THE RELATIONS OF TEMPERATURE TO THE POTASSIUM EFFECT
AND THE BIOELECTRIC POTENTIAL OF VALONIA*†

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The influence of temperature upon bioelectric potential has been occasionally investigated, both to initiate action potentials,1,2 and to indicate the nature of the controlling mechanisms,3-8 e.g. whether simple diffusion potentials are adequate to account for the effects, or whether metabolic, surface active, or other phenomena are also involved. Before conclusions can be drawn from the temperature data alone, it is desirable to combine their study with those of other conditions or agents known to be electrically effective. In Valonia the two most studied of these are the seawater (NaCl) dilution effect4 and the potassium concentration effect.5 In the earlier work on these, temperature was not experimentally varied, the measurements being made over a narrow seasonal range (16-23°). Wider alterations are here described, especially upon the potassium effect. (The NaCl dilution effect is much less sensitive to temperature.)

The methods are essentially those of previous studies,5-8 the cells being supported on glass rings, and impaled from above with fine glass capillaries, sap-filled and leading to calomel electrodes via KCl-agar salt bridges. Cells were employed only after good healing of the impalement wound had occurred—from several days up to several weeks after impalement. For the normal sea water effects it was found necessary to renew the sea water frequently, since small amounts of KCl diffusing from the

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1 Umrath, K., Protoplasma, 1930, 9, 576; 1934, 21, 329.
3 For a discussion of the earlier work, see Bélehrádek, J., Temperature and living matter, Berlin, Borntraeger, 1935, 113–4, 170, 186–8, 223, 226.
8 Blinks, L. R., J. Gen. Physiol., 1930–31, 14, 139.

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external salt bridge can markedly influence the magnitude of the temperature effects, as shown below. The measurements were mostly made by an automatic recording potentiometer (Micromax) compensating every 3 seconds. Current flow was therefore insignificant, and no appreciably different results were obtained when a vacuum tube interstage was used to render the instrument electrostatic. The time curves given are usually direct tracings of such Micromax records. In most cases the solutions were gently stirred with a fine jet of air, which also speeded temperature equilibration. When quick changes of temperature were desired, vials containing the sea water or other solutions were brought in advance to the desired temperature, and rapidly substituted for the previous vial. In all cases the experimental vials were immersed in larger vessels, either thermostated or manually adjusted to within 1/2° of the indicated temperature during the exposure. A small thermometer projected directly into the vial, immediately next the cell. All measurements here reported were performed in a darkened room, with generally less than 1 foot-candle of light in the vicinity of the cell. The vessels were also usually jacketed with black paper.

Most of the measurements were performed during the winter months in Florida, where the two species *V. ventricosa* and *V. macrophysa* yielded essentially similar results. *V. ventricosa* displays on the whole a larger potassium effect which correlates with both its larger normal P.D. in sea water and its greater change with temperature. Some of the earlier exploratory experiments were performed with *V. macrophysa* in Bermuda, and with cells brought from there, with concordant results.

**Effects of Temperature upon the Normal Potential**

Fig. 1 shows some of the characteristic changes of P.D. of a cell immersed in sea water. On cooling the cell from 23° to 13° there is first a slight depression of P.D., but this soon recovers and increases well above the warmer value. On warming to 25° there is first an increase, but a later fall; cooling to 15° repeats the rise at 13°, to even higher values. And again a warming to 25° causes an initial slight increase, but a pronounced later fall, which only slowly recovers, not however, to the 15° value. Still greater warming, to 35°, produces not only a large initial cusp, but an appreciably higher final level, sometimes, though not always, as high as at 15°. The exact time curve of these effects varies somewhat, as seen in the rest of Fig. 1, but the main points remain: essentially, the P.D. is lower in the range around 25° than it is either at lower or higher temperatures. The same overall effect is likewise seen in Fig. 2, where instead of quick 10° changes, the cells were slowly warmed from 10° up to 36° in the course of about 2 hours. Here the P.D. rose to higher values at the warm end than at the cool, in cell B; but cell A, when cooled finally to 8°, showed at least as high a P.D. as at 36°, after an initial drop.

