BIOELECTRIC POTENTIALS IN HALICYSTIS

VII. THE EFFECTS OF LOW OXYGEN TENSION*

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Previous work1 has shown that the existing or experimentally altered gradients of inorganic ions or other substances between the environment and the vacuolar sap of impaled cells of Halicystis have little influence upon the large and steady potential difference measurable across the protoplasm. The rôle of the latter, with its inherent gradients and asymmetries was therefore emphasized in the earlier title of this series: “protoplasmic potentials.” Although it seems desirable to alter this to the more general term now used, it is particularly the “protoplasmic” aspects which will be considered in the next several papers. Since the cell's E. M. F. can drive an appreciable current of 5 to 10 microamperes through a completed circuit for many days, a source of energy, eventually metabolic, is indicated. Agents affecting metabolism, such as oxygen tension, temperature, light, inhibitors, and stimulants, will therefore be discussed in the next few papers, with a view to indicating how metabolism may produce or alter the bioelectric potential.

The present paper is restricted to the effects of altered oxygen ten-

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This refers to the steady potential maintained when the vacuole is perfused with sea water, or when vacuolar sap is placed outside the cell. There are transient decreases of P.D. produced by dilution of sea water, and by potassium and other salts, but the P.D. soon recovers much of its original value, probably because penetration or loss of the salts concerned soon equalizes the disturbed gradient. But recent work with sulfates has produced a nearly constant alteration of P.D. due to the very low mobility and slow penetration of the sulfate ion. The presence of other slow moving anions in the protoplasm may account for the normal P.D.
sion. Some of the results have been described in a preliminary note; time curves are now given for these, as well as for experiments involving the concurrent effects of other agents affecting the potential, such as KCl, sulfates, ammonia, and current flow, applied with a view to analyzing the mechanism and locus of the changes produced by low oxygen tension.

METHODS

The methods are essentially the same as those previously employed. Electrical measurement was by compensation with a sensitive galvanometer as null instrument; more recently records have been taken with a specially adapted Leeds and Northrup "Micromax" automatic recording potentiometer making an adjustment every 3 seconds. Both of these methods involve a slight current flow during brief periods of unbalance when the potential is changing rapidly, but the results differed in no respect when records were taken with a vacuum tube electrometer. This identity could be predicted from the detailed study of current flow effects in this organism. Indeed, there is usually no difference between the P.D. as read by compensation and that from the deflection of the galvanometer when compensation is removed, except under certain conditions of low oxygen tension which will be mentioned below. In this case it was actually the inability of the cell to maintain a corresponding uncompensated deflection, that indicated increased polarizability, and was tested in the D.C. bridge.

For altering oxygen content of the sea water bathing the cells, gas mixtures were bubbled through closed vials of about 25 cc. volume, all openings except a small vent being vaselined. Tank oxygen, air, and two different commercial nitrogens were used, one (Linde) with about 2 per cent O₂ content, the other (Ohio Chemical Co.) with about 0.2 per cent O₂. The latter two were particularly convenient, giving close to the upper and lower P.D. limits respectively with many cells. Mixtures were made by counting the bubbles of the two gas streams emerging in separate vessels from equal sized orifices, as shown in Fig. 1, and mixing in a Y connector. Purification to lower O₂ content, when necessary, was done by bubbling the Ohio nitrogen through an absorber ("Oxsorbent," or acid chromous sulfate plus amalgamated Zn which do not absorb CO₂ or produce CO₂, like alkaline pyrogallol). The actual O₂ content of the sea water was also directly tested by Winkler titration of a sample carefully withdrawn, with a minimum of contact with air. Still more convenient, as being applicable in one of the closed

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vials of the chain itself, was the polarographic O₂ method of Vitek; this has been employed for several years in respiration studies in the Stanford department of Physiology, and it deserves wider use in biology.

For altering solutions in equilibrium with low O₂ tensions, without admission of air during the change, the train of vessels shown in Fig. 1 was employed. The cell in vessel I was preceded by several other vials, each with a sintered glass or fine nozzle bubbler, and containing a desired solution e.g., KCl. When the

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6 Baumberger, J. P., and Müller, O., reported at the winter meeting of the Western Society of Naturalists, Stanford University, December, 1934, and the XVI International Physiological Congress, Zürich, 1938.

A modification of this method, using a stationary electrode for following very rapid changes of oxygen tension during photosynthesis, has been described recently (Blinks, L. R., and Skow, R. K., Proc. Nat. Acad. Sc., 1938, 24, 420). Just after the present paper was submitted, Professor Farrington Daniels informed us that he and his co-workers at the University of Wisconsin had also been applying the method of Vitek for photosynthetic measurements, reporting it at the Dallas meetings of the Electrochemical Society, 1938, the Photochemical Conference at Stanford University, 1938, and the American Chemical Society, Milwaukee, 1938. Their description is in press (J. Am. Chem. Soc., 1938).
P.D. had dropped in the cell to a constant level, it was assumed that the previous vessels were also in equilibrium with the gas mixture. Vessel I was then emptied by closing vent V with a glass plug (P), the pressure of the incoming gas forcing the sea water out through siphon S. When I was empty, vessel II was inverted and its solution was forced in through the bubbler. Sea water, meanwhile held in vessel III, was restored by similar manipulation. Where less alteration of the original sea water was necessary, e.g. where a mixture of 1 part sea water and 1 part 0.6 M KCl was desired, vial I had to be only half-filled; then no siphoning out of the first solution was needed, merely its filling from vessel II. For still smaller modifications, e.g. acidification, or addition of ammonia, small amounts of solution could be safely injected with a hypodermic syringe through the vent V, insufficient air being admitted to cause any change in p.d. (as tested with sea water injection). The study of current flow effects during low O₂ exposures presented no difficulties, all necessary connections being made in advance.

