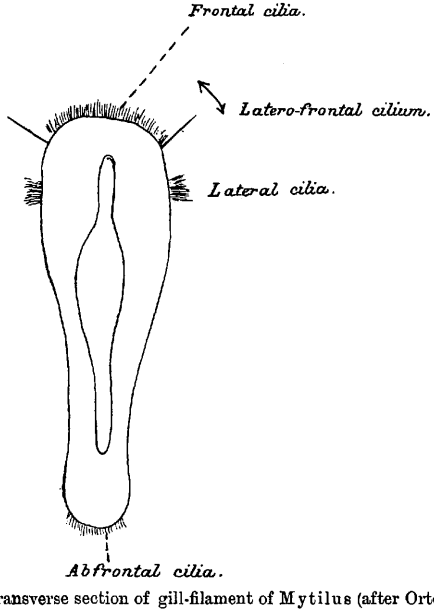
TEXT-FIG. 1.




Lateral view of a living filament of the gill of Mytilus edulis (after Orton).

The latero-frontal cilia are the most conspicuous cilia on the whole gill (ex. the terminal cilia). They stand out stiffly from the filament and move relatively slowly on a line parallel to the transverse axis of the filament. Each cilium arises from

TEXT-FIG. 2.



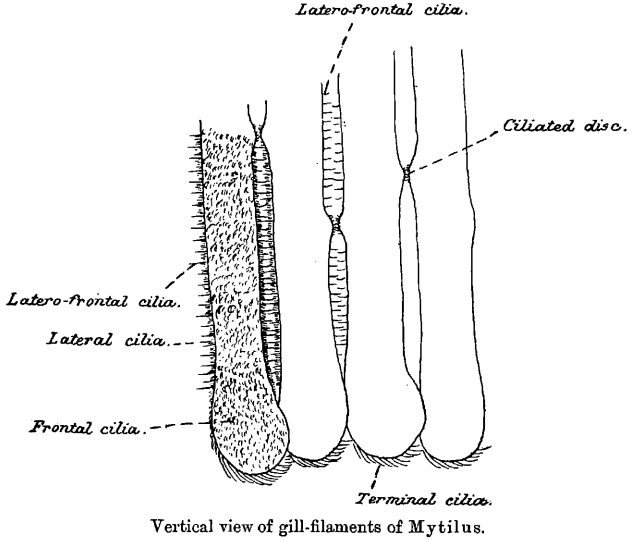

Transverse section of gill-filament of Mytilus (after Orton.)

a single cell (Text-fig. 5). These are straining cilia and assist in passing food from the main current on to the frontal cilia. The frontal cilia pass the food down the face of the gill on to the ciliated terminal groove. The cilia of the terminal groove lash in an oral-aboral direction and pass the food in a long line of mucus up to the mouth.

In studying the effects of various ions on ciliary movement it was therefore decided to select the terminal cilia and the latero-frontals. These cilia have distinct advantages.

(1) The terminal cilia are absolutely free in the solution, and are extremely easy to observe under the microscope.

TEXT-FIG. 3.



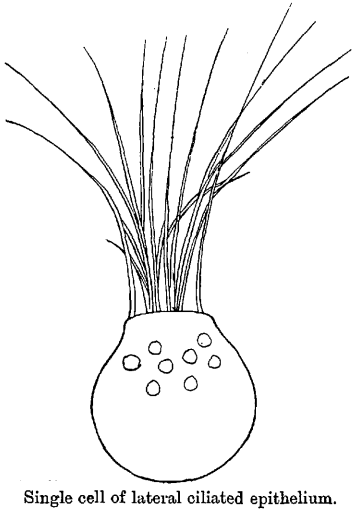

Vertical view of gill-filaments of Mytilus.

(2) The latero-frontal cilia are very obvious, as they are large cilia, and each cilium arises from a single cell.

It is advisable to repeat the recommendation of Orton to the effect that "in the case of all the cilia on gill-filaments, it is necessary to see them living to obtain an accurate idea of their size and function." This is particularly the case with the latero-frontal cilia of Mytilus. In the living gill it is impossible to confuse these

cilia with any others—they are much more distinct and move more slowly than any of the others. In preserved specimens they are almost indistinguishable from the lateral cilia. When a portion of the gill is removed the whole of this ciliated mechanism continues—even the co-ordination and transport of the food columns up to the oral corner of the terminal groove.

TEXT-FIG. 4.



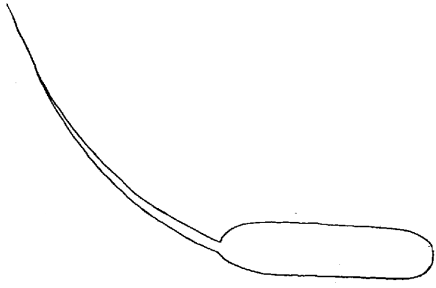
Single cell of lateral ciliated epithelium.