The difference in behavior of these two cells is characteristic at the lower temperatures. Thus while the P.D. is high in the range 10-15°, it often tends to fall off sharply below about 8°; when it does, the P.D. often does not recover rapidly on warming. This state also coincides with a greatly decreased or
negligible potassium effect (contrary to the response at 10–15°, as seen below). There is quite possibly a change in physical state at the region 4–8°—perhaps a solidification or rupture of the protoplasmic surface—which is not readily reversible. (There is also a drop in P.D. following exposure to temperatures over 40° C.) Fig. 3 shows a similar experiment, plotted against temperature instead of time. The saddle-shaped curve is hardly compatible

Fig. 1. Time course of P.D. change of impaled Valonia ventricosa in sea water. The temperatures were as indicated, changes being made at the arrows by substituting new vials already brought to the indicated temperature and maintained by water jacket. On changing from 23° to 13° there is a slight fall, followed by a rise; warming to 25° produces a rise then a fall, cooling to 15° a rise. Warming to 35° produces a sharp increase above 25° however, either as a cusp, or a maintained plateau. The curves are actual tracings of a continuous recording potentiometer record (Micromax). Time intervals of 10 minutes shown by vertical marks at bottom of records. Ordinates are potential difference across the protoplasm in millivolts, the sign being that of the exterior (here negative).

with either a thermodynamic potential (as at an electrode) or with a process closely related to metabolism. (The respiration of Valonia has been investigated over this temperature range⁹ and shows a regular increase from low to high temperature, with a Q₁₀ of around 2.) It is therefore desirable to inquire whether any other phenomena more closely parallel these P.D. changes, and if so, whether these may be responsible for the anomalous temperature course of the latter. Such parallel and control appear to be at hand in the potassium ion effects, next taken up.

⁹ Unpublished experiments.
The Temperature Effect at Various Potassium Concentrations

The first suggestion that potassium might be implicated in the temperature effect came from the observation that occasional cells displayed larger changes than those shown in Figs. 1, 2, and 3. This often occurred with cells which were exposed after impalement to sea water from a dish where they had previously been kept for some time (to avoid osmotic or other changes at the time of impalement and healing). Such water often had an increased KCl content, due to release of KCl from the vacuoles of other cells which had died in the vessel. Enhanced temperature effects also occurred with cells when salt bridges had been left in the sea water for some time, permitting slow diffusion of KCl from the agar (see paragraph on methods, above). On renewing the sea water in both these cases, the temperature effect usually decreased

10 Such increased KCl concentration is not injurious to the remaining cells: indeed it seems to aid their survival, probably by supplying sufficient potassium for normal accumulation, to keep pace with growth or slight evaporation.
markedly, although sometimes with characteristic changes due to removal of higher potassium concentrations (notably a temporary reversal of P.D. to a large positive value).

These observations led to controlled experimental changes of the KCl content of the sea water, with the results shown in Fig. 4. When the normal sea water value of 0.012 M KCl was halved (by adding an equal volume of van't Hoff artificial sea water made up without KCl), the temperature effect was greatly diminished (Fig. 4 a). It was practically abolished in potassium-free sea water, but there are complications here due to the tendency for reversal of P.D.; in such a positive state the P.D. is very sensitive to a variety of agents, including temperature, but its time course is extremely unpredictable.

Increase of KCl concentration, on the other hand, back to 0.012 M restores the normal temperature effect, while doubling the sea water value, to 0.024 M KCl, enhances the response, which becomes greatly exaggerated at 0.1 M KCl (Fig. 4 c). Concentrations higher than this give not only a complex time course themselves but also induce an irregular temperature response,
with exaggeration of the initial cusps, but no great alteration of permanent level. This is consistent with an "alterative" effect of these higher concentrations, found by Damon, and probably occurring even at lower concentrations, though with subsequent recovery. (See Discussion.)

NH₄Cl is even more effective in these respects than is KCl; sufficient ammonia can enter the sea water from tobacco smoke in the room to increase the effects of temperature (as well as of light⁹). Dilution of the NaCl and other

![Graph](image)

Fig. 4. The influence of KCl concentration on the temperature effect. The cell of *V. ventricosa* was changed from sea water (containing 0.012 M KCl) to half this value, 0.006 M KCl, at the first arrow. Very little change of p.d. thereafter occurred either on warming to 35° or cooling to 15°. On restoration to normal sea water (S.W.) the normal temperature effect is regained (cf. Fig. 1); and on increasing the KCl content to 0.024 M and 0.1 M respectively in the next two panels, the temperature effect is correspondingly increased. Tracings of continuous Micromax records, the time marks being 10 minutes apart.

salts of the sea water (by isotonic glycerol) does not, however, greatly alter the temperature effect, provided the KCl concentration is kept constant by proper additions.