A strong agar gel in the impaling capillary helped prevent collapse of the cell during the changes of solution by the gas pressure developed in the vials.

Most of the measurements here reported have been made on H. ovalis, of California, but consistent results have been found in many cases with H. Osterhoutii in Bermuda.

The experiments were made in darkness, or in very dim light (1 foot-candle or less) too weak to affect the p.d. Greater illumination, although with negligible effect on the normal p.d. of Halicystis, may become highly effective under low O₂ tension by restoring oxygen through photosynthesis. These and other light effects are reserved for another paper. Similarly reserved are the effects of temperature, which are again not very great upon the normal p.d. of Halicystis, but may markedly influence the level reached under low O₂ tension, or the speed with which it is attained. (Indicated in a preliminary note.) The present experiments were performed at room temperature, usually between 15 and 20°C. and not varying more than 1°C. in a given experiment.

For comparison with the effects of low O₂ on p.d., respiration measurements were made on single Halicystis cells in a Fenn respirometer, and on groups of four or five cells in Warburg manometer vessels. On the whole, the respiration rate fell off at about the same O₂ tensions as did the p.d. It was also found that oxygen consumption was markedly enhanced for some time (even for several days), after the cells were impaled, later falling off to nearly the rate found before impalement. This agrees with the greater sensitivity of the cells to lowered O₂ tension soon after impalement, and with other bioelectric properties at this time. Detailed report of the respiration measurements will be given in another paper.

**General Effects of Oxygen**

It has been realized for a long time that large plant cells like Valonia and Halicystis have a very small respiration in relation to their volume; it is not inappreciable, however, amounting to 1 or 2 mm.
O₂ per hour per cell of 0.5 cm³ volume. Considering that the immense vacuole may occupy 99 per cent of the cell's volume, this is probably as high per unit volume of protoplasm as in many marine algae. Nevertheless, the actual O₂ consumption is so small that the cells may be kept for many days in a sealed vial of 25 cc sea water without effect upon the P.D., which remains also much the same whether the solution is stirred, aerated, or bubbled with pure O₂ gas.

It is only when cells are confined in a very small volume, e.g. of 1 cc. or less, that the P.D. may fall overnight due to O₂ exhaustion. If, however, a more actively respiring organism, such as Ulex or yeast is also included in the vessel, the P.D. of Halicystis may drop very quickly on standing, to be restored by aeration (or light). Although these experiments suggest that the P.D. is sensitive to reduced O₂ tension, they are not free from the possibility that CO₂ or other products of the cell itself or of other organisms may be responsible for the fall of P.D., these being blown out or oxidized on aeration.

Experiments involving definite O₂ content of the sea water, in equilibrium with known gas mixtures, were therefore undertaken. The first gas employed, "Linde" nitrogen containing about 2 per cent O₂, held the P.D. at practically the same value as in air as long as it was actively bubbled. When bubbling was stopped, the P.D. began to drift downward (Fig. 2). The speed with which this occurred, and the eventual level reached were not perfectly constant from cell to cell. The sex of the plant (as indicated at its previous reproductive period) may be of influence here, female plants being apparently more sensitive, and having a higher respiratory rate, possibly because of the heavier masses of unliberated gametes often remaining in the vacuole. The length of time after insertion of the capillary is also important; freshly impaled cells are more sensitive to partial lowering of O₂ tension than are those which have stood for a longer time. This again is correlated with an increased respiratory rate soon after impaling (see Methods above), which would drive the O₂ level down faster in the vicinity of the cell. It might be thought that the freshly healed region in the vicinity of the capillary is more readily injured by low O₂ tension than the rest of the cell surface, and so introduces a short circuit to the remaining potential source, but resistance measurements (see below) do not indicate this.
After recovery, the effects of fresh impalement may be simulated by twisting the cells upon their capillary again.

As shown in Fig. 2, restoration of higher \( O_2 \) tension around the cell restores the P.D., whether the sea water is merely agitated by stirring (with a gas-tight stirrer) or by resumption of bubbling of 2 per cent \( O_2 \) in nitrogen. The fall and recovery can be repeated many times with excellent reproduction of the time curves. The downward cusp at the re-introduction of higher \( O_2 \), which carries the P.D. lower before it restores it, is a characteristic which will be discussed later.

Since the fall of P.D. on cessation of bubbling does not give exact information on the \( O_2 \) tension adjacent to the cell when the P.D. has dropped to various levels, active bubbling was continued with various mixtures of 2 per cent and 0.2 per cent \( O_2 \) in nitrogen, by means of

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**Fig. 2.** Graph showing the fall of potential difference across the protoplasm of an impaled cell of *Halicystis ovalis*, when bubbling of 2.0 per cent \( O_2 \) in \( N_2 \) is stopped (at first arrow). When the solution is quiet the cell consumes oxygen faster than it can diffuse in, and the P.D. drops to 10 mv. positive. On stirring the sea water (with a gas-tight stirrer), the P.D. first falls abruptly (or even slightly reverses), then rapidly recovers. On cessation of stirring it falls again; at 40 mv. stirring is resumed, producing a small downward cusp, then a rapid recovery. (Bubbling of 2 per cent \( O_2 \) has the same effect as stirring.)

