THE EFFECTS OF CURRENT FLOW ON BIOELECTRIC POTENTIAL

I. VALONIA

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A description will be given of the effects of the flow of direct current, of controlled direction and density, upon the potential difference displayed across the protoplasm of impaled Valonia cells. The results are presented as string galvanometer records of the bioelectric potential before, during, and after current flow. Incidentally it is hoped that these will make more graphically evident some of the phenomena previously described in terms of resistance measurements with this organism.1,2

It was concluded early in the study of these phenomena that the apparent resistance of the living protoplasm was largely due to the appearance of a counter E.M.F. ("polarization") opposing the applied potential, and that the observed variations with applied potentials were due to the magnitude and speed of development of this counter E.M.F. depending upon the direction, density, and duration of current flow. It is obvious that such a counter E.M.F. constitutes a change (increase, decrease, or reversal) of the P.D. previously existing across the protoplasm. While this change can be calculated from the change of apparent resistance, and was so derived in a previous paper, the P.D. is better measured and recorded in its own terms, e.g. as millivolts. The present records are designed to show this.

Furthermore, the counter E.M.F. often develops in a complex fashion, following a sigmoid or cusped time course during the first few seconds of current flow, in a manner difficult or impossible to follow by manual resistance settings. Finally, the form of the depolarization curve, occurring after the current has ceased to

1 Blinks, L. R., J. Gen. Physiol., 1929–30, 13, 793.
flow, is often as interesting and characteristic as the polarization itself; this, of course, entirely escapes measurement in terms of apparent resistance.

A few string galvanometer records of the course of changing potential during and after current flow were included in previous papers; these were, however, oriented to show increased "resistance" by a galvanometer deflection in a given direction regardless of whether the current passed inward or outward across the protoplasm. It was therefore not immediately apparent whether the normal p.d. was being increased or decreased by current flow. The present records show this directly. In addition they present some new findings not previously described.

**Method**

The principles of recording bioelectric potentials during current flow possibly call for brief discussion since such potentials are usually measured statically, with avoidance of current flow (at least in the measuring instrument). They merely involve balancing the purely ohmic resistance of the system (here largely that of the capillary inserted into the cell) so that the IR drop produced along this during current flow is compensated by an equal IR drop in the adjacent arm of the bridge, leaving only the changes of bioelectric potential to be recorded. This is conveniently done in the direct current bridge previously described, with vacuum tube detector. The latter may be regarded as an electrometer connected in series with the cell and one arm of the bridge, and shunted by the other two arms. In a bridge with equal ratio arms as here used, the shunt is equal to the series resistance, which reduces the electrometer sensitivity to half its open circuit value. Direct calibration of sensitivity is obtained under any given conditions by introducing a known E.M.F. in series with the cell. This is frequently included in the records published, and from it is derived the effective millivolt sensitivity, marked as ordinates at the beginning of each record strip.

The records are photographs of the deflections of an Einthoven string galvanometer balanced into the plate circuit of the vacuum tube (201A worked at free-grid potential). The deflections of this instrument are essentially proportional over the field employed, and are practically rectangular at the film speeds used. This is shown by the calibrations taken with non-reactive circuits (e.g. dead cells, or those in the state of delayed polarization). With actively polarizing cells the deflections may be curved due to the change of current produced through the cell by calibration.

Since the bridge is necessarily a completed circuit, the cell's own potential can, of course, discharge continuously through it, in a "residual current." Although this might produce disturbing effects (as in *Nitella*, where special precautions are required), the p.d. of *Valonia* is so small (usually less than 10 mv.) that its discharge through the high resistances of the bridge (usually 50,000 to 100,000 ohms in series with the cell) produces only a small current, usually 0.1 or 0.2

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*Blinks, L. R., J. Gen. Physiol., 1929-30, 13, 361.*
microampere, or in a cell of the average surface of 2 cm.² a current density of 0.1 μa/cm.², or less. This was in some cases abolished entirely by compensating the cellular E.M.F. to zero by an opposed series E.M.F. Since the results taken in this manner differed in no appreciable way from those taken uncompensated, the residual current was allowed to flow during the experiments here reported. Its value is given in the legend of each figure. Such records have the advantage of showing the existing bioelectric potential in its positive or negative value at all times, before, during, and after current flow.

The current densities are derived from the estimated surface of the nearly spherical cells employed, an attempt being made to use cells of uniform size throughout, with a surface of about 2 cm.². There was necessarily, however, some variation in size and shape, so that the estimated surfaces are probably not correct to better than 10 or 20 per cent, with consequent variability of the current densities. Absolute values of these, however, except to orders of magnitude, are not of very great importance, since the threshold densities for various effects differ from cell to cell and from time to time. Much more significant are the relative values for the same cell; these are accurate to the agreement of the individual dry-cells of the bridge input battery, which were checked from time to time and replaced when their voltages became unequal. The relative densities are therefore accurate to 1 or 2 per cent.