**Influence of Temperature on the Potassium Effect**

It seems hardly possible that KCl alters in strictly proportional fashion some other process controlling the p.d. so that it still seems best to ascribe its effects to its high diffusion potential in the protoplasmic surface, as was assumed by Damon and by Osterhout. The time curves show characteristically an increased negativity when KCl concentrations are increased, due to the high relative mobility of K⁺, compared to the Cl⁻ (and of course to
Na⁺). For the higher KCl concentrations, this ordinarily takes the form of an initial sharp cusp, followed by a depression, and a later rise of negativity. Damon has interpreted such curves as due to an advancing wave of KCl which first establishes a diffusion potential across the outer surface (X), later abolishes this as its concentration builds up inside that surface, and finally produces a new negativity as it arrives at the inner surface (Y). These curves are duplicated in V. ventricosa at the normal laboratory temperatures, being most cusped at 0.1 M KCl or higher, but showing at least rudimentary cusps with a slight fall and later rise even when the sea water value was doubled from

![Graph showing influence of temperature on potassium effect in V. ventricosa.](image-url)
0.012 M to 0.024 M KCl (Fig. 5). This is generally true at 25°, although sometimes repeated later exposures give a flatter topped curve than the first, as seen in several cases in Figs. 5 and 6. A definite control is exercised by temperature upon the shape and magnitude of these time courses. Cooling to 15° generally gives perfectly regular, smooth curves, soon reaching a constant value, with scarcely an indication of cusp or later rise, even at 0.1 M KCl. This constant value may be 50 to 100 per cent higher than the average increase at 25°. However, at 35°, coinciding with the increased P.D. in sea water, the potassium effect is again increased, even over that at 15°. The time course is usually cusped, but not as markedly as at 25°, both the initial and final values being much higher, with a smaller depression between. (Repeated exposures may entirely smooth out these curves also, but their magnitude remains much greater than at 25°, as seen in several instances in Figs. 5 and 6.)

To a considerable degree, the same differences obtain when the potassium concentration is decreased, as for example when potassium-free sea water is substituted for normal sea water, as in Fig. 5 c. The P.D. change is greater
both at 35° and at 15° than at 25°, and the magnitude of the change closely parallels the P.D. value in sea water. (The tendency to reversal of P.D. at 35° is evident in Fig. 5 e, with potassium-free sea water.)

**DISCUSSION**

The close parallel at the different temperatures studied between the magnitude of the normal P.D., and of its increase by KCl, suggests that the two may have the same basis. Indeed, the practical abolition of both the P.D. itself, and of any temperature effect upon it at very low potassium concentrations, raises the question whether any other potential except that due to the external KCl concentration contributes toward the total P.D. across the protoplasm of *Valonia*. It is, of course, possible that the high KCl content of the sap sets up a large potential across the vacuolar surface of the protoplasm, which is in turn closely balanced by some other potential (perhaps due to organic ions of the protoplasm\(^{11}\)). It has not yet proved possible to perfuse the vacuole of *Valonia* with new solutions, such as sea water, to determine the response of the vacuolar surface to potassium salts, but such experiments with *Halicystis*\(^{12}\) suggest that the vacuolar surface is practically indifferent to them. If so, it is perhaps not surprising that the P.D. of *Valonia* is very slightly influenced by temperature when there is only 0.006 M KCl in the sea water—there may then be no other potential present to be altered. (Possibly two balanced potentials are being altered by temperature in exactly equal and opposite degree, but if so, this would still leave the external potassium concentration effect as the experimentally controlling factor.)

The simplest hypothesis is that a change in the mobilities of the ions K\(^+\) and Cl\(^-\) occurs in the protoplasmic surface, over the temperature range studied. The values of the initial cusps suggest that this does indeed change somewhat. But mostly it is the succeeding time course that differs; and here we may have several possible hypotheses to choose between:

(a) We may use the simple picture advanced by Damon\(^{7}\) to account for the cusped time courses, namely an advancing concentration wave of KCl. Thus we may say that at 15° KCl enters very slowly, so that the original gradient is well maintained across the outer surface (X), hence the potential is steady. At 25° sufficient KCl enters to decrease the gradient across X fairly soon, hence the cusp, but the wave reaches the inner surface (Y) only slowly, hence sets up a second potential there only slowly and incompletely. At 35°, however, although there is rapid entrance across X, the wave also reaches Y very soon, and reestablishes a potential there. This attractive picture suffers

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\(^{11}\) Blinks, L. R., Cold Spring Harbor symposia on quantitative biology, Cold Spring Harbor, Long Island Biological Association, 1940, 8, 204.

from the uncertainty as to whether there actually is any potassium effect at Y, as discussed above.