It will be noticed that the ciliary complex of the gill of Mytilus forms a highly co-ordinated system of vital importance to the animal, for on the rate of ciliary movement will depend the amount of food available for nutrition. Such a system one would normally suppose to be closely dependent upon the nervous system of the animal, and that as soon as a portion of the tissue is removed, the mechanism of co-ordinated movement would be speedily deranged. This, however, is

not the case. If a single gill-filament be removed and kept in normal sea-water, the whole of the ciliary movement continues unchanged for a very long period. If a portion of the gill-plate $\frac{1}{8}$ in. or more in width be removed, the cilia continue to beat normally for at least fourteen days, with the exception of the lateral cilia, which often cease to show their characteristic waves of pulsation, and may even stop. The latero-frontal, frontal and terminal cilia invariably remain normal.

The reorganisation and regeneration of the ciliated tissue

TEXT-FIG. 5.



Single cell of latero-frontal ciliated epithelium.

which takes place in isolated gill-fragments after about four days will be described in a subsequent publication.

During the progress of this work two distinct phenomena have arisen: (1) The action of certain ions upon the activity of individual ciliated cells—i.e. upon ciliary movement as such; (2) the action of certain ions upon the tissue complex. Thus the absence of certain ions results in the break-up of the ciliated epithelium into its constituent cells, although the cilia of the individual cells continue to beat strongly when the cells are entirely detached from the gill and from other cells. It is only in the case of the hydrogen-ion that direct action upon ciliary movement can be studied without injury to the general structure of the gill.

In these pages the following abbreviations will be used:

| | |
|---|---|
| Terminal cilia . . . | Terminals. |
| Latero-frontal cilia. . . | Latero-frontals. |
| Lateral cilia . . . . | Laterals. |
| Hydrogen-ion concentration . | $P_H$. |

For the sake of simplicity the hydrogen-ion concentration will be referred to as "high" or "low," according to the numerical value of the exponent $P_H$—for example, a solution of $P_H = 9{\cdot}0$ will be regarded as having a higher hydrogen-ion concentration than a solution for which $P_H = 7{\cdot}0$. This, of course, involves a contradiction in terms, but it greatly simplifies the text for any reader not intimately conversant with these particular problems.

The term "disintegration" will be used to denote the separation of the individual ciliated cells of the gill epithelium which takes place in many solutions.

## Acids and Alkalis.

The sea-water used for these experiments was obtained from the English Channel, some miles south of Plymouth; the hydrogen-ion concentration was constant about 7·85 during the whole period of the experiments. The hydrogen-ion concentration of the various solutions used was determined by Sørensen's and Palitzsch's method, and the salt correction of these authors was applied in each case.

The effect of lowering the $P_H$ of the sea-water on the ciliary movement of the gills is shown in the following table.

The table shows clearly the profound effect of the hydrogen-ion on ciliary activity. In solutions whose $P_H$ is below about 6·0 the cilia very rapidly came to rest. There is no sign of the ciliated epithelium breaking up, and, as will be shown later, the stoppage of the cilia is entirely reversible when the $P_H$ of the water is raised.

In solutions of $P_H$ 6·7 there appears to be a certain amount of regulation to altered conditions. Thus in sea-water of $P_H$ 6·7 the rate of ciliary movement is greatly depressed after about twenty minutes, after which the rate gradually

TABLE I.

| 50 c.c. sea-water + c.c. N/10 HCl. | $P_H$. | Ciliary movement after— | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 5′. | 10′. | 20′. | 30′. | 40′. | 60′. | 90′. | 120′. | 18 hrs. |
| ·14 . . | 7·34 | ++ | ++ | ++ | ++ | ++ | ++ | ++ | ++ | ++ |
| ·28 . . | 6·9 | ++ | ++ | ++ | ++ | +⊕ | ++ | ++ | ++ | ++ |
| ·42 . . | 6·7 | ++ | ++ | +⊕ | + | ⊕ | +⊕ | +⊕ | +⊕ | ++ |
| ·56 . . | 6·6 | ++ | +⊕ | ⊕ | ⊕ | very slight | very slight | ⊕ | +⊕ | ++ |
| ·70 . . | 6·2 | ++ | +⊕ | ⊕ | ⊕ | very slight | very slight | very slight | slight | ++ |
| ·98 . . | 5·5 | + | ⊕ | ⊕ | 0 | 0 | 0 | 0 | 0 | 0 |
| 1·19 . . | 4·2 | very slight | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1·33 . . | 3·8 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1·40 . . | 3·6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

++ Very active. + Considerable movement.
+⊕ Active. ⊕ Slight.

increases until after sixteen hours the rate of movement is equal to that of the control. In the more acid solutions no recovery takes place, however, and the tissue eventually loses its translucent appearance and dies. With different individuals the critical $P_H$ differs, but in each case the critical value is well marked; for example, in the case of one gill, movement persisted in 50 c.c. sea-water + 1·36 c.c. N/10 HCl for twenty minutes, whereas it stopped instantly in 50 c.c. sea-water + 1·4 c.c. N/10 HCl; in the case of another gill, movement persisted in 50 c.c. sea-water + 0·7 c.c. N/10 HCl, and stopped in 50 c.c. sea-water + 0·84 c.c. N/10 HCl. Such extreme values are rare, and the average critical value for the N/10 HCl is about 1·12 c.c. to 50 c.c. sea-water.

