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Effects of Oxygen Tension and Temperature Change on the Bio-Electric Potential of Halicystis.*

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Previous experiments (external application of sap, perfusion of vacuole with sea water[1, 2]) have indicated that the large bio-electric potential (P.D. = 70 to 80 mv. outside +) across the protoplasm of impaled *Halicystis* cells (coenocytic marine alga) is mostly independent of inorganic ions or other gradients between cell sap and environment. Internal gradients or assymetries in the protoplasmic film itself were suggested. Since the P.D. can produce a measurable current for long periods through a completed circuit, a source of energy, eventually metabolic, must be available. Experiments have therefore been directed toward analyzing the relation between P.D. and metabolism in this organism. The electrical methods are those previously employed,[1, 2] the cells being kept in the dark to avoid photosynthesis. Manometric measurements of O_2 consumption by the Warburg method were made on groups of several intact cells under comparable conditions (except for impalement). Concurrent P.D. and respiration measurements on identical impaled cells are now under way.

Oxygen tension. Over the range where respiration is unchanged, the P.D. is also unaltered. This holds for pure O_2, air, and for mixtures of O_2 with N_2 down to about 2% O_2, brought into equilibrium with sea water by continued bubbling. If bubbling of the 2% O_2 mixture is stopped, the P.D. drifts downward as O_2 is consumed in the vicinity of the cell, eventually falling to 10 or 15 mv. +. Stirring of the sea water, or rebubbling of 2% O_2 promptly restores the P.D. This downward drift and recovery may be repeated almost indefinitely. Further lowering of O_2 tension, *e. g.*, to 0.2% O_2 in N_2, reduces the P.D. to 10 mv. or less while bubbling or stirring continues. Respiration also falls to low values in this mixture. Recovery of P.D. again occurs if 2% or higher O_2 is bubbled, unless the cells have been kept for many hours at low O_2 tensions. Curious downward cusps of P.D. often precede the recovery curve. In this respect, as well as in the general linkage of P.D.

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1 Blinks, L. R., *J. Gen. Physiol.*, 1932, **16**, 147.

2 Blinks, L. R., *J. Gen. Physiol.*, 1935, **18**, 409.

with respiration, these findings agree with those of Lund,[3] Francis,[4] and others on other organisms. The exact nature of the linkage is not demonstrated however; respiration may be necessary to maintain either surfaces or other structures, or a supply of organic ions. An O_2 gradient, *as such* across the protoplasm is not responsible, for the P.D. is equally well restored, in its original sign, by perfusion of aerated sea water through the vacuole while 0.2% O_2 is bubbled outside.

Temperature, of course, markedly influences respiration, which has the usual Q_{10} of 2.0 or more in the temperature intervals 5° to 15° (normal), and 15° to 25°C. (in intact *Halicystis* cells; preliminary work with impaled cells shows similar values). The P.D., however, is lowered only 10 or 15% at most when the cells are cooled from 15° to 5°, even for several days ($Q_{10} = 1.1$ to 1.2). There is still less increase of P.D. when cells are warmed from 15° to 25° or higher ($Q_{10} = 1.1$ or less). Relatively small temperature effect on P.D. was also found by Hill in *Nitella*.[5]

These small temperature effects may indicate that some physical process such as diffusion limits the P.D., the supply of ions being more than sufficient to maintain the gradients. Independent evidence,[6] however, indicates that in *Halicystis* 2 large discrete potentials, + and —, summate to give the observed P.D. across the protoplasm. If these had large but slightly different individual Q_{10} values, their algebraic sum might remain nearly unchanged with temperature. The somewhat larger cusps obtained on the sudden immersion of cells in warmer or cooler sea water might represent the momentary lagging of the inner surface behind the outer while a radial temperature gradient existed. Larger temperature effects in other organisms[7, 8] might represent the lessening or absence of such summation, or very different Q_{10} values for the component potentials.

Under altered conditions, large temperature effects may also be obtained in *Halicystis*. Thus the downward drift of P.D. after bubbling of 2% O_2 is stopped, occurs only very slowly or not at all at 5°C., probably because diffusion can now supply O_2 as fast as it is respired. Warming to 15° then causes it to fall, while at 25°

[3] Lund, E. J., *J. Exp. Zool.*, 1928, **51**, 265.

[4] Francis, W. L., *J. Exp. Biol.*, 1934, **11**, 35.

[5] Hill, S. E., *J. Gen. Physiol.*, 1935, **18**, 357.

[6] Blinks, L. R., Rhodes, R. D., and McCallum, G. A., *Proc. Nat. Acad. Sci.*, 1935, **21**, 123.

[7] Umrath, K., *Protoplasma*, 1934, **21**, 329.

[8] Marsh, G., *Carnegie Inst., Washington*, 1936, Pub. No. 475. 1.

it may even fall during bubbling or stirring. A large apparent Q_{10} results, but with negative sign; a higher respiratory rate correlates with a lower P.D. and *vice versa.*

Other conditions facilitate large temperature effects. 5 to 10% CO_2, mixed with air and bubbled through sea water, depresses the P.D. of *Halicystis*, probably by raising the protoplasmic acidity. The CO_2 tension at which this occurs is a function of temperature. A similar situation holds for dilute ammonia, which causes reversal of P.D.[9] At 15° the threshold for this lies around 0.002 M NH_4Cl in sea water; the threshold concentration is increased by cooling the sea water, and decreased by warming (probably by altering the dissociation and solubility of ammonia). As a result, a 10° change may alter the P.D. by 100 to 150 mv., giving very large apparent Q_{10} values. The sign is negative, warming causing more negative values, cooling more positive ones (*cf., Valonia*[8]). Temperature also facilitates the reversal of P.D. by vacuolar perfusion of alkaline sea water[2]; this occurs in *H. ovalis* at 25° but not at 15°. Possibly other large temperature effects are similarly conditioned by physiological alterations of O_2, CO_2, ammonia, and pH.

Time curves of these effects, as well as those of other agents influencing metabolism and P.D., will be reported elsewhere.

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Zinc Sulphate as a Chemoprophylactic Agent in Experimental Poliomyelitis.*

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In earlier notes[1] we reported observations on the prophylactic action exercised by picric acid and certain other compounds. In this note we are reporting results which indicate that zinc sulphate applied to the nasal mucosa is even more effective than picric acid. These observations are set forth in Table I, in which the relative efficiency of the respective agents is compared in terms of the ap-

[9] Blinks, L. R., *J. Gen. Physiol.*, 1933, **17**, 109.

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[1] Schultz, E. W., and Gebhardt, L. P., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 133; *Calif. and West. Med.*, 1936, **45**, 38.