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TEMPERATURE EFFECTS ON VALONIA BIOELECTRIC POTENTIAL*

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SUMMARY

This investigation concerned detailed effects of temperature on bioelectric potential. Previous measurements of algal potentials were made at irregular, widely spaced, temperature intervals; this study recorded temperature continuously from 10 to 36°. The current models predict relatively simple continuous temperature dependencies for bioelectric potentials; yet in this investigation abrupt changes in potential were observed near 15 and 30°. Below 15 and above 30° the potentials changed sharply while between 15 and 30° temperature had only slight effect on the potential.

INTRODUCTION

Bioelectric potentials in algal cells have been studied for more than 40 years, yet one variable – temperature – has been almost entirely neglected. This is not justified as it is one of the most important environmental factors for most organisms[1-3]. Before biophysical data can be related to the macro-physiology and ultimately the ecology of organisms, quantitative temperature effects must be studied. Also, testing "laws" at other than standard temperatures may show that these hold only fortuitously. For example, the apparent NaCl molal volume obeys the Debye–Hückel law at temperatures near 25°; however, large deviations from the limiting law occur near 0° and 55° (ref. 4).

In addition to neglecting the details of temperature, workers assumed that bioelectric potentials exhibit a smooth continuous behavior with respect to temperature. The NERNST equation[5] for describing passive ionic equilibrium has been used as a first approximation. This is:

$$E = \frac{RT}{zF} \ln \frac{c_o}{c_i}$$

where E is the potential in mV across the membrane system, R is the gas constant, F is the Faraday constant, T is temperature in degrees Kelvin, z is the algebraic valence of ionic species, and c_o and c_i are ionic concentrations outside and inside the membrane. (These should be replaced by ion activities.)

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The GOLDMAN equation[6] expresses potentials which are caused by membrane permeation of more than one ion. That is:

$$E = \frac{RT}{zF} \ln \frac{P_{\mathrm{K}}[\mathrm{K}_0] + P_{\mathrm{Na}}[\mathrm{Na}_0] + P_{\mathrm{Cl}}[\mathrm{Cl}_\mathrm{i}]}{P_{\mathrm{K}}[\mathrm{K}_\mathrm{i}] + P_{\mathrm{Na}}[\mathrm{Na}_\mathrm{i}] + P_{\mathrm{Cl}}[\mathrm{Cl}_0]}$$

where E, R, z, T and F represent the same terms in the NERNST equation; P_{K}, P_{Na} and P_{Cl} are ionic permeabilities, and the bracketed symbols indicate concentration. If permeability and concentration are not temperature dependent, the potential is proportional to T. Most workers question the assumptions on which the GOLDMAN equation is based; often it is treated as semiempirical[7]. Furthermore, both equations apply to one membrane rather than two in a series (as occur in giant algal cells); also they refer to passively moving ions.

Considerable attention has been directed at these equations but temperature has been essentially neglected, and its dependency generally assumed to be smooth and monotonic.

However, upon re-evaluating the relative few existing membrane data[8–13] the writer found that temperature dependence probably was not represented by a smooth monotonic function. Since the existing evidence was not conclusive, experiments were carried out to determine the real temperature effect on bioelectric potential. Continuous measurements of bioelectric potential *versus* temperature were made on 55 *Valonia macrophysa* and *Valonia ventricosa* cells over the ecologically relevant range 10–36°.

METHODS

Experimental success required that the Valonia cells be in an excellent condition; thus they were held in as nearly natural a situation as possible. Upon collection, they were transferred to a large, running seawater outdoor tank containing sand and rocks. The cells grew, reproduced and remained in a healthy state for several years.

To measure the bioelectric potential across the combined cell membrane system of Valonia, a glass capillary tip (0.2 mm diameter) was inserted into the vacuole; both membranes were pierced. Large diameter tips lessen the problem of tip resistance. This minimizes potential changes to large molecules being trapped in the capillary tip. Modification of the classic methods of OSTERHOUT[14] and BLINKS[15] made it possible to maintain healthy impaled cells for as long as a year, thus providing excellent control conditions. This remarkable property was probably due to the protoplasm rapidly forming a tight seal around the impaling pipette.

Controls included tests for random variations in resting potential of each cell (biological noise); this consisted of determining the variation in the potential of each cell at 25° over time periods of 0.5–4 h. Other variations were also measured. One cell could be recycled three times through a temperature series, before the cell appeared non-responsive to light–dark tests. If allowed to rest for 1 day, the cell then could repeat the temperature cycles.