Before dealing further with the effect of acids, it is convenient to consider the effect of alkalis. If strong alkali is added to sea-water only a comparatively small change is effected in the $P_H$ of the solution before magnesium and calcium begin to be precipitated. At this point a coloration is given with thymolphthalein, equivalent to a $P_H$ of about 9·6. In all such solutions ciliary movement continues actively

and usually more actively than in the control experiments. The alkali, however, causes marked disintegration of the tissues, and the individual ciliated cells break away with their cilia still beating strongly. Disintegration occurs in 50 c.c. sea-water + 1·68 c.c. N/10 NaOH or in 50 c.c. Van't Hoff solution + 0·30 c.c. N/10 NaOH.

It is therefore impossible to determine the duration of ciliary movement in hyperalkaline solutions, owing to the fact that the movement continues longer than the tissue retains its normal condition. Even when disintegration is far advanced individual cells continue to move actively. Such isolated cells are in an entirely different environment to that afforded by their normal position in the tissues, and therefore the duration of movement of their cilia can hardly be compared to that of cells remaining in their normal positions. It is interesting to note that individual cells rapidly become spherical in shape; eventually the cilia disappear and the cell cytolyses.

The cessation of movement caused by acid is entirely reversible by alkali if the acid treatment has not been too severe.

Portions of a gill were placed in 50 c.c. V. Hoff's solution + 0·32 c.c. N/10 $H_2SO_4$ for two minutes; examination under the microscope showed that all movement had stopped. They were then transferred to the following solutions and examined after twenty minutes:

TABLE II.

| 50 c.c. Van't Hoff's solution. + | Behaviour of cilia after 20 minutes. |
|---|---|
| 0 | Not moving. |
| 0·05 c.c. N/10 $H_2SO_4$ | ,, ,, |
| 0·10 ,, ,, | ,, ,, |
| 0·05 ,, N/10 NaOH | Active normal movement. |
| 0·10 ,, ,, | ,, ,, ,, |
| 0·20 ,, ,, | ,, ,, ,, |
| 0·40 ,, ,, | ,, ,, ,, |

This experiment was repeated many times with the same

results except that in certain cases recovery took place in normal Van't Hoff's solution, while in others recovery did not take place so completely in the weaker concentrations of alkali as it did in the stronger solutions. One point was noticeable, however, namely, that when recovery took place in any solution eventually the rate of ciliary movement nearly always became absolutely normal.

It was found that in the case of a certain gill ciliary movement stopped in less than two minutes in 50 c.c. sea-water + 1·26 c.c. N/10 HCl, and that no recovery took place in this solution. Portions of this gill were left in the acid solution for various periods of time and were then transferred to normal sea-water and the time required for recovery noted.

TABLE III.

| Time of exposure to acid solution. | Time required for full recovery in normal sea-water. |
|---|---|
| 2 mins. | 40 mins. |
| 5 „ | 55 „ |
| 10 „ | 55 „ |
| 15 „ | 50 „ |
| 20 „ | 40 „ |
| 30 „ | 30 „ |
| 40 „ | 30 „ |
| 60 „ | Disintegration set in, only partial recovery. |
| 90 „ | „ „ „ |
| 120 „ | Only a few cells recovered. |
| 180 „ | No recovery. |
| 200 „ | „ |

From the experiments performed it is not safe to draw any definite conclusions except that prolonged exposure prevents ultimate recovery, but it would seem that the length of time required for recovery in sea-water is not dependent upon the length of exposure to the acid solution, and it would seem that the acid produces its maximum reversible effect almost at once, after which a certain amount of regulation takes place within the cell, so that cells exposed to the acid solution of thirty minutes recover just as quickly, if not more so, as cells exposed to the acid for only two minutes.

In their rate of recovery different gills also exhibit differences.

TABLE IV.

| | Duration of movement in 50 c.c. sea-water, + 1·26 c.c. N/10 HCl. | Rate of recovery in sea-water after exposure to acid solution for 20 mins. |
|---|---|---|
| Gill A . . | < 2 mins. | 40 mins. |
| Gill B . . | 7 „ | 20 „ |

In other words, Gill B seems to have been more resistant to the acid solution than Gill A.

