

PROTOPLASMIC POTENTIALS IN HALICYSTIS

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I

The capillary technique used for *Valonia macrophysa*¹ is logically available for other large cells of similar structure. I have recently applied it with success to the closely related *V. ventricosa* of Florida² and to *Halicystis*³ of Bermuda (long confused with *V. ventricosa*⁴). *Halicystis* is a multinucleate cell which superficially resembles *Valonia*, but differs markedly in details of morphology and in the constitution of the vacuolar sap.⁵ Its study by the methods developed for *Valonia* is therefore of importance from a comparative standpoint, and its strikingly different behavior must be interpreted in any general bioelectrical theory.

II

Mechanically, *Halicystis* is distinguished by having a more elastic, extensible wall than *Valonia*. The cells are not firm and hard but rather resilient to the touch. They are capable of more shrinkage and swelling without injury to the protoplasm. The tendency to shrink makes the cells more difficult to impale; most of the sap may be lost by spurting through the opening around the capillary before the wound closes. Neither may this loss be made up by sap from the capillary, since hydrostatic pressure through the latter causes a sufficient stream to wash away protoplasm opposite its opening. By the use of sharpened capillaries, however, and by twisting the cells slightly as they are pushed on, most of them promptly form a seal. They then live as long as two or three weeks thus impaled, resting upon cut corks as described by Damon. They may shrink noticeably in

¹ Damon, E. B., *J. Gen. Physiol.*, 1929-30, **13**, 207.

² Blinks, L. R., *Carnegie Inst. Washington Year Book No. 28*, 1928-29, in press.

³ Probably *H. ovalis*. A descriptive paper on this organism is in preparation.

⁴ Blinks, L. R., *Science*, 1927, **65**, 429.

⁵ Cooper, W. C., Jr., and Blinks, L. R., *Science*, 1928, **68**, 164.

size during this time, apparently by loss of sap into the capillary. Since the sap of *Halicystis* is less dense than sea water, the larger cells tend to float, and are easily dislodged from the capillary by sudden jar. Cells having a bit of the substrate (calcareous *Lithothamnion*) still adhering to the holdfast remain more surely seated. The most conveniently handled cells are those about 1 cm. in diameter. The capillary may not be pushed far enough into smaller cells to ensure a firm seal, and larger cells tend to collapse on impalement.

The capillaries, drawn on the ends of quarter inch glass tubing, are usually about 0.5 mm. in outside diameter, and from 0.5 to 1.0 cm. in length. They project into the vacuole of the cell 2 or 3 mm. They, and the bottles into which they connect, are filled with artificial *Halicystis* sap made to correspond to the analyses previously published. (The sap of the small cells used in these experiments was

TABLE I
Molar Composition of Saps Expressed as Per Cent of Halide

	A	B	C	D	E
	Sea water Bermuda	<i>Halicystis</i> Bermuda	<i>V. macrophysa</i> Bermuda	<i>V. macrophysa</i> Tortugas	<i>V. ventricosa</i> Tortugas
Cl + Br.....	100.00	100.00	100.00	100.00	100.00
K.....	2.15	2.58	86.24	82.33	94.74
Na.....	85.87	92.80	15.08	18.55	5.73
Ca.....	2.05	1.36	0.288	0.02	Trace
Mg.....	9.74	2.49	Trace ?	0.08	Trace
SO ₄	6.26	Trace ?	Trace ?	0.04	Trace

Analyses: B by Dorcas.

A, C by Van der Pyl.

D, E by Cooper.

essentially the same in composition as that of the large floating cells, according to analysis by Mr. Jacques.) Sodium, potassium, and calcium chlorides are present about as in sea water, with magnesium less concentrated and sulfate absent. (Cells which have formed zoospores and recovered may show sulfate.) The sap thus differs strikingly from that of the *Valonias*, as shown in Table I.⁵

Connection to the outside of the cell was made according to Damon's method,¹ the desired solution flowing down a strip of filter paper in contact with the top of the cell. Certain measurements were likewise made with the cell completely immersed in solution. These p.d.'s were essentially the same as with a flowing contact. Wet strings or salt-agar bridges formed the connection to lead chloride or calomel electrodes. The measuring instruments were a Compton electrometer, and a calibrated vacuum-tube electrometer.

III

Immediately on impalement, the cells of *Halicystis* showed almost no P.D. across the protoplasm. This was the case no matter what solution the cell was bathed in: sea water, artificial sap, or various single salt solutions. The impalement probably caused temporary injury.