These tests were carried out during both heating and cooling processes at selected temperatures ranging from 8 to 38°. To detect and eliminate cells which were dying or injured, light–dark responses were measured by placing the impaled cells in the dark for 1 min. In healthy cells this causes a drop in the potential; sudden exposure to light then produces a rapid increase potential (at least 1.0 mV), then a return to a resting potential. As a further measure against recording data from unhealthy cells, a cell which did not survive for at least 2 days after the completion of the experiment was discarded.

The experiments on bioelectric potential-temperature measurements were carried out in complete darkness and at three artificial light intensities, all less than 78 foot candles. Fifteen rates for heating and cooling were used, ranging from 0.05–1°/min. The electric potentials were measured to ± 0.05 mV by using a Keithley high impedance potentiometer 660 A and the temperature was controlled to ± 0.01°. The impaled cell was immersed in a 250-ml seawater bath suspended in a water-filled, insulated, 20-l glass chromatography jar (see Fig. 1). This was temperature controlled by opposing a refrigeration unit maintained at −1.0°, accuracy ± 0.001°, *via* a twelve turn copper coil submerged in the bath with two 250-W heaters controlled by a Sargent thermonitor. Very efficient stirring was effected in both the Valonia, *via* a submerged electromagnetic stirrer with a teflon coated stirring bar, and in the chromatography jar *via* a motor. Temperature variation within the Valonia bath was ± 0.05°. Temperature was recorded on an X–Y recorder *via* a thermistor bridge with a probe immediately next to the cell; a microthermistor mounted in a 26-gauge needle was impaled into the center of the cell. The temperature inside the cell was obtained from resistance measurements on a Wayne Kerr autobalance bridge and recorded on a strip chart recorder. There was no apparent measurable difference in temperature between the Valonia internal fluid and the bathing media at the fastest or slowest heating and cooling rates.

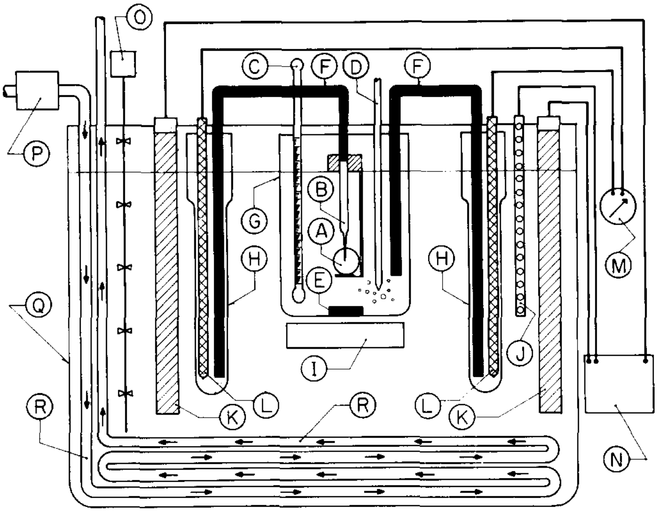

Fig. 1. Schematic diagram of the experimental apparatus. (A) Valonia cell; (B) impaling pipette; (C) thermometer; (D) bubbler; (E) teflon-covered magnet; (F) salt bridges; (G) container for seawater; (H) containers for salt bridges and electrodes; (I) electromagnetic stirrer; (J) thermistor for heating control; (K) heaters; (L) electrodes; (M) potentiometer; (N) Sargent thermonitor; (O) stirring motor; (P) cooling unit; (Q) water bath; (R) cooling coils. (The internal thermistor and conductance bridge are not represented here.)

The capillaries used to impale the cells were filled with agar containing 0.5 M KCl and were connected to a calomel electrode by a salt bridge of 0.5 M KCl. A second calomel communicated to the bathing media by another 0.5 M KCl salt bridge.

All this was contained in the temperature bath. The containers for salt bridges and calomel half cells were also suspended in this bath. The thermal coefficient for the whole system was tested by running each part separately. The thermal coefficient of the system without the Valonia was 0.1 mV/° between 10 and 36°. The dead Valonia had almost exactly this same coefficient. Drift of the entire system without the Valonia cell was less than 0.05 mV for the time period of the experiment.