## EFFECTS OF "NEUTRAL" SALTS.

The fact that the hydrogen-ion has a profound effect upon ciliary movement at once raises a complication when dealing with artificial solutions of the various salts contained in sea-water. For example, a solution of pure sodium chloride in distilled water gives a bright red colour with neutral red, whereas sea-water is usually orange. The reason for this is, of course, to be found in the $CO_2$ of the air. In the absence of any buffer there is a sufficient concentration of carbonic acid in the sodium chloride solution to give an acid reaction to neutral red or to methyl red. The following table shows the wide variation in hydrogen-ion concentration of the various single salts found in sea-water:

TABLE V.

| Salt. | Indicator. | |
|---|---|---|
| | Methyl red. | Neutral red. |
| NaCl . . . . | Bright red . . . | Bright red. |
| „ after boiling . | Orange red . . . | „ |
| KCl . . . . | Red, with orange tinge . | „ |
| „ after boiling . | Orange . . . . | „ |
| $MgCl_2$. . . . | Orange red . . . | „ |
| „ after boiling . | Orange . . . . | „ |
| $CaCl_2$ . . . . | Yellow . . . . | Red. |
| „ after boiling . | „ . . . . | Yellowish-orange. |

The salts used were as follows:

| | |
|---|---|
| NaCl . . . | Kahlbaum (for analysis). |
| „ . . . | Hopkins & Williams (puriss). |
| KCl . . . . | Merck's extra pure. |
| $CaCl_2$ (anhydrous) . | Merck's. |
| „ ( „ ) . | Kahlbaum (for analysis). |
| $MgCl_2$ . . . | „ „ |

It is clear, therefore, that solutions of the above salts possess hydrogen-ion concentrations widely different from each other and from sea-water, and that in order to compare the physiological effects of such pure solutions it is first necessary to adjust these differences in their $P_H$.

In the case of pure NaCl or KCl the problem presents very little difficulty, as they can readily be buffered by the presence of a very small quantity of sodium or potassium bicarbonate; further, the addition of these salts adds no fresh ions to the solutions. By adding the requisite amount of bicarbonate, solutions can be obtained of the required hydrogen-ion concentration and such solutions can be directly compared with sea-water of the same $P_H$.

The case of magnesium and calcium chlorides is, however, different. Thus, the addition of sodium bicarbonate to a solution of $MgCl_2$ (such as was used in these experiments) never increases the $P_H$ sufficiently to give an orange colour with neutral red. Again, whereas the addition of strong alkali to a solution of NaCl rapidly gives a bright blue coloration with thymol-phthalein, with $MgCl_2$ only a very faint coloration is produced, and the addition of excess of alkali only causes the precipitation of magnesium hydrate without increasing the hydroxyl-ion concentration of the solution. Similar difficulties arise when dealing with mixtures of NaCl and $MgCl_2$ which never possess a $P_H$ of more than about 7·8 when carbonates are present.

The case of $CaCl_2$ is still more difficult. When the pure anhydrous salt was dissolved in distilled water the solution was found to be very strongly alkaline—in fact, 50 c.c. required 8·25 c.c. N/10 HCl to neutralise to neutral red.

Every sample of anhydrous $CaCl_2$ obtainable gave similar results; this alkalinity is doubtless due to the presence of calcium carbonate.

There is, however, a further complication to be considered in the case of magnesium and calcium chlorides. As is well known, the stabilisation of the hydrogen-ion concentration of a solution containing carbonate depends upon the ratio $\frac{HCO_3'}{CO_3''}$; the higher the value of this ratio the greater the alkalinity of the solution. Now magnesium carbonate, and to a much greater extent calcium carbonate, is much less soluble and less ionised than the respective bicarbonates. Hence the presence of magnesium- or calcium-ions in the solution profoundly affects the number of free $CO_3''$ ions which can exist in solution, so that these metals also affect the hydrogen-ion concentration of the solutions to which they are added. By increasing or decreasing the number of magnesium- or calcium-ions the hydrogen-ion concentration of the solution is unavoidably altered.

This complication of the problem is by no means simple, and at the present moment it is only possible to bring forward evidence as to the effect of the various metallic ions between fairly wide limits of $P_H$, and until it is found possible to stabilise the $P_H$ of artificial solutions containing varying amounts of calcium or magnesium, it is impossible to state with exactness the qualitative properties of these ions on physiological processes.

## A. Isotonic Solutions of Single Salts.

### Effects of Solutions of Pure Sodium Chloride.

(1) Pure sodium chloride quickly depresses the rate of ciliary movement, but before cessation of movement is complete disintegration of the tissues begins. The cilia do not appear to dissolve.

(2) Disintegration is well marked in solutions whose $P_H$ is as low as 7·0.

(3) The duration of movement depends partly on the hydrogen-ion concentration of the solution.

TEXT-FIG. 6.