Current densities are marked in μa/cm.², the direction being indicated as "in" for positive currents passing inward across the protoplasm from sea water to sap, and "out" for those in the opposite direction. Sometimes upward arrows are used to designate inward currents, downward arrows for outward currents (on the records the counter E.M.F. develops counter to these arrows).

The current densities are to be regarded with two reservations:

(a) They are really increments or decrements produced upon the residual currents as a base; the latter are so small in Valonia, however, that they are negligible in comparison with most of the experimental densities employed.

(b) They are the densities at the beginning of current flow, therefore subject to decrease as the counter E.M.F. develops. The circuit conditions, however, render the extent of this decrease rather small, since to produce the necessary currents through the high resistances of the bridge (100,000 ohms or more), external potentials of 1 to 10 volts are often applied. The counter E.M.F. seldom exceeds 200 mv., so that the maximum reduction of current due to it is at most 20 per cent and usually much less; i.e., 2 or 3 per cent.

Positive potentials are shown below the zero line, negative ones above, in accordance with the convention followed in most of the bioelectric work from this laboratory. The sign is that of the outer surface of the protoplasm, a positive potential tending to produce positive current outward across the protoplasm.

The cells were impaled and supported in the arrangements previously described.¹⁴ KCl-agar salt bridges led to calomel electrodes, non-polarizable at

the currents used. The basal ohmic resistances, used to balance the bridge, were
determined from the values before impalement, and changed but slightly during
the runs. They were further verified by A.C. measurements at 5,000 to 10,000
cycles (where the protoplasmic impedance was negligible) and in some cases,
where polarization was completely delayed, by the resistance values of the impaled
cell for low inward or all outward currents.

The results as given are believed to be typical of the genus *Valonia*. Both
*V. ventricosa* and *V. macrophysa* were studied, the former in Florida, the latter in
Bermuda, with essential agreement. The Bermuda species appears to be somewhat
more hardy, living longer in the laboratory, particularly when impaled
(e.g. Fig. 9).

Measurements were usually made on cells which had stood at least a day or two
after impalement in order to insure manifestation of the full bioelectric potential
and effective resistance, by healing of the wound inflicted by insertion of the glass
capillary. The necessity for this precaution is obvious and has been repeatedly
emphasized. The results obtained soon after impalement will depend upon at
least two factors: the previous state of the cell (i.e. whether displaying delayed or
regular polarization as defined below); and the extent of the leaks around the
capillary, which will short circuit the transprotoplasmic circuit and reduce the
apparent magnitude of both the P.D. and the polarizations to an extent depend-
ing upon the relative areas of injured and uninjured protoplasm. As the wounds
heal (for migration of chloroplasts, and other morphological details in this process,
see the recent reports of Kopac) an intact surface is sooner or later re-established.
There are, however, secondary changes usually supervening, apparently involving
the whole protoplasmic surface, and producing if it was not already present, the
state known as variable resistance or delayed polarization. This will be taken up
below. Whether this state (or any state of the impaled cells for that matter),
is “normal,” seems increasingly futile to discuss. It might be concluded that the
“constant” state, with its prompt and regular polarizations and high effective
resistance, is a normal one, reflecting, for example, the relative impermeability of
the cell to electrolytes. This is borne out by its almost invariable occurrence in
other plant cells so far studied (5 other genera). But it has now proved possible
to produce or destroy this state at will in *Valonia*, by proper chemical treatment,
without having to wait for time, “injury, recovery” etc., to accomplish the changes.
This is more likely to throw light upon the structural and metabolic factors
involved than academic discussions of normality. It seems reasonable to assume
instead that both states are functional, occurring spacially or temporally in the
same cell according to the prevailing internal or external milieu, especially, as
shown below, acidity. The ease with which one state passes into the other may
have an important bearing upon other problems, e.g. the accumulation of elec-
trlytes, since instead of a constant surface for penetration, there may be one

* Kopac, M. J., *Carnegie Institution of Washington, Year Book No. 32, 1932-33,
273; No. 33, 1933-34, 253.*
which changes its properties depending upon which type of ion, acidic or basic, strikes it at a given place.

The essential point is, however, that the regular and delayed polarization states differ, not merely quantitatively (depending for instance on the relative areas of injured and uninjured surfaces), but fundamentally and qualitatively in their time relations, in a manner to be distinguished only by control of current density, and by continuous records of the time course of polarization. These differences are best shown by the actual records which will now be presented.