The ordinates are P.D. in millivolts, the positive sign that of the cell exterior.
the bubble counting mixer of Fig. 1. With a 1/1 mixture, giving
about 1.1 per cent O₂, the P.D. was usually no longer maintained, but
fell off to around 2/3 its value in air, as shown in Fig. 3. 0.5 per
cent O₂ dropped the P.D. to a still lower level, almost to that produced
by 0.2 per cent O₂. And still lower O₂ tensions, produced by ab-
sorbing most of the O₂ from "Ohio" nitrogen, or allowing the cells
to respire it, did not decrease this P.D. much below 5 or 10 mv., at
least for periods of an hour or longer. From all these exposures,
good recovery occurred when O₂ tension was again raised, either to
2 per cent O₂, or to 20 per cent (air), still almost invariably with the
preliminary downward cusp (Fig. 4).
It should be pointed out that equally prompt and complete recovery of P.D. is obtained if oxygen is restored, not in the sea water around the cell, but by perfusion of aerated solution (sap or sea water).

**Fig. 4.** Graphs showing fall of P.D. in impaled cells of *H. ovalis*, when 0.2 per cent O₂ in N₂ is substituted for 2 per cent O₂ (each bubbling continuously). In A the fall of P.D. in 0.2 per cent O₂ is rather slow the first time, possibly because air had not been thoroughly exhausted from the vacuole, although 2 per cent O₂ had been bubbled for ½ hour previously (without reducing the P.D. below the air value). After recovery in 2 per cent O₂, the second fall in 0.2 per cent O₂ is faster, and reaches a lower level. Note the downward cusps preceding recovery in each admission of 2 per cent O₂.

In graph B, another more sensitive cell is shown. This had 70 mv. P.D. in aerated sea water, which fell to 60 mv. in 2 per cent O₂. The fall on bubbling with 0.2 per cent O₂ is much faster and the P.D. drops to 10 mv. Readmission of 2 per cent O₂ promptly restores the P.D. The reproducibility of the curves and of the levels reached in each case are noteworthy.

Ordinates are P.D. in millivolts, the sign, outside of cell positive. Arrows indicate change of the gases bubbled.
water) in the vacuole (Fig. 5). This indicates that an oxygen gradient, as such, is not concerned in the maintenance or restoration of the p.d., since O₂ can be supplied from either side of the protoplasm. Some other gradient or structure must be responsible for the electrical asymmetry of the cells.

Fig. 5. Graph showing course of p.d. across protoplasm of cell of H. ovales, impaled on a double capillary permitting perfusion of solutions in the vacuole. At the start of the graph 0.2 per cent O₂ in N₂ has been bubbled for 15 minutes outside the cell, and the p.d. has begun to fall. This continues until the p.d. reaches about 8 mV., at which time (P) aerated sea water is perfused in the vacuole of the cell; the p.d. quickly recovers, although 0.2 per cent O₂ continues to bubble outside the cell. At (Q) perfusion is stopped and the vacuolar solution remains quiet; the p.d. promptly drops again, as oxygen is consumed from the vacuole. At (P) perfusion of aerated sea water is again started, but bubbling is stopped outside the cell; the p.d. rises again, although the normal oxygen gradient is now reversed. At (Q) the perfusion is again stopped and the p.d. falls. At (A) the outside sea water is aerated, and the p.d. rapidly rises to 82 mV.

The designations and horizontal arrows at the bottom of the graph refer to the state of the sea water outside the cell. Ordinates are p.d. in millivolts, outside of cell positive. Time intervals 10 minutes.

It is perhaps desirable to discuss at this point the rôle of "injury" in the fall of p.d. during exposure to low O₂ tension. There is no doubt that injury eventually occurs when cells are deprived too long of oxygen. There may be visible evidence such as small holes in the
protoplasm, or its pulling away from the inserted capillary. In such cases it is easy to see why the P.D. may be lowered by short circuits through these visible, or invisible leaks. Usually there is no recovery when oxygen is restored to these cells, their P.D. soon drops close to zero (not 5 or 10 mv. constantly maintained as in 0.2 per cent O₂), and their effective resistance is very low.

It is quite otherwise with cells showing reversible depression of P.D. in low O₂ tensions. Cells have been so kept for many hours or even overnight, showing the same low positive P.D. of 5 to 10 mv., and then recovering promptly when aerated. It is not likely that a partial short circuit due to injury would remain so long constant. Furthermore, as seen in a later section, the electrical resistance of such cells is higher, not lower, than normal. Finally, they maintain this low positive value in low O₂ under conditions when they may display a negative P.D. when oxygen is restored. This could not possibly be due to a reduction of the “aerated” value by partial short circuits. This decisive behavior is seen in the next section.

**Combined Effects of Ammonia and Low Oxygen Tension**

An earlier paper⁴ has described the reversal of P.D. produced by small concentrations of NH₄Cl in the sea water. Sharp thresholds characterize both the reversal and recovery of positive P.D., and the reversal may be repeated many times, or continued an hour or more at a time without apparent injury of any sort. It became of interest therefore to combine this treatment with that of low O₂ tension, seeking answers to the following questions:

1. Does the presence of sub-threshold ammonia concentration in any way influence the sensitivity to low O₂, either in the effective tensions for fall of P.D., the speed of fall, or the P.D. level reached?

2. Conversely, does low O₂ tension alter the sensitivity of the cells to ammonia; i.e., increase or decrease the threshold, or change the level of P.D. reached?