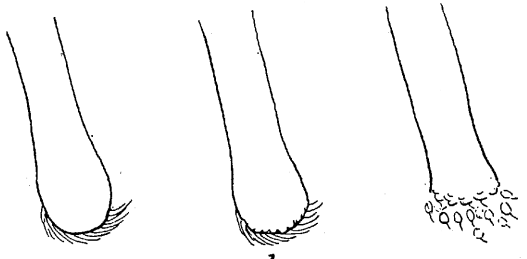



Diagram showing progressive disintegration of the terminal ciliated epithelium in pure sodium chloride.

TABLE VI.

| Solution. | $P_H$. | Duration of ciliary movement. | Remarks. |
|---|---|---|---|
| 50 c.c. ·6 M. NaCl | 7·0 | 30–120 mins. | In about 10 mins. movement much slowed down; in less than 30 mins. disintegration begins. After 1½–2 hours the latero-frontals and terminals show advanced disintegration, although a few cilia are still moving. After 16 hours the gill is completely disintegrated and all movement of cilia has ceased. |
| 50 c.c. ·6 M. NaCl + (·07–·7 c.c. N/10 $NaHCO_3$) | 7·9 | 30–120 mins. | After 16 hours gill completely disintegrated (a few cells twitching). |
| | 9·0 | 30–120 mins. | Disintegration well marked after < 30 mins. (a few cells twitching). |
| ·6 M. NaCl + acid (N/10 HCl) | 4·0 | 30 mins. | Movement stops completely. No disintegration. |
| | 3·3 | 10 „ | Ditto. |
| | 2·9 | 4 „ | „ |

(4) In solutions of $P_H$ 7 and higher it is impossible to determine the duration of ciliary movement, as disintegration sets in before the cilia have ceased to move.

(5) The higher the alkalinity of the solution the more intense is the disintegration of the tissues.

(6) The depressant effect of sodium chloride was not removed by subsequent addition of magnesium chloride (cf. Mayer (3)).

## Effects of Pure Potassium Chloride.

(1) The effects of potassium chloride solution appeared to be almost identical with those of sodium chloride, except that the depressant effect on the rate of movement was less marked.

(2) Disintegration occurs as in sodium chloride, but the individual cells exhibit stronger ciliary movement than in sodium chloride.

TABLE VII.

| Solution. | $P_H$. | After 2 hours. | 5 hours. | 16 hours. |
|---|---|---|---|---|
| ·6 MKCl | 7·0<br>7·4<br>7·6<br>8·1 | Active movement, but progressive disintegration with increasing $P_H$ | Cilia still moving, but disintegration well advanced. Individual cells active | No movement, and complete disintegration in all solutions. |

## Pure Magnesium Chloride Solution.

As mentioned on p. 357, when sodium bicarbonate is added to a solution of magnesium chloride the $P_H$ never rises sufficiently to give an orange colour with neutral red. Even the addition of strong alkali only produces a faint colour with phenolphthalein. The observations given below must be interpreted in the light of these facts.

TABLE VIII.

| Solution. | $P_H$. | Remarks. |
|---|---|---|
| ·6 M. $MgCl_2$ . | 6·8 | Fairly strong movement after 1½ hours. Disintegration began after 2 hours. |
| „ +alkali | 7·8 | Strong movement for 1½ hours. A few cilia moving after 16 hours. Advanced disintegration after 16 hours. |
| „ „ | 8·0 | Ditto. |

(1) Normal movement continued for about one hour, after which it slowed down. A few cells continued to move after 16 hours.

(2) Disintegration sets in after about two hours.

(3) In solutions of $P_H$ 6 and less movement rapidly stopped. On transference to Van't Hoff's solution ($P_H$ 8·0) recovery rapidly took place. No recovery took place on addition of NaCl. $P_H$(7·0).

## Solutions of Pure Calcium Chloride.

In calcium chloride solutions movement usually ceases within one hour, but in solutions whose $P_H$ is about 7·8 movement may persist for more than sixteen hours. In solutions of $P_H$ above 6·0 there is a distinct tendency for individual filaments to separate from one another.

TABLE IX.

| Solution. | $P_H$. | Duration of movement. | Remarks. |
|---|---|---|---|
| ·6 M. $CaCl_2$ | 10·5 | < 1 min. | — |
| | 8·3 | 5 mins. | Gill-filaments separated from each other. |
| | 7·8 | > 45 „ | Tendency for gill-filaments to separate. Some movement after 16 hours. |
| | 7·6 | 12 „ | Gill-filaments separated from each other. |
| | 6·0 | < 1 min. | Recovered in Van't Hoff, $P_H$ 8·0. No recovery in „ „ $P_H$ 6·0. |

As mentioned above, the $P_H$ of calcium chloride solutions do not remain constant for more than a few hours owing to reaction with the $CO_2$ of the air, so that critical experiments are not possible.