Types of Response to Current Flow

Several stages of electrical behavior were distinguished in *V. ventricosa*, where an attempt was made to relate them to degrees of injury and recovery after collecting, impaling, etc. Most of the same characteristics are displayed by *V. macrophysa*, but further study has shown that they may be controlled to a large extent by experimental treatment, which will be described below.

Two principal states are distinguished: (A) “regular,” and (B) “delayed” polarization, the latter term being used for convenience, without prejudice as to the real nature of the counter E.M.F. (i.e., whether it has the character of a static or a polarization capacity; there is evidence, partly given below, that it is largely polarization). These may be defined as follows:

(A) Regular Polarization.—There is an immediate and regular development of counter E.M.F., beginning at the instant of current flow, and being proportional, at least for the smaller currents, to the current density, in either direction across the protoplasm. This gives rise to an effective resistance which is high and uniform for these current densities, and corresponds to the state of “constant resistance” earlier described.

(B) Delayed and Non-Proportional Polarization.—There is little or no counter E.M.F. developed with small currents in either direction across the protoplasm, nor with very large ones passing outward, but a large counter E.M.F. develops suddenly, with a sigmoid curve, when a critical or threshold density of inward current is reached. After this has occurred, larger currents produce only slightly greater counter E.M.F.’s; but the protoplasm has become thereby “conditioned” so that polarizations occur thereafter, not only with considerably smaller
EFFECTS OF CURRENT FLOW. 1

Fig. 1
inward currents, but even, temporarily, with outward currents. This correspondence to the state of "variable resistance" previously described.¹

These two states merge into each other, delayed polarization passing spontaneously over into regular polarization through intermediate stages where some of the characteristics of each are displayed. Each sometimes occurs, however, in a pure form which will first be described.

**Regular Polarization**

This condition is found in a few freshly gathered cells, and in most of those which have stood undisturbed in the laboratory for some days.

**FIG. 1. Effects of current flow on the P.D. of a cell of Valonia ventricosa**

which had reached the constant resistance level and was then impaled, the records being taken within a few minutes after impalement. The P.D., about 5 mv. negative (inwardly directed), is low, as is also the effective resistance (the latter being only 750 ohms higher than that of the capillary alone); this is due to the electrical leak around the insertion of the capillary, still unhealed.

- Records a to e inclusive were taken with the bridge balanced to this effective resistance; the string image, after a deflection at "make" (m), returns to zero in the steady state; it does the same again after a deflection at "break" (b). The deflections are seen to be regular and symmetrical for both inward (in) and outward (out) currents up to some 30 μA/cm² of cell surface. Above this (Records c, d) there is an increasing recession after a cusp, the steady state during current flow being away from the zero line, indicating bridge unbalance (cell resistance reduced). It should be noted that the recession is somewhat larger for outward currents (c) than for inward (d); this is practically the only difference between them in this state.

- Record e was taken with the bridge balanced as before to the effective resistance, but 6 equal increments of 5 μA each were made without a break, then 6 equal decrements down to zero again. The essential similarity of each response is evident.

- Records f and g were then taken with the bridge balanced only to the ohmic resistance of the system (largely the capillary) here about 14,000 ohms. The counter E.M.F.'s now build up as positive or negative potentials respectively for inward and outward currents, in f with a break (to r) between each value of current density, followed by a return to zero ("m" and "b" are omitted here): in g without a break between the increments and decrements. Sensitivity about 10 mv. per division, zero and 50 mv. + and − being indicated on each record. Residual current, r (due to discharge of bioelectric potential when no external E.M.F. is applied), about 0.15 μA/cm². Time marks 1 second apart.

- Current densities in μA per cm² of cell surface, the experimental densities being shown with figures, the residual current, on cessation of flow, by r.
or weeks after cleaning and separating. When such a constant cell is impaled, it usually continues to display for a while its regular polarizability, although reduced in magnitude because of the short circuiting introduced by the injured zone around the capillary. An example is shown in Fig. 1, taken immediately after the impalement of such a cell. When the bridge is balanced to the effective resistance of the system (here only 750 ohms higher than the capillary), the polarizations appear as “kicks” upward or downward at make and break of current, approaching the zero line in the steady state except with too high currents, when they show a cusp and a recession (c and d, Fig. 1). When the bridge is balanced only to the ohmic resistance involved, then the polarizations appear in their true light as alterations of the existing (here very low) bioelectric potentials. Reccessions from the highest value begin to appear slightly sooner for outward currents than for inward ones; this is practically the only difference between them. The regularity and symmetry of curves in charge and discharge, and in successive polarizations, should be noted.