RESULTS

The initial experiments relating temperature to the resting potential of *V. ventricosa* and *V. macrophysa* showed abrupt changes in bioelectric potential for all 55 cells near 15 and 29.5°. A typical example, as shown in Fig. 2, demonstrates that the temperature coefficient of the Valonia resting potential is not simply related to the RT/F term in either the NERNST[5] or GOLDMAN[6] equation.

If the temperature regions of 10–15° and 30–36° are considered separately, there appear to be distinct regional temperature coefficients. The mean resting potential of $\psi_i - \psi_o$ (vacuole to external media) was 9.6 mV (± 1.1 mV) for 30 *V. ventricosa* cells at 25° and, below 15°, showed a temperature coefficient of −1.1 mV/°; between 15 and 29.5°, a coefficient of less than 0.05 mV/° while above 30° a coefficient of + 1.2 mV/°. These mean resting potentials and variabilities from the means were obtained in the following manner: the resting potential at 25° was assumed to be the normal resting potential. (The low variability bears out this assumption.) From the original continuous recording of potential *versus* temperature, the potential of each

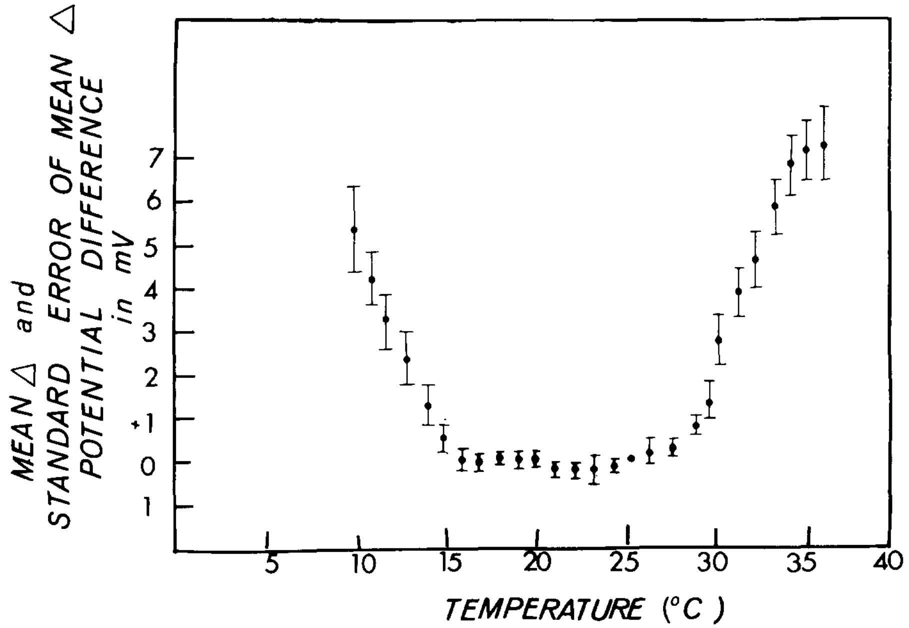

Fig. 2. Change of mean potential (closed dots) from resting potential at 25° and standard error of mean potential (vertical lines) across the protoplasm of 30 *V. ventricosa* cells *versus* temperature ($E = \psi_i - \psi_o$).