### B. Binary Mixtures.

#### (i) Mixtures of Sodium Chloride and Magnesium or Calcium Chloride.

(1) The tissue remains more healthy than in solutions of pure sodium chloride, and ciliary movement remains strong when disintegration is relatively far advanced. Freed individual cells move strongly for some time.

(2) The higher the $P_H$ the more marked is the disintegration.

(3) No apparent difference in effect is noticeable when the relative concentrations $\frac{Na'}{Mg'' \text{ or } Ca''}$ is varied from $\frac{50}{10}$ – $\frac{50}{2}$.

(4) Occasionally cilia remain twitching after forty-eight hours.

Table X.

| Solution. | $P_H$. | Remarks. |
|---|---|---|
| 50 c.c. ·6 M. NaCl | 7·3 | After 16 hours cilia active; some disintegration of lateral epithelium. Gill otherwise normal. |
| 6 c.c. ·6 M. $MgCl_2$ + Sodium bicarbonate | 7·3 | After 24 hours some disintegration, but cilia still moving; after 48 hours, no movement. |
| Ditto . . . . | 7·5 | After 16 hours, very active movement.<br>„ 24 „ disruption far advanced.<br>„ 48 „ complete disruption. |
| Ditto . . . . | 7·6 | „ 24 „ „ „ |

#### (ii) Mixture of Potassium Chloride and Magnesium or Calcium Chloride.

These solutions resemble binary sodium mixtures in all respects.

## Mixtures of KCl and $MgCl_2$.

TABLE XI.

| Solution. | $P_H$. | Remarks. |
|---|---|---|
| 50·6 M. KCl . | 8·0 | 2 hours, active movement; some disruption. |
| 4·6 $MgCl_2$ . | ,, | 24 hours, complete disruption. |
| Ditto . . | 7·6 | 2 hours, active movement; some disruption. |
| | | 24 hours, complete disruption. |
| Ditto . . | 8·6 (by addition of KOH) | 2 hours, considerable disruption. |
| | | 24 hours, complete disruption; a little movement. |

## Mixtures of KCl and $CaCl_2$.

TABLE XII.

| Solution. | $P_H$. . | After 2 hours. | After 16 hours. |
|---|---|---|---|
| 50·6 M. KCl + 2·6 M. $CaCl_2$ | 7·6 | Normal | Some movement; some disruption; no separation of filaments. |
| + 4 $CaCl_2$ . | 7·3 | ,, | Fairly active movement; some disruption; no separation of filaments. |
| + 6 $CaCl_2$ . | 7·0 | ,, | Ditto. |
| + 8 $CaCl_2$ . | 6·8 | ,, | Ditto. |
| + 10 $CaCl_2$ . | 6·5 | Very active; some tendency of filaments to separate | Very little movement; tendency for filaments to separate. |
| 50 KCl + 2 $CaCl_2$ [1] . | 10·4 | Filaments rapidly separated, but cilia moved strongly for some time. Complete disruption and no movement after 24 hours | — |

[1] In this experiment the original $CaCl_2$ solution was used, in the others the solution was previously neutralised to neutral red.

### c. Tertiary Mixtures.

#### (i) NaCl, KCl, $MgCl_2$, or $CaCl_2$.

These solutions support ciliary movement for as much as seventy-two hours.

Table XIII.

| Solution. | $P_H$. | Movement after— 12 hours. | 24 hours. | 48 hours. | 72 hours. | Remarks. |
|---|---|---|---|---|---|---|
| 50 c.c. ·6 M. NaCl . 4 c.c. ·6 M. $MgCl_2$ . ·9 c.c. ·6 M. KCl . | 5·9 | Normal | Slow movement | Slight movement | Slight movement | No disintegration. |
| Ditto + 5 c.c. N/10 $NaHCO_3$ | 7·3 | „ | Medium movement | Medium movement | Medium movement | Progressive disintegration after 24 hours with increasing $P_H$. |
| Ditto + 1 c.c. „ | 7·7 | „ | Normal | Normal | Normal | |
| Ditto + 3 c.c. „ | 8·0 | „ | Normal, some disintegration | Total disintegration | — | — |

#### (ii) Mixtures of NaCl or KCl with $CaCl_2$ and $MgCl_2$.

These are less efficient than other tertiary mixtures. Movement ceases in less than twenty-four hours, and disintegration is more marked.

### d. Mixtures containing KCl, NaCl, $MgCl_2$, $CaCl_2$, with Sodium Bicarbonate.

A solution containing these salts in the proportions in which they exist in sea-water, and which contains the requisite amount of N/10 $NaHCO_3$ to adjust the hydrogen-ion concentration to about 7·8, maintains gill-fragments in a healthy normal condition for more than five days, after which the experiments were discontinued.