On standing in sea water, however, the impaled cells soon displayed a larger and larger P.D. Within 1 hour the value might reach 30 to 40 millivolts, with the *outside positive* to the measuring instrument (*i.e.* positive current tending to flow from outside through the electrometer to the vacuole by way of the capillary; thus the positive current if allowed to flow would be across the protoplasm from inner surface to outer surface). Then more slowly the P.D. continued to rise, usually reaching in a day a maximum which was maintained more or less steadily for as long as two weeks. The highest P.D. found under any condition was 90 millivolts, the lowest steady value in sea water about 50 millivolts. The average value for some 50 cells measured was about 70 millivolts. There was often a fluctuation between 60 and 80 millivolts with the same cell from time to time. All of these were in the direction *outside positive*, and it was not possible to reverse the P.D. by any treatment so far administered.

Exposure of the cells to concentrated and dilute sea water had little of the expected effect on the P.D. A drop of about 10 millivolts was produced by $\frac{1}{3}$ sea water (made isotonic by glycerine) but in one case of long exposure the P.D. returned and rose above the original value; $\frac{2}{3}$ sea water produced a variation of 5 millivolts without permanent effect.

On the contrary, solutions of each of the more important salt constituents of sea water produced an immediate effect, abolishing the P.D. completely. 0.6 M NaCl, 0.6 M KCl, 0.4 M CaCl₂, 0.6 M MgSO₄, 0.4 M MgCl₂, each caused the P.D. to fall to zero in a minute or two, and to remain zero during the exposure. There was occasionally a slight rise just after the preliminary fall, amounting to 5 to 10 millivolts and lasting 2 or 3 minutes (see Fig. 1).

Except CaCl₂, which is quite toxic, these solutions did not cause permanent alteration of the cells, even by exposures up to $\frac{1}{2}$ hour.

The P.D. was restored remarkably soon upon re-exposure to sea water. This recovery is shown in Fig. 1 for a typical cell, after exposure to 0.6 M KCl. The recovery was delayed after long exposures but was rapid when once initiated. There was characteristically an "over-shooting" by which the P.D. went to a higher level than before treatment, and

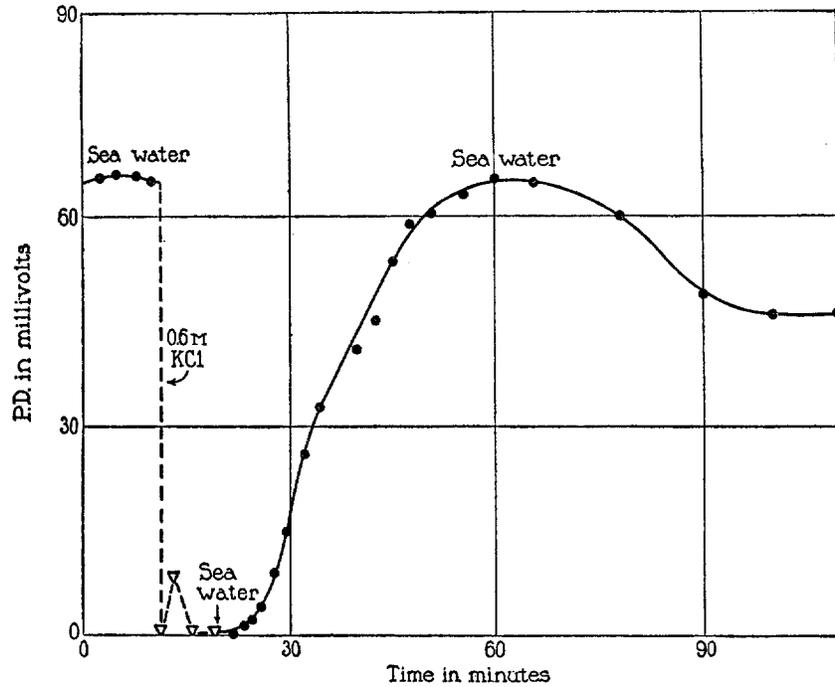


FIG. 1. P.D. in millivolts of an impaled cell of *Halicystis* exposed to 0.6 M KCl; with subsequent recovery in sea water. Time in minutes.

then descended slowly in 1 or 2 hours to a steady value. Occasionally there was only a partial recovery, quickly followed by death.