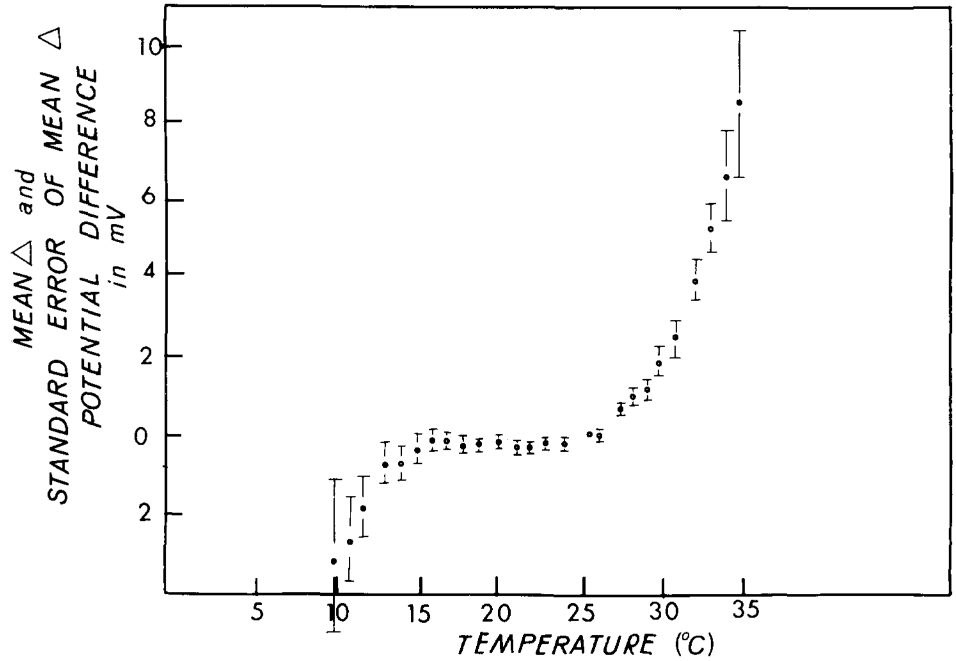

Fig. 3. Change of mean potential (open dots) from resting potential at 25° and standard error of mean potential (vertical lines) across the protoplasm of 25 *V. macrophysa* cells *versus* temperature ($E = \psi_i - \psi_o$).

cell was plotted at 1.0° intervals. The mean resting potential of 25 cells of *V. macrophysa*, which was 10.0 mV (± 1.1 mV) at 25°, showed a temperature coefficient below 15° of + 0.5 mV/° (Fig. 3). Between 15 and 30° the cells tended to remain very near their resting potential. However, both the abrupt change at 15° and the temperature coefficient between 15 and 30° were similar to that exhibited by *V. ventricosa*.

Further examination shows distinct variabilities for the three temperature regions. The variability (calculated as standard error of the mean) from the resting potential between 15 and 29.5° is very low; ± 0.3 mV for *V. ventricosa* and ± 0.4 mV for *V. macrophysa*. This low SE_m was thought to be due to the low tip potential and the excellent condition of the cell after weeks of impalement. Such low values are not normally attained in other cell preparations. At and below 15°, as well as at and above 30°, the standard error becomes markedly greater, in both species. This indicates that individual cellular responses are quite stable between 15 and 30°. In fact, the variation of potential of Valonia with time is ± 1.1 mV for 2 h for both species, and ± 0.2 mV for 30 min with both species. The variation between the repeated cycles of the same cell was ± 1 mV. No potential difference appeared to be due to the large tip diameter since other workers using much smaller tip diameters have very similar time variation results[19,24].

When cooled from 10 to 5°, cells showed essentially the same mean potential increase and thus the same temperature coefficient as those exhibited by cells exposed to the 10 to 15° range. Few experiments were conducted below 10° since the probability of cells existing in such temperatures under normal environmental conditions is extremely low. From 30 to 36°, the mean increase in potential was + 1.2 mV/°. Above 36° the behavior was similar to that between 30 and 36°. Rates of heating and cooling in the range stated did not change these effects.

There was a tendency in *V. macrophysa* for the potential to change more sharply above 30° than below 15°. These cells decreased (became more negative) in potential below 15°, behavior similar to that observed in previous studies of *V. utricularis* by the author[16]; by comparison, *V. ventricosa* showed a more positive potential in this low range, as did the earlier results by BLINKS[8], who reported a single *V. macrophysa* cell's variation with temperature at approximately 5° intervals. (It should be noted that only two *V. macrophysa* examined by the writer behaved similarly to this cell of BLINKS.) Above 30°, the two species behaved in a similar manner*.

For both species the temperature effect in the presence of potassium was more marked than without added potassium as found by BLINKS[8].

DISCUSSION AND CONCLUSIONS

Perhaps the most important conclusion from these experiments is that living biological membrane systems differ sharply in behavior from what might have naively been expected. When the bioelectric potential of Valonia cells was measured continuously over the temperature range of 10–35°, the RT/F factor of the NERNST or GOLDMAN equations did not adequately describe the temperature–potential relations of these cells. In addition to the slight magnitude of the temperature coefficient, there was little change in bioelectric potential and a very low variability among cells. A most surprising result of the present work was the magnitude of the temperature coefficient below 15° and above 30°, and also the difference in the response of the two Valonia species below 15°. The temperate squid axon (*Loligo forbesi*) was reported[17,18] to show almost no potential change over its normal ecological range (2–16°). However, such behavior is in contrast to that reported for Nitella, which has a high temperature coefficient (1.6 mV/°) over its natural range of temperature by HOGG *et al.*[12], and to the behavior of the giant lobster axon[10], which also has a large response in its natural range between 2 and 16°. Recent work on the squid axon of *Dosidicus gigas* by LATORRE AND HIDALGO[13] showed a large decrease in resting potential with temperature in the range 3–20°, which is markedly different from behavior of *Loligo forbesi*.