3. Especially, when definitely superthreshold ammonia concentrations have reversed the P.D., does lowering the O₂ tension merely lower the reversed, negative P.D. to about the same extent as it does the normal positive P.D., or does it abolish the negativity altogether?

In answer to the first question, very small amounts of NH₄Cl were
added to the sea water, in concentrations from 0.0001 to 0.0005 M, which are too low to affect the P.D. appreciably. Thereafter, lowering the O₂ tension produced much the same effects as in untreated sea water, the P.D. falling off at the same rate, and reaching the same low levels, except that the ammonia appeared to exaggerate the "hump" sometimes found (Fig. 6, dotted curve) which carries the P.D. to a somewhat lower level than it finally attains, during continued bubbling of 0.2 per cent O₂. Also, recovery in air tended to give a more pronounced downward or even reversed "cusp" on first admission of air, preceding the rapid recovery of P.D. The significance of these changes is seen when slightly higher ammonia concentrations are applied, and they suggest the rôle played in these anomalies of the P.D. curves by the small amounts of ammonia often found in the vacuoles of Halicystis cells.

At higher ammonia concentrations, close to threshold for reversal, a more complicated interplay of the two treatments occurs, which may be due to acidity. An example is shown in Fig. 6. A cell exposed to ammonia just insufficient to reverse its P.D. alone, is often found to undergo temporary reversal when 0.2 per cent O₂ is first bubbled; but continued bubbling brings the P.D. back to positive values, about those reached in the absence of ammonia. Reintroduction of 2 per cent O₂ especially if slowly bubbled, may again produce temporary reversal (as shown in Fig. 6), but continued bubbling, at this or higher O₂ tensions, restores positive P.D.

This remarkable behavior is not always found, since conditions have to be adjusted so that the ammonia is close to threshold and remains near there during the bubbling (it was tested at the end of the run by Nessler reagent and found very little lowered in concentration). The suggested interpretation is as follows: In air, slightly sub-threshold ammonia is unable to alter the pH of the protoplasm quite enough to produce its effects; but when respiration is decreased by lowering the oxygen supply, less CO₂ is produced, and the ammonia now becomes effective, with reversal of P.D. This is only temporary, however, possibly because sufficient anaerobic acidity develops to counteract the ammonia alkalinity, but more likely because later anaerobic changes inhibit the potential mechanism upon which the ammonia normally operates. The P.D. therefore
returns to the low positive value characteristic of 0.2 per cent O₂. On restoration of 2 per cent O₂, the reverse cycle occurs as the ammonia becomes again effective upon the recovering potential mechanism, or as anaerobic acidity disappears. Then as CO₂ is produced in

![Graph showing course of P.D. across the protoplasm of impaled cell of H. ovalis when 0.2 per cent O₂ in N₂ is bubbled in the presence of 0.0005 M NH₄Cl in the sea water (solid line). All gases including air, were bubbled through KOH previously to remove CO₂ and avoid complications due to changing the pH. This low concentration of ammonia scarcely influences the normal P.D. (in sea water, S.W.), reducing it by 8 mv., to about 70 mv. (positive). On bubbling with 0.2 per cent O₂, however, the P.D. not only begins to fall, as would be expected with low oxygen tension, but temporarily reverses in sign, becoming 25 mv. negative before returning to the usual low positive value. On bubbling with 2 per cent O₂, the usual downward cusp becomes exaggerated into another temporary reversal (25 mv. negative again), before recovering, with an overshooting, to its original value in air. Sea water restores normal P.D.

For comparison, the dotted line indicates a characteristic time course when a very little ammonia is added to the sea water, showing residual signs of the reversal, in the "hump" during the fall, and the cusp preceding the recovery of P.D. It seems possible that such residual effects are often due to the small amount of ammonia present in the vacuole and protoplasm of many cells. See text.

Ordinates are P.D. in millivolts, the sign being that of the cell exterior. Arrows indicate change of solution or of gases bubbling.
greater quantity, the ammonia is no longer effective, and positive P.D. is regained.

Another interpretation of the effects is that ammonia is used up, possibly in the formation of proteins or other nitrogen compounds, during normal respiration, so that very low NH₄Cl concentrations are not effective in raising the internal pH of the protoplasm. When respiration is slowed up, however, such utilization of ammonia is also decreased and the internal pH is raised, causing P.D. reversal. Then the potential source is affected in turn by the low oxygen tension, and the P.D. falls away to its usual low positive value. When oxygen is restored, the reverse cycle is produced, the potential mechanism first recovers from anaerobic inhibition, whereupon the ammonia reversal becomes effective; then the ammonia is in turn attacked, and the P.D. recovers positive values.

Whether or not either of these explanations is correct, the experimental fact is clear that oxygen tension can control the ammonia reversal, and at the threshold give rise to time courses more complex than those obtained with either ammonia or low O₂ alone. The importance of interacting factors is thus demonstrated.

With slightly higher ammonia concentrations, the same tendency exists for reversal to be favored by low O₂ tension, before anaerobic inhibition sets in. But reversal, not recovery, occurs on readmission of air, as shown in Fig. 7. 0.001 M NH₄Cl was not quite sufficient to reverse the P.D. alone, but bubbling of 0.2 per cent O₂ caused temporary reversal, after which the usual low positive anaerobic P.D. appeared. Readmission of air or of 2 per cent O₂ caused the ammonia negativity to appear, without later recovery to positive aerobic values. Evidently the ammonia is now sufficient to maintain negativity, even though it was not originally able to induce it in air. Such a hysteresis has been noted before, both with altered ammonia concentrations, and with current flow assisting reversal close to the ammonia threshold. It will also be seen in the effects of temperature in another paper. Here it might be pointed out that the P.D. is able to become negative again even after the slight positive P.D. of low O₂ tensions. This probably indicates that the anaerobic P.D. is of another sort from the normal, upon which ammonia can operate.