(b) We may invoke instead, "alterations" in the outer protoplasmic surface, induced by the potassium itself. There is considerable evidence pointing to this as the cause of the depression following the initial cusp, for if one changes solutions, either back to sea water or up to higher KCl values, at the depth of this depression, the p.d. change is greatly diminished, instead of being merely displaced downward around a new and lower position, which would be expected in scheme (a). There is a real loss, partial or complete, of the potassium effect during the trough; it recovers rapidly if sea water is restored, or more slowly if the KCl remains. The cause of this "alteration" by potassium itself is not clear, but it is conditioned among other things by the pH of the sea water, being almost entirely abolished at high pH. Clearly, temperature also conditions it, for there is no trough, and no corresponding loss of potassium effect at 15°; while it becomes prominent at 25°. The situation at 35° is then interesting: what causes the very slight trough and final very large potassium effect? Apparently the higher temperature greatly speeds up the spontaneous recovery from the alteration, which occurs more slowly and incompletely at 25°. This may well be some metabolic response to the altered pH due to the entrance of KOH; it may even cause the curve after recovery to
rise higher than at 15°. Fig. 7 shows a striking example of these alterations at 20°, which were prevented at 10°. The picture for pH is very similar to this and will be reported elsewhere.

(c) A third possibility involves the accumulatory mechanism, which carries the entering potassium over across Y into the vacuole. This is clearly conditioned by metabolism, and might therefore be expected to have a high temperature coefficient. We may assume that (as in a) little KCl moves across X at 15°, so that the original gradient is maintained, hence a high P.D. At 25°, however, considerably more KCl enters X, but is not very rapidly moved out of the protoplasm across Y to the sap by the accumulatory mechanism. At 35°, finally, despite a large entrance across X, the accumulatory mechanism is very active, and keeps the protoplasm well depleted of the entering potassium, hence the P.D. is high. There is evidence that in Nitella radioactive potassium does enter the protoplasm readily (across X), but only slowly accumulates in the sap.13 The entrance is also rhythmic, somewhat like the later rises and falls of the KCl time curves in Valonia. The behavior of radioactive potassium in the protoplasm of Valonia at different temperatures would be interesting to study in connection with the bioelectric curves.

Choice between (a), (b), and (c) is difficult, but the author inclines toward (b) ("alteration") as having the most experimental proof at the moment. By permitting a temporary approach or equality of mobility for K⁺ and Cl⁻, this could govern the effects described, although the other possibilities (a) and (c) cannot yet be dismissed.

It is evident that (b) might account for the temperature effects in ordinary sea water since the P.D. in the steady state after "recovery" would vary in the manner observed. In (a), however, any change in the required direction might be only temporary and the steady state after "recovery" would not be predictable without additional assumptions. In (a) and (c) it would be necessary to assume that in ordinary sea water there is enough inward movement of K⁺, due to growth, to account for the observed effects.

These considerations emphasize again the remarkable lability of the protoplasmic surface, which Osterhout has demonstrated with a variety of chemical agents in Valonia and other plant cells.14 Temperature, in influencing the alteration of the surface by potassium itself, is therefore but another agent in producing this "Osterhout effect."15

15 The large positive P.D. of Halicystis, which has its probable origin in the organic salts of the protoplasm,13 is only slightly influenced by temperature.4 If, however, the KCl content of the sea water is increased, the temperature effects are considerably enhanced.11 The mechanism is probably that of (b) described above, and will be discussed at length in another paper.
The effect of temperature upon the bioelectric potential across the protoplasm of impaled Valonia cells is described. Over the ordinary tolerated range, the P.D. is lowest around 25°C., rising both toward 15° and 35°. The time curves are characteristic also. The magnitude of the temperature effect can be controlled by changing the KCl content of the sea water (normally 0.012 M); the magnitude is greatly reduced at 0.006 M KCl, enhanced at 0.024 M, and greatly exaggerated at 0.1 M KCl.

Conversely, temperature controls the magnitude of the potassium effect, which is smallest at 25°, with a cusped time course. It is increased, with a smoothly rising course, at 15°, and considerably enhanced, with only a small cusp, at 35°. A temporary "alteration" of the protoplasmic surface by the potassium is suggested to account for the time courses. This alteration does not occur at 15°; the protoplasm recovers only slowly and incompletely at 25°, but rapidly at 35°, in such fashion as to make the P.D. more negative than at 15°. This would account for the temperature effects observed in ordinary sea water.