That the value was dependent on a balanced solution is evident from experiments with mixed salts. Thus the P.D. did not drop to zero when artificial sap was applied to the exterior of the cell, but remained for a long time at about 35 millivolts (Fig. 2). In one case recovery to over 60 millivolts occurred during such exposure. Injury ensued in another experiment, with disappearance of P.D. It is evident that

here again is an example of radial asymmetry in the protoplasm, since similar solutions applied to both sides of the protoplasm still may produce a high P.D. There is apparently a delicate balance at about this composition, since the value with sap is variable.

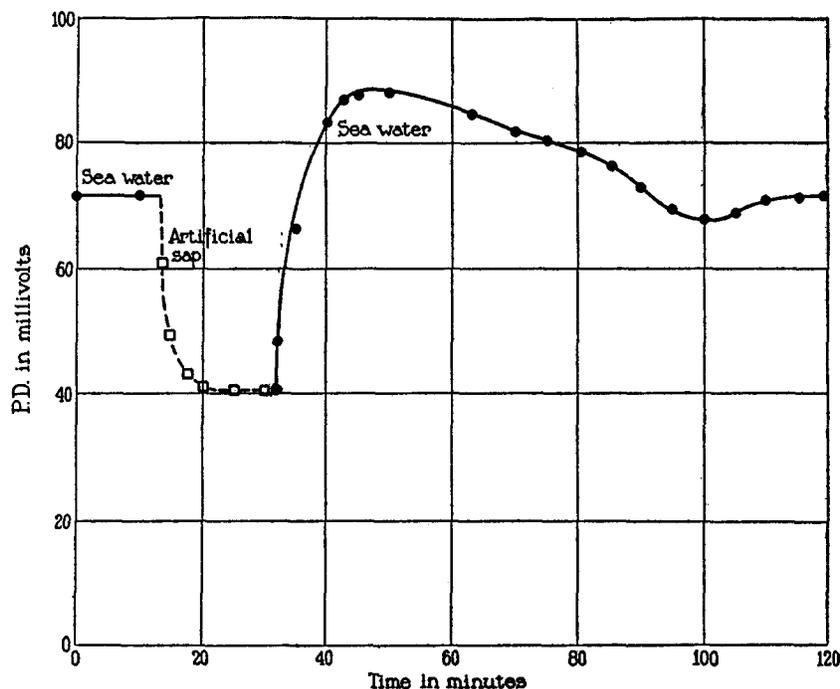


FIG. 2. P.D. in millivolts of an impaled cell of *Halicystis* exposed to artificial sap; with subsequent recovery in sea water. Time in minutes.

The P.D. was still less stable in simpler mixtures, as was exemplified by cells exposed to 0.6 M NaCl, 97.5 parts, + 0.4 M CaCl₂, 2.5 parts. The graph of Fig. 3 shows the course of P.D. variation in a cell exposed to this mixture. There is striking evidence here of alternate breakdown and recovery, which suggests the balance of processes dependent not on a single salt, but on several in proper proportion.

IV

It is not possible to draw full theoretical conclusions from the data so far available for *Halicystis*. Two striking facts stand out distinguishing it from *Valonia*.