The same inhibition of the ammonia effects is seen with higher
NH₄Cl concentrations, sufficient in air to reverse the P.D. and maintain it negative. If reversal has already occurred during aeration, bubbling of 0.2 per cent O₂ brings the P.D. back to the usual low

**FIG. 7.** Graph showing course of P.D. across protoplasm of impaled cell of *H. ovalis* when 0.2 per cent O₂ in N₂ is substituted for air in the presence of 0.001 M NH₄Cl in sea water. (Gases washed free of CO₂ with KOH to avoid complications due to pH change.) This ammonia concentration is just below the threshold for reversal with this cell, dropping the P.D. from 83 mv. to about 70 mv. (where it would stay indefinitely in air). On bubbling of 0.2 per cent O₂, the P.D. not only falls, as would be expected, but first undergoes a strong reversal to nearly 40 mv. negative before returning to the usual low O₂ level of 10 mv. positive. Restoration of air then induces a strong reversal, to 50 mv. negative, which is again counteracted by 0.2 per cent O₂. 2 per cent O₂ is also able to induce negative P.D. Only a lowering of ammonia concentration is able to abolish this negativity with higher oxygen tensions, although the negativity is only latent at 0.2 per cent O₂.

Ordinates are P.D. in millivolts, the sign being that of the cell exterior. Arrows indicate change of solution or of gases bubbling.

anaerobic levels; while bubbling of air again restores large negative values (much as in Fig. 7). Or if the ammonia is added when the P.D. has been already lowered by 0.2 per cent O₂, then it has practically no effect, until oxygen is restored, whereupon the P.D. reverses.
There is merely a "latent" negativity during low O₂ exposure, which requires oxygen for its development, and becomes latent again when O₂ tension is reduced.

This latent negativity holds true even for rather large additions of NH₄Cl, such as would produce almost instant reversal of P.D. in air (0.01 M or higher). There is not even the initial cusp which is found at low pH values, hence attributable to ammonium ion rather than to the penetration of undissociated base. This indicates that low O₂ has not only abolished the secondary effects of ammonia but also its primary ionic mobility effects. This suggests an alteration of the ionic permeability of the surface, which will be further discussed in the next section.

If ammonia is added to the sea water when low O₂ tensions have had time to carry the P.D. down only part way, e.g. from 80 to 40 mv. positive, then there is usually a prompt reversal of P.D., although not to its expected negative value, after which the P.D. drifts downward and becomes again positive at its usual anaerobic level. In other words the ammonia response is lost about in proportion to the loss of positive P.D.

It should be noted that the direction of the responses to low and high oxygen tensions, in the presence of superthreshold ammonia, is the opposite of that in the absence of ammonia. The curves are nearly mirror images of those in normal sea water. Ammonia has reversed the oxygen response.

**Effects of KCl during Exposure to Low Oxygen Tension**

Increased KCl concentration was chosen as an agent which alters the potential almost certainly via the differential mobility of K⁺ and Cl⁻ ions in the surface of the cell. Its effect on the P.D. has been described (for both species of Halicystis) as an initial sharp cusp which decreases the positive P.D. (or even temporarily reverses it slightly), after which all or nearly all of the positive P.D. is regained during 5 or 10 minutes. The recovery no doubt represents the entrance of potassium into the protoplasm, largely equalizing the initial gradient; the entrance appears to be more than simple diffusion, and prob-

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ably the normal accumulatory mechanism for potassium is concerned. As such, the duration of the cusp is sensitive to metabolic influences (e.g., temperature, in a way to be described elsewhere). The cusp probably reflects the high mobility of the K\(^+\) ion with respect to the Cl\(^-\) ion, and therefore is dependent on the special properties of the cell surface producing this. It was clearly desirable to study the effect of lowered O\(_2\) tension upon the cusp, and if it persisted, upon the recovery curve.

The results are shown in Fig. 8. A KCl cusp and recovery curve,

![Fig. 8. Tracings of automatic potentiometer ("Micromax") records of P.D. changes in impaled cells of *H. ovalis* when sea water containing 0.2 M KCl (1 part 0.6 M KCl to 2 parts sea water) is substituted for normal sea water (S.W.) under aerobic conditions and when the P.D. has been lowered to various values by bubbling with 0.2 per cent O\(_2\). Curve A shows a complete experiment where 0.2 M KCl is first applied in aerated sea water, decreasing the original P.D. from 77 mV to 40 mV, followed by a recovery to 65 mV. Replacing sea water then produces a reverse cusp and a return to normal P.D. 0.2 per cent O\(_2\) is then bubbled through the apparatus of Fig. 1, carrying the P.D. down to about 12 mV. At this time sufficient 0.6 M KCl (also in equilibrium with 0.2 per cent O\(_2\)) was run into vial I to bring the KCl concentration to 0.2 M. A greatly reduced cusp ensues, with about half the height as with aerated KCl, and a partial recovery. When air is bubbled through the system, the P.D. recovers to about the value reached formerly with KCl in air, and readmission of aerated sea water restores it to 78 mV. After 20 minutes delay (shown by the interrupted curve), duplication of the first KCl cusp in air is then seen, indicating the cell has recovered its large response to KCl. The response is smaller if KCl is applied earlier.

