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Protoplasmic Potentials in Halicystis. VI. Rôle of Ammonia in Potential Reversal by Perfusion.*

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Earlier papers[1] have described the reversal of the normally positive potential difference across the protoplasm of impaled cells of *Halicystis osterhoutii* (Bermuda) both by exposure to ammonia (or other weak bases) and by perfusion of the vacuole with more alkaline sap or sea water. The author was then inclined to explain the former effect by the latter, since penetration of ammonia raised the vacuolar pH. One difficulty of this interpretation was that the Californian species, *H. ovalis,* did not show such reversal on perfusion with sea water at pH 8.0 or higher, although reversing well on exposure to ammonia. This difference between the species now seems reconciled by referring the effects in both cases to the penetration of ammonia into the protoplasm. This is shown in *H. ovalis* by introducing into the perfusing fluid a trace of NH_4Cl; at pH 5.0, the normal acidity of the sap, no effect is observed, but if sea water now is perfused at pH 8.0, reversal of potential often results. Then as perfusion is continued, and removes the added ammonia, recovery of positive P.D. may result, even at pH 8.0.

Reëxamination of *H. osterhoutii* in Bermuda shows similar results. Very clear, pale cells, with no evidence of recent reproduction, also do not show reversal of P.D. when perfused with sea water at pH 8.0. This behavior was sometimes found in earlier work, but was then ascribed to imperfect perfusion; such is now definitely not the case, since rapid flow, good mixing in the vacuole, (as shown by tracer dyes), and long continued perfusion were carefully maintained. Certain other cells at first may reverse on such treatment, but later recover positive P.D. on continued perfusion. In both of these cases, addition of a trace of NH_4Cl to the perfusing fluid promptly causes reversal, which can be counteracted by lowering the pH to 6.0 or less:—the effect originally ascribed to pH alone. In such a state also, the P.D. responds to light, in a manner analo-

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[1] Blinks, L. R., *J. Gen. Physiol.*, 1933, **17**, 109; 1935, **18**, 409. The methods employed are described in these papers.

gous to that produced when ammonia is externally applied. This effect, which will be described elsewhere, seems wholly attributable to the pH changes accompanying photosynthesis, acting upon the ammonia dissociation. In the absence of ammonia, the effects of light are much smaller.

Examination showed, however, many cells of *H. osterhoutii* like those originally reported, which display continued reversal of P.D. during perfusion of sea water for long periods. These are generally dark green in color, and have dark masses, the remains of unliberated gametes from previous reproductive periods, floating in the vacuole. Ammonia can be detected in the vacuolar sap of such cells up to 0.005 M or higher by Nessler test; it is possibly derived from the breakdown of the gametes. Obviously considerable dilution of the sap must be necessary to reduce this value below threshold concentrations for reversal, which in this species occurs on external application of 0.0005 to 0.001 M NH_4Cl at pH 8.0. Since perfusion is less certain in these very cases, due to repeated clogging of the outlet capillary by the floating masses, incomplete elimination of the "native" ammonia appears to be the explanation of the earlier results; thus an imperfect perfusion results in reversal, rather than in failure to reverse.

A corrected interpretation for both species is now suggested: Reversal of P.D. is not produced by high pH alone, either in sea water or vacuole; *i. e.,* strong bases are not effective in the physiologically tolerated pH range. But if a weak base, such as ammonia (or amines) is present, either externally or in the vacuole, then raising the pH at that place causes the P.D. to fall or to reverse. This is doubtless due to the penetration of undissociated weak base into the protoplasm, with an expected rise in the protoplasmic pH. How this internal acidity change operates upon the potential mechanism is not known, but it may be upon the charges either of organic ions responsible for the potential, or of surfaces necessary for its manifestation.