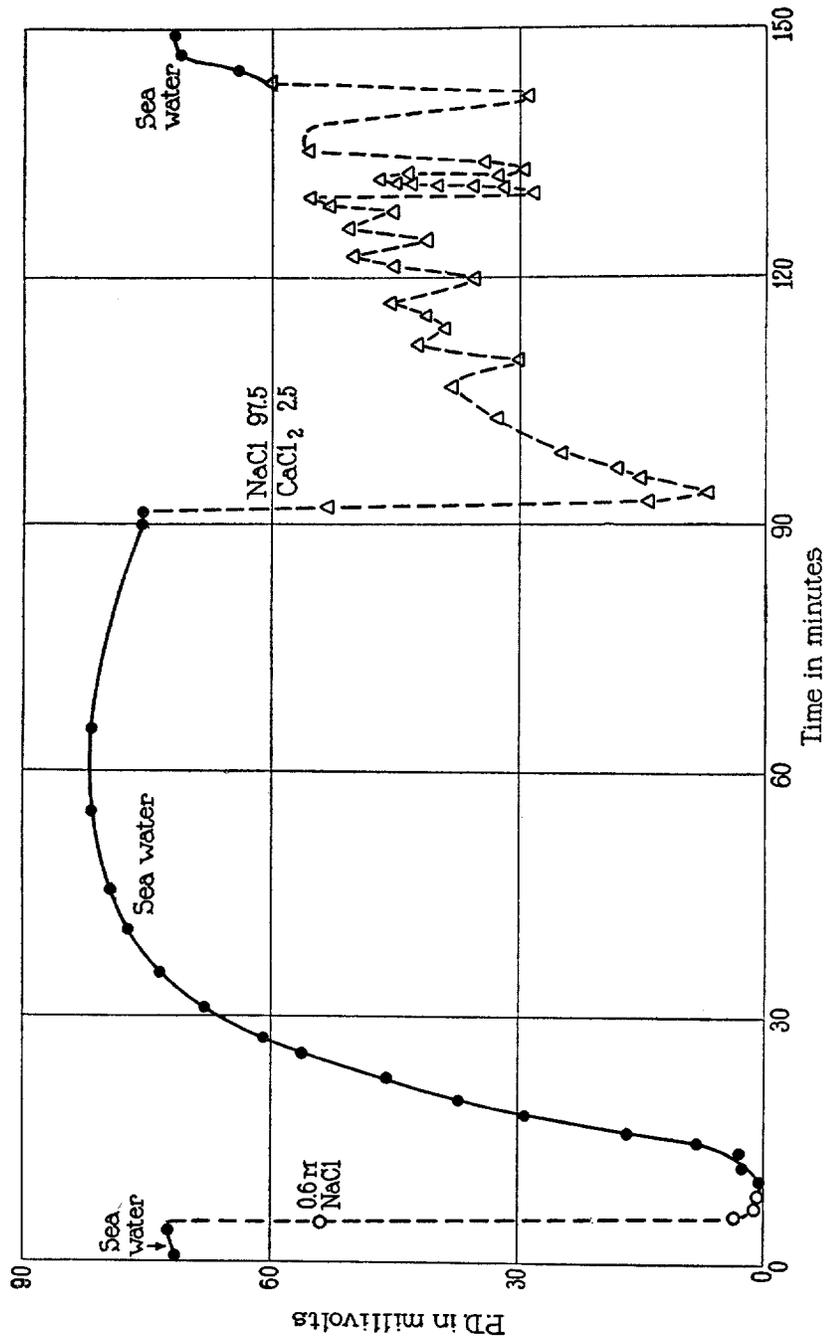


FIG. 3. P.D. in millivolts of an impaled cell of *Halicystis* exposed to (a) 0.6 M NaCl; (b) a balanced solution of 0.6 M NaCl 97.5 parts, and 0.4 M CaCl₂ 2.5 parts; with recovery in sea water. Time in minutes.

1. It shows a P.D. nearly ten times as large as does *V. macrophysa*, and four or five times as large as *V. ventricosa* (both being similarly impaled and immersed in sea water), and directed in the opposite sense. In these respects it is much more like *Nitella* in tap water both as to direction and magnitude of the P.D. The P.D. produced when sap is applied necessarily implies an asymmetric protoplasm.

2. Identification of the ions responsible for the E.M.F. appears difficult in view of the fact that a balanced solution is necessary for the production of any P.D. whatever. Systematic variation of the sea water composition is thus of doubtful value. Except for H^+ and SO_4^- there are no abundant ions of the sea water sufficiently different from those of the sap to give rise to an E.M.F. of 70 to 80 millivolts by concentration effect. That these two are probably not concerned was shown by changing their relative concentration in the sea water. Sulfate ion was doubled by the addition of Na_2SO_4 without effect on the P.D. The pH was changed from 8.2 to 6.0 without immediate effect. (Lower pH produced permanent alteration.)

Further study of these effects will be carried on. It is possible that the slow rise of P.D. observed after impalement is not due to a recovery but to an alteration such as a permanent lowering of E.M.F. at the outer or *X* layer. (This might be produced by the diffusion of salts into or out of the aqueous layer *W*.) Bridge measurements of intact cells show that they have a greater polarization response than the impaled cells, and we have increasing evidence of the expected correlation between polarizability and the bioelectric P.D. It is hoped that the study of *Halicystis* in conjunction with *Valonia* will assist in a general critique of the method of impalement.

SUMMARY

The cells of *Halicystis* impaled on capillaries reach a steady P.D. of 60 to 80 millivolts across the protoplasm from sap to sea water. The outer surface of the protoplasm is positive in the electrometer to the inner surface. The P.D. is reduced by contact with sap and balanced NaCl-CaCl₂ mixtures; it is abolished completely in solutions of NaCl, CaCl₂, KCl, MgSO₄, and MgCl₂. There is prompt recovery of P.D. in sea water after these exposures.