If the P.D. is not allowed to drop to as low a level, through shorter bubbling or in a less sensitive cell (curve B), the KCl effect is much greater than in curve A. The cusp is also lengthened, an effect discussed in the text.

On the other hand, in curves C and D where low O\(_2\) tensions have reduced the P.D. to still lower values than in A, the KCl cusp is also much smaller, and the restoration of sea water gives a smaller recovery. The apparent mobility of K with reference to Cl is lower when the P.D. drops in low O\(_2\).

Arrows indicate change of solution or of gas bubbling, the duration of the latter being also indicated at the bottom of the graph for curve A. The fall of P.D. in the other curves has been omitted to avoid confusing the graph, but was much like those in Figs. 2, 4, etc.

Ordinates show P.D. in millivolts, the sign being that of the cell exterior. Time intervals 10 minutes. Automatic balance of the potentiometer every 3 seconds obviates the necessity of showing observed points; the lag of the instrument is usually not over 30 seconds, in following curves like these.
with the replacement of sea water afterward are first shown in air (A). Then the cell was exposed to low oxygen tension after which the KCl content was again raised to 0.2 M by means of the series vials shown in Fig. 1. If this is done before the p.d. has fallen very low, a large response still obtains (curve B). Possibly the long duration of the cusp is due to decreased accumulation of potassium in the protoplasm, even though the K+ ion mobility is still large in the surface. If the p.d. is first carried still lower, however, the KCl cusp is considerably reduced, as in curve A; if allowed to reach its constant anaerobic level, the cusp is greatly flattened indeed, and recovery is very slow (C and D).

This result clearly shows that the response of the cell, and probably the nature of its surface, has been altered as the result of low O₂ tension, in a direction to diminish the mobility or the concentration of K⁺ with respect to Na⁺. It is shown later that \( U_{Na} = V_{Cl} \) and is not affected by low oxygen tension and since the concentration of Cl is presumably constant in the protoplasmic surface, the most probable assumption is that \( U_K \) is lowered.

On re-admission of oxygen, the KCl effect also reappears (Fig. 8 A), but not as rapidly as the p.d. itself recovers. This lag both in the falling phase (Fig. 8 B), and in the recovery may well indicate that K⁺ vs. Cl⁻ is not largely responsible for the normal p.d., and contrasts with the sulfate effects next described.

**Effects of Sulfate vs. Chloride during Low Oxygen Exposure**

It would be desirable to supplement the potassium ion effects with others, especially with those of dilute sea water which gives in *Valonia* such beautifully reproducible effects almost certainly to be referred to the much greater mobility of Cl⁻ than of Na⁺ (If \( V_{Cl} = 1.0 \), then \( U_{Na} = 0.2 \)).

But in *Halicystis*, and especially in *H. ovalis*, there is often little

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*In *H. Osterhoutii*, there is often a transient reduction of p.d. when dilute sea water is applied; this is dependent upon the isotonic solution used as diluent, mannitol, glycerol, glucose, and other non-electrolytes giving different values (Osterhout, W. J. V., *Proc. Nat. Acad. Sc.*, 1938, 24, 75). They possibly alter the surface in different manners, but it would be interesting to see what the effect of low O₂ would be, and it is planned to do this in the future on this species. In*
effect of diluting sea water with isotonic glycerol, etc., so that \( U_{Na} \) may be assumed to equal \( V_{Cl} \). This indifference persists under low \( O_3 \) tensions, so that little can be said about the changed mobilities of \( Na \) and \( Cl \). Either they are not altered at all, or they are both reduced equally by low \( O_3 \). The latter seems more likely in view of the increased electrical resistance, and of the sulfate effects now to be described.

It was recently found that large, proportional and extremely reproducible alterations of P.D. could be produced in \( H. ovalis \) by substitution of sulfate for chloride in an artificial sea water. The changes are almost certainly to be referred to the much greater mobility of \( Na^+ \) than of \( SO_4^{--} \) in the outer protoplasmic surface. (Vacuolar perfusion of sulfates has little effect.) When the P.D. is lowered by low \( O_3 \) tension, and the sulfate substitutions made, a greatly decreased effect is obtained, indicating an approach of \( U_{Na} \) and \( V_{SO_4} \). The resistance also rises, as shown in the next section, arguing for a decreased mobility of \( Na^+ \), rather than an increase of \( SO_4^{--} \), in this approach.

On re-admission of air, the sulfate effect reappears pari passu with the recovering P.D. (as it also decreases in low \( O_3 \)). This contrasts with the more lagging KCl effect described above, and makes it seem likely that Na, and some slow moving anion like sulfate (possibly of an organic acid) may be responsible for the normal P.D.

Further details of the sulfate effect are reserved for another paper.

**Electrical Resistance during Exposure to Low Oxygen Tension**

Intact cells of \( Halicystis \) show an appreciable resistance to the flow of continuous current, although not as high as in \( Valonia \), and much below that of \( Nitella \). (The lower resistance may correspond with the appreciably higher NaCl content of the saps, the protoplasm allowing penetration of \( Na^+ \) and \( Cl^- \) ions.) The impaled cells have even less resistance; this has been shown in terms of "polarization" by the application of very dilute sea water, or the pure isotonic non-electrolytes mentioned, produces a very drastic and irregular effect on the P.D., which is also believed to be due to alteration of the surface, and is partly counteracted by adding calcium salts. But smaller dilutions usually have little effect.
or counter-E.M.F. by string galvanometer records which indicate that currents of 5 or 10 microamperes per cm.² of cell surface change the P.D. but slightly, and in a very slow time course (= large effective capacity).