One simple caveat from these results is that data on bioelectric potential and other biophysical parameters may be misleading when measured over widely spaced temperature intervals. If adjacent points are at intervals of 5–10° and a single curve fitted to the resulting data, as has been done by past investigators[9,19], the variability of the data may not represent experimental error or "biological noise". Instead, the data may reflect regions of differing temperature coefficients. Thus, interpolation between

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* The possibility of the pipette becoming unsealed due to different coefficients of thermal expansion of the glass and protoplasm, which could result in a shunt of potential, was considered. When the cell was first impaled, there was a leak around the pipette; consequently, the potential was near 0 mV. The process of sealing was observed microscopically with a simultaneous increase in potential. If a leak occurred during the experiment, the potential would have dropped to approx. 0 mV.

points would be misleading. Also, since extrapolation of data below 15° and above 30° is not a valid procedure for Valonia bioelectric potential, it may not be valid for other biophysical parameters. Obviously, studies should not be carried out near the points of transition between two temperature coefficients, and temperature controls should be included data in all experiments*.

When considering the possible causes of the abrupt changes in bioelectric potential, it should first be recalled that the effect must be a property of the living cell; dead or dying cells did not show such a temperature response. The GOLDMAN equation, which may more closely approximate the membrane events in Valonia, can only be applied to a single membrane, not two membranes in series as found in Valonia. This results in an immediate theoretical difficulty in locating the site of the temperature changes. Even when considering a single membrane, the potential is affected directly by the temperature factor (T), and indirectly by the temperature dependencies of quantities such as permeabilities; such factors as diffusion rate, ion activities, or ion concentrations may be modified by temperature. However, changes in concentrations can probably be ruled out as the cause of the abrupt changes in potential, since AIKMAN[20] has shown that the exchange time for cytoplasmic potassium is about 1 h, and the abrupt potential change occurred in a matter of minutes. If electrogenic pumps exist in Valonia, they may be strongly temperature dependent and may not vary smoothly with temperature; for instance, studies on the activity of ATPase from animal cells[21,22] have shown changes in slope of activity of ATPase *versus* temperature near 15 and 30°. Next, since the P.D. is measured across the cytoplasm, cytoplasmic events such as changes in pH or endoplasmic reticulum could occur. Finally, the possibility cannot be excluded that morphological changes are reflected in the abrupt temperature changes. Examples of this are modifications in the lipid (as discussed by BEAN AND CHAN[23]) or protein structure, or, as has been suggested by THORHAUG and co-workers[16,24] changes in the structure of water in the membrane.

Assuming we are indeed measuring a membrane phenomenon, it is reasonable to question whether this abrupt temperature response occurs at one or both Valonia membranes. GUTKNECHT[25] has stated that the potentials across the plasmalemma and the tonoplast are both high, and the resulting measured potential is the sum of these two potentials. This indicates that relevant information could be obtained by impaling the cytoplasm and the vacuole at the same time and determining the temperature effect on both membranes. The measurement of relative fluxes of ions and water across the plasmalemma compared to the tonoplast would be a fruitful study.

A further suggestion would be to examine the effect of temperature on the structure of the membrane. If structural effects occur, they are reversible over short time periods. However, when cells are exposed to temperatures below 15 and above 31° for periods of 72 h or more, a profound change in the cell occurs resulting in irreversible plasmolysis and death[26]. Therefore, the abrupt change in the potential, as well as the marked increase in variability, may be interpreted from the physiological point of view as reaction to stress in order to maintain a steady state.

The implications of this abrupt change in bioelectric potential may be far-reaching. A. KATCHALSKY (personal communication) has proposed that the organisms are living in steady states far from equilibrium, whereby it is possible to see

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[*] These conclusions do not question any experimental information for Valonia obtained at a single well-controlled and defined temperature within the optimum range (20–27°).

abrupt changes in properties of living systems with small environmental changes. The data presented here appear to substantiate this notion. This may be very pertinent to ecological considerations such as thermal pollution in the tropics.

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