The optimum composition of this Van't Hoff's solution was found to be:

TABLE XIV.

| Salt. | Molecular concentration. | Volume in c.c. |
|---|---|---|
| NaCl . . . . . | ·6 | 500 |
| KCl . . . . . | ·6 | 9 |
| $CaCl_2$ . . . . . | ·6 | 9 |
| $MgCl_2$ . . . . . | ·6 | 36 |
| $NaHCO_3$ . . . . | N/10 | 5 |

SUMMARY OF EFFECTS OF METALLIC IONS IN SEA-WATER.

As far as the action of metallic ions is concerned, the most definite point established is that an efficient physiological solution must contain sodium, potassium, magnesium and calcium. In such a solution, whose $P_H$ is identical with sea-water, gill-filaments will remain healthy and exhibit active ciliary movement for more than five days. If any one ion be omitted from the solution, the ciliated epithelium exhibits signs of disentegration after some hours, and the tissue gradually breaks up, although some cilia may continue to beat for as long as three days. If two ions are omitted, the duration of active ciliary movement is from sixteen to twenty-four hours, but the phenomenon of disintegration is very marked. Finally, in solutions containing only one metallic ion, disintegration rapidly sets in and the tissue is very unhealthy after twelve hours; also ciliary movement is usually markedly affected in less than two hours.

It will be noted that very little evidence is obtained on the qualitative value of any particular metallic ion. Sodium appears to be more toxic than potassium. The presence of excess of calcium appears to cause separation of the gill-filaments, and it is possible, therefore, that this ion affects the ciliated junctions or discs. No clear evidence is obtained of antagonistic action between ions; the action of the various

metals appears to be additive. Solutions of pure salts are more toxic than binary mixtures, binary mixtures more toxic than tertiary mixtures, while solutions containing Na, K, Mg and Ca form satisfactory physiological solutions.

A. G. Mayer (3) has described the effect of various ions upon ciliary movement, using annelid larvæ and veligers. He came to the conclusion that ions act upon ciliary movement in exactly the reverse way to their action on neuro-muscular activity. His results may be summarised as follows:

TABLE XV.

| Ion. | Nature of action on ciliary movement. |
|---|---|
| $Na'$ . . | Most powerful of all ions as inhibitor of ciliary movement. |
| $Mg''$ . . | ,, ,, stimulant ,, ,, |
| $K'$ . . | Primary depressant, but recovery afterwards takes place. |
| $Ca''$ . . | Weak stimulant in presence of $Na'$. |
| Weak acids | Primary depressant, but recovery takes place. |
| $NH'_4$ . . | Ciliary movement first stops and then recovers. |

Mayer points out that these conclusions do not apply to ciliated plant cells.

This author only deals very shortly with the effects of acids, and these were all weak acids—e.g. $CO_2$, lactic acid, uric acid —and he does not deal with the effect of alkalis except by the statement that NaOH is a primary depressant. He does not mention whether the artificial solutions used were acid or not.

Had the hydrogen-ion concentration of the solutions of the various pure salts been ignored in the experiments described in this paper, it will be noted that the order in which these salts would maintain ciliary movement was as follows:

Magnesium chloride.
Potassium chloride.
Sodium chloride.
Calcium chloride.

It is interesting to note that on this basis one would have agreed with Mayer's conclusions that magnesium is the most efficient ion for maintaining ciliary action, that sodium is a powerful depressant, that pure calcium rapidly stops ciliary movement, and that calcium inhibits the action of sodium. Finally, potassium occupies an intermediate position between magnesium and sodium.

R. S. Lillie performed numerous experiments with isolated gill-filaments of Mytilus edulis. As I became aware of these experiments after the present work had been completed, it is interesting to note that the results of actual experiments with Na', K', Ca'' and Mg'' agree very closely. Lillie also mentions the disintegration phenomena in various solutions, which he observed was inhibited by the hydrogen-ion and accelerated by the hydroxyl-ion. He also observed that movement in pure sodium chloride solution was prolonged by the addition of hydrochloric acid. The results of the present experiments tend to support the conclusion that for each salt there is an optimum hydrogen-ion concentration for ciliary movement; at the same time, exception must be taken to the conclusion that this is evidence of an antitoxic effect between the acid and the salt, since the hydrogen-ion concentration of the original salt solution was not investigated. For the same reason, Lillie's experiments with many di- and trivalent salts cannot be regarded as critical, since the hydrogen-ion concentration of these salts differs profoundly, according to their degree of hydrolytic dissociation.

## Note on Antagonistic Ion Action.

A very large amount of work has been performed on the physiological action of various ions. In very few cases, however, has any attention been paid to the hydrogen-ion concentration of the solutions used. In view of the fact that solutions of pure salts or mixtures of salts are never of exactly the same $P_H$ as sea-water (or other external medium of the cell), it would appear that many results which have been

attributed to the action of metallic ions may possibly be due either wholly or in part to hydrogen or hydroxyl-ions. One or two examples may be quoted. Loeb (2) showed that by exposing fertilised Echinoid eggs to various solutions, on subsequent transference to normal sea-water "twins" were often produced. The following table (from Loeb) shows the percentage of twins produced in various solutions.