![Diagram showing the concurrent change of P.D. across the protoplasm of impaled cell of Halicystis, and the counter-E.M.F. developed by a flow of 20 micro-amperes passed inward across the protoplasm in a d.c. bridge. (Ohmic resistances of capillary, etc. balanced out.) The high P.D. is very little altered by this current flow in aerated sea water, only about 4 mv. counter-E.M.F. developing (equivalent to 200 ohms effective resistance). But when 0.2 per cent O₂ in N₂ is bubbled, the P.D. begins to fall, and the counter-E.M.F. rises reaching its highest value, 140 mv. (equivalent to 7,000 ohms effective resistance), when the P.D. has fallen to its lowest value (6 mv.). It then begins to fall slightly as the P.D. rises.

When air is re-admitted, the counter-E.M.F. in turn drops off as the P.D. recovers. The cycle is repeated on second bubbling of 0.2 per cent O₂, and readmission of air.

Left-hand ordinates are P.D. and counter-E.M.F. in millivolts. At the right the effective resistance, calculated from the counter-E.M.F., is also given. P.D. is black circles, counter-E.M.F. or resistance open circles. Arrows indicate the time of admission of nitrogen or air.

It would be difficult to detect an increased ionic permeability under these conditions, since the effective resistance is already so
low that reductions might be insignificant, and within the limits of error. However, it was early discovered that the uncompensated P.D. of Halicystis was much less able to produce a steady galvanometer deflection during low O₂ tensions, than when well aerated. This indicated an increased polarizability, which was directly tested in a D.C. bridge during nitrogen bubbling. It was found that the resistance rose remarkably as the P.D. fell, and eventually the cells were able to give large, prompt polarizations of 100 mv. or more, as compared with a few millivolts when aerated.

The course of such a resistance change is shown in Fig. 9, plotted both in terms of counter-E.M.F. developed, and in ohms effective resistance. An increase of 30-fold or more occurs during low oxygen exposure, with recovery of low resistance on aeration. It is clear that a decreased, rather than increased passage of ions occurs under low O₂ tension. This is true both in the presence of sea water, KCl-rich sea water, and sulfate sea water. An exclusion of ions would thus seem to be indicated, and could account for both the resistance rise and the P.D. fall.

The possibility is not excluded that a supply of internal ions, either organic or inorganic, did most of the current carrying during aeration, but decreased during exposure to low O₂ tension. But there is no direct evidence on this point, while the altered ionic mobilities of K, Na, etc., are clear.

DISCUSSION

The foregoing data show beyond question that many of the electrical properties of Halicystis cells are dependent upon the maintenance of small, though definite oxygen tensions (e.g., 1 to 2 per cent O₂ in N₂) outside the cells. It is reasonable to presume that this involves respiration, since oxygen consumption remains constant under higher O₂ tensions, but falls off when the oxygen tension falls below a certain value and the electrical properties are also affected in this region. This is hardly surprising, since the output of electrical energy is appreciable, and there must be some metabolic source for it. Furthermore, anaerobic respiration, if present, is not able to maintain more than about 20 per cent of the P.D. found in air. The important point for discussion is the mechanism by which respiration
and P.D. are related. At least two main possibilities suggest themselves (or with their combination, a third):

A. Respiration maintains a gradient of ions, either organic (intermediate metabolites) or inorganic (possibly in connection with electrolyte accumulation) which is responsible for the P.D. When oxygen is lacking, these gradients are reduced or abolished, to re-appear on aeration.

B. Respiration maintains the remarkable protoplasmic surfaces which are responsible for many of the unique properties of the cell. When these properties are lost or altered, the P.D. drops, disappears, or reverses.

C. Both gradients and surfaces are altered, simultaneously or in succession.

It is easier to speculate concerning A than to adduce definite proof. Until we are able to identify some specific ionic gradient as the source of the P.D., we shall not be able to say much concerning its alteration under metabolic influences. Formally, the disappearance of such active ions would account satisfactorily for both the fall of P.D. and the rise of effective resistance (there being fewer ionic carriers of the current which could pass the surface). However, it is difficult to see why the reduction in numbers of internal ions should so strikingly influence the effects of ions applied externally. Since there is definitely some alteration in the response of the cell toward external ions, referable to changes in the surface properties, the principle of least causes (Occam's razor) suggests that we explore this possibility fully before postulating an unknown, internal change in addition.

If surface changes be accepted as adequate to account for the altered P.D. and resistance, it is important to know by what mechanism these are effected under reduced oxygen tension. Here again we can only speculate, concerned as we are with the very nature of the surface, and its origin, maintenance, and alteration under metabolic influences. That it should bear such a relation is not surprising, since it is without doubt a complex lipoid in some manner built up by the cell itself, perhaps also constantly disappearing during metabolism. There is no reason to think it is a stable secretion like cellulose or chitin (although these also may have their intimate relation with the protoplasm). Its ease of formation when the cell is cut,
etc., suggest a large reserve and ready mobilization of surface materials, but what the relation of this mobilization may be to metabolism is not known. Evidently the surface is not destroyed by low O₂ tension; on the contrary, the rise of resistance indicates that it becomes a more effective barrier, at least to the passage of ions. This might be due to thickening, dehydration, or lowering of the dielectric constant of the surface; or to a change in charge, number or size of “pores.” In any case the cell is apparently protected against the loss of accumulated ions, or entrance of excluded ones, during temporary anaerobic conditions (cf. Cowan⁹).

Probably the surface does not actually alter mechanically (surface tension studies would be valuable under anaerobic conditions), but some constituent is gained or lost. The hypothetical substance “R” of Osterhout,⁹ necessary for the manifestation of high K⁺ mobility in Nitella, may be a product of respiration and tend to disappear under anaerobic conditions. Or it may be that the surface gains rather than loses something; thus the addition of guaiacol or cresol alters many of the electrical properties of Valonia, including both K⁺ mobility,¹⁰ and polarizability.¹¹ While it is not suggested that a specific phenolic substance like these is produced anaerobically in Halicystis, it is quite likely that some metabolite may accumulate with similar effects. In general, any weak acid which can penetrate the cell tends to lower the p.d., and increase the polarizability of Halicystis; lactic, pyruvic or other acids known to be involved in metabolism may affect the surface similarly.

It was partly to test the possible rôle of increased anaerobic acidity that some of the ammonia experiments were performed (although the suppression of reversed p.d. may be merely another manifestation of reduced permeability to the ionic species involved). The increased sensitivity to ammonia during part of the anaerobic drop of potential scarcely agrees with increased acidity, although the later inhibition might be so explained. But it was found that previous exposure of the cells to weak acid (e.g. acetic) did not render them more sensitive,

or quicker to lose their P.D. in low O\textsubscript{2} tensions. Probably the surface effects, though they resemble in some respects those produced by acidity, are due to some other anaerobic alteration. The nature of this must wait for future investigation.

It may be that some of the electrical effects of low oxygen tension correspond to the effects on permeability described with other organisms. A slightly decreased permeability to glycerol was found by Hunter\textsuperscript{12} in erythrocytes under anaerobic conditions; a greater difference might be found with more polar substances. Collander and Holmström\textsuperscript{13} describe a decreased absorption of several sulfonated dyes by plant cells under decreased oxygen tension. Decreased absorption of salts as well as of water by roots and other tissues when deprived of oxygen is well known. It is planned to test the permeability of Halicystis under low O\textsubscript{2}.

Whether the surface alterations here invoked can account for the bioelectric changes produced by altered O\textsubscript{2} tension in other organisms (Lund,\textsuperscript{14} Francis,\textsuperscript{15} Taylor,\textsuperscript{16} Cowan,\textsuperscript{17} Gerard,\textsuperscript{18} and others\textsuperscript{19}) must wait for future testing of altered ionic effects in those cases. Theories based upon oxygen effects should not overlook this possibility however.

**SUMMARY**

The potential difference across the protoplasm of impaled cells of Halicystis is not affected by increase of oxygen tension in equilibrium with the sea water, nor with decrease down to about 1/10 its tension

\textsuperscript{12} Hunter, F. R., *J. Cell. and Comp. Physiol.*, 1936–37, 9, 15, where a few other papers are also quoted.


\textsuperscript{17} Cowan, S. L., *Proc. Roy. Soc. London, Series B*, 1934, 116, 216. The increased resistance in Halicystis agrees with Cowan's finding that potassium was not lost from crab nerve during brief asphyxia, although the injury and action potentials were depressed. Long asphyxia, however, caused its loss.


in the air (2 per cent O₂ in N₂). When bubbling of 2 per cent O₂ is stopped, the P.D. drifts downward, to be restored on stirring the seawater, or rebubbling the gas. Bubbling 0.2 per cent O₂ causes the P.D. to drop to 20 mv. or less; 1.1 per cent O₂ to about 50 mv. Restoration of 2 per cent or higher O₂ causes recovery to 70 or 80 mv. often with a preliminary cusp which decreases the P.D. before it rises. Perfusion of aerated seawater through the vacuole is just as effective in restoring the P.D. as external aeration, indicating that the direction of the oxygen gradient is not significant.

Low O₂ tension also inhibits the reversed, negative P.D. produced by adding NH₄Cl to seawater, 0.2 per cent O₂ bringing this P.D. back to the same low positive values found without ammonia. Restoration of 2 per cent O₂ or air, restores this latent negativity. At slightly below the threshold for ammonia reversal, low O₂ may induce a temporary negativity when first bubbled, and a negative cusp may occur on aeration before positive P.D. is regained. This may be due to a decreased consumption of ammonia, or to intermediate pH changes.

The locus of the P.D. alteration was tested by applying increased KCl concentrations to the cell exterior; the large cusps produced in aerated solutions become greatly decreased when the P.D. has fallen in 0.2 per cent O₂. This indicates that the originally high relative mobility or concentration of K⁺ ion has approached that of Na⁺ in the external protoplasmic surface under reduced O₂ tension. Results obtained with sulfate seawater indicate that Na⁺ mobility approaches that of SO₄²⁻ in 0.2 per cent O₂. P.D. measurements alone cannot tell whether this is due to an increase of the slower ion or a decrease of the faster ion. A decrease of all ionic permeability is indicated, however, by a greatly increased effective resistance to direct current during low O₂. Low resistance is regained on aeration.

The resistance increase resembles that produced by weak acids, cresol, etc. Acids or other substances produced in anaerobiosis may be responsible for the alteration. Or a deficiency of some surface constituent may develop.

In addition to the surface changes there may be alterations in gradients of inorganic or organic ions within the protoplasm, but there is at present no evidence on this point. The surface changes are probably sufficient to account for the phenomena.