TABLE XVI.

| Nature of solution. | Length of exposure to solution before transfer to sea-water. | | |
|---|---|---|---|
| | 2 hours. | 2½ hours. | 3 hours. |
| | Per cent. | Per cent. | Per cent. |
| Na, K | 50 | 90 | 90 |
| Na, Ca | 5 | 80 | 90 |
| Na, Mg | 40 | 80 | 90 |
| Na, K, Ca | 0 | ½ | 5 |
| Na, K, Mg | 50 | 90 | 100 |
| Na, Ca, Mg. | 1 | 20 | 20 |
| Na, K, Ca, Mg. | 0 | 0 | 0 |

Further, Loeb showed that the effect of the simpler solutions was inhibited by alkali.

TABLE XVII.

| Solution. | Per cent. of twins produced. |
|---|---|
| Na, K | 50 |
| 50 Na, K, ·1 M/5 $NaHCO_3$ | 0 |
| 50 Na, 5 Mg. | 98 |
| „ + ·1 M/5 $NaHCO_3$ | 0 |
| 50 Na, 5 Ca | 15 |
| „ „ + ·1 $NaHCO_3$ | 0 |
| 50 Na, 5 Mg, 1·1 KCl | 60 |
| „ „ „ + ·1 $NaHCO_3$ | 0 |

Now, when it is remembered that $CaCl_2$[1] may be distinctly alkaline, that $MgCl_2$ is more alkaline than KCl, and that NaCl is distinctly acid, the above results are capable of a very simple explanation. If the production of "twins" is due to exposing the eggs to a solution of low $P_H$, then in Table XVI we would expect to find that all those solutions which lack calcium produce a high percentage of twins, whereas solutions containing calcium do not. This is actually the case except in the case of eggs exposed to Na and Ca for three hours; but Table XVII shows that the effect of this solution also is removable by raising the alkalinity.

Again, in the case of Osterhout's (5) results on plant growth :

TABLE XVIII.

| Solution. | Duration of life. | Solution. | Duration of life. |
|---|---|---|---|
| NaCl . . . | 23 | $CaCl_2$ . . . . . | 58 |
| KCl . . . | 56 | NaCl, $CaCl_2$ . . . . . | 65 |
| $MgCl_2$ . . . | 19 | NaCl, $MgCl_2$, $CaCl_2$ . . . | 45 |
| NaCl, KCl . . | 23 | NaCl, KCl, $CaCl_2$ . . . | 88 |
| NaCl, $MgCl_2$ . | 25 | NaCl, KCl, $CaCl_2$, $MgCl_2$, $MgSO_4$ | ∞ |
| NaCl, $MgCl_2$, KCl | 30 | | |

In these experiments no mention is made of the hydrogen-ion concentration or of the effects of alkalis. In each case the solutions containing calcium support life very much better than those without this metal.

It is not necessary to enlarge upon the critical effects of very small changes in the hydrogen-ion concentration of the medium with which an organism or cell is in contact. The work of Mines, Moore, etc., amply illustrate the point. It is therefore necessary to question the conclusions drawn from experiments dealing with antagonistic ion action in which the

[1] While anhydrous calcium chloride tends to be alkaline, the crystallised salt is usually more acid than sodium chloride solutions, and the above explanation would not apply.

hydrogen-ion concentration of the various solutions has not been controlled. It is, however, not suggested that changes in hydrogen-ion concentration afford a complete explanation of such experiments, or that true antagonistic ion action does not exist.

## Summary of Results.

(1) With the exception of the wave action of the lateral cilia, isolated fragments of the gills of Mytilus continue to function normally in sea-water for many days.

(2) Ciliary activity is dependent upon a certain minimal concentration of hydroxyl-ions ($P_H$=5·5–6·0).

(3) Stoppage of the cilia by acid is reversible by raising the $P_H$ by means of alkali.

(4) If the $P_H$ of the medium is above 9·0 the ciliated epithelium rapidly breaks up into its constituent cells, but ciliary movement does not stop either in the isolated cells or in those which remain in sitû.

(5) The breaking up or disintegration of the ciliated epithelium takes place in all solutions which do not contain potassium, sodium, magnesium and calcium.

(6) Solutions containing only one metallic ion are highly toxic to the tissue, causing marked disintegration even at low values of $P_H$. Solutions containing two ions are less detrimental than solutions containing only one. Solutions containing three ions support the tissue in a comparatively healthy state for as much as seventy-two hours; but it is only when the four metallic ions are present that the tissue remains normal and healthy as in sea water.

(7) Little evidence was obtained of qualitative effects of single metallic ions or of antagonistic ion action. Sodium chloride forms the most toxic salt in sea-water.

(8) Attention is drawn to the necessity of controlling the hydrogen-ion concentration in all solutions used in the investigation of antagonistic ion action.

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