In a few cases (particularly where a very fine capillary has been inserted) this regular polarization persists through the life of the cell while impaled, the polarizations merely becoming larger, and the effective resistance higher, as the injured area heals. Usually, however, the cells pass over into the variable or delayed state, which will next be described.

Delayed Polarization

Although this may not be a normal condition (e.g. it may reflect a heightened permeability to electrolytes) it is nevertheless almost universally found in freshly gathered cells, and in most impaled ones, which are able to survive for a long time in this condition. To be sure, the state is not always pure, since there is often a small polarization to low currents, becoming disproportionately larger at a certain threshold of inward current. An example of this is shown in Fig. 2, taken with the same cell used for Fig. 1, but 10 days after impalement. This may be taken as a representative of the transitional state, referred to again later.

There are, however, many cells which display a pure state of delayed polarization, showing no polarization at all with small currents. These
cells are like purely ohmic conductors, without reactance, and with a resistance practically that of dead cells to all outward currents, and to inward currents up to the threshold density. An example is shown in Fig. 3, where it is impossible to distinguish make and break of current except by the rectangular deflection due to a slight ohmic unbalance in the bridge. (This serves, by the way, to show the absence of spurious reactances in the system, such as electrode polarizations.) Other, even better examples of the lack of polarization at low current densities are seen in Fig. 13.

If the inward current be sufficiently increased, however, a critical density is reached at which a large polarization is suddenly produced. The threshold for this effect varies somewhat from cell to cell, and with time in the same cell. It often lies at about 25 μa/cm², but may be much higher, especially with the first flow of current after a long rest. In Fig. 3, for example, it is between 40 and 45 μa/cm², about as high as it ever goes. The time course of the response is distinctly sigmoid, with a slow start and an inflection to a very rapid rise of positive p.d. There may or may not be a cusp at the apex of this curve before it flattens out to a steady value; the cusp is lacking in Fig. 3, but is shown in other records (Figs. 5d, 8, 9, 15). Obviously the original bioelectric potential has been reversed, and remains reversed as long as the current continues to flow. When the current is stopped, this positive p.d. rapidly decreases, then more slowly returns to a negative value in a smooth curve.

This performance may be repeated any number of times, essentially the same positive potential being reached each time; but the curve changes shape, becoming progressively faster with equal current flows in rapid succession, so that the inflection may smooth out and practically disappear. Examples of this are shown in Fig. 4. On the other hand if a long wait intervenes between flows, the curve again becomes slower (Fig. 5). The duration of current flow also affects the speed of subsequent depolarization, negativity being regained more quickly after short flows than after long ones. This is shown in Fig. 4. The polarization curve is also usually different in shape from the depolarization.

In all these respects the response at the threshold differs from either condenser discharges or electrode polarizations, since in neither of these
FIG. 2
is there such an effect of one current flow upon the next. This is the first of the conditioning effects to be met with in the delayed state. Another will now be taken up.

If the current density be changed from the threshold value, several interesting results occur. An increase usually produces a still higher positive P.D., as seen in Fig. 3 for example. But the extent of this increase is not at all proportional to that produced at the threshold and two or three current increments usually cease to increase the positive P.D., at least beyond a temporary cusp, which is followed by a recession, sometimes even to lower values than at the threshold density itself (Fig. 7e).

On the other hand decreased inward current densities now produce a much larger effect than before the threshold flows, good polarizations

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Fig. 2. Effect of current flow on the P.D. of Valonia ventricosa, the same cell as used in Fig. 1, but 10 days later. At the start the bioelectric potential is about 10 mv. negative (i.e. inwardly directed). (Marks as in f and g, Fig. 1, but r is omitted in some cases.)

Outward currents, in 6 equal increments (without breaks) up to 60 μA/cm.$^2$, increase this P.D. to about 35 mv. negative; this is followed by 6 equal decrements. Larger outward currents have little further effect. Inward currents first decrease the negative P.D., driving it to zero at 20 μA/cm.$^2$ and at a threshold of 30 μA reversing it to large positive values, but with a delay, giving a sigmoid time course. Recovery to negative values occurs on interruption of current (to residual current, r). Further increments of inward current increase the P.D. up to about 150 mv. positive. An outward current of the same density produces a much larger counter E.M.F. than in Record a, but this rapidly recedes to a lower value, which is duplicated on succeeding outward flows. After two interrupted inward flows of 60 μA the current is decreased in 6 equal decrements without interruption, giving the effects of Records e and f.

It should be noted that 20 μA maintains a much higher positive P.D. than it originally produced (b) and that even 10 μA sustains positive P.D. for some time although steadily falling off in value, and becoming still less on a succeeding flow after short interruption.

The non-proportionality of response to equal decrements should be noted in contrast to the regular state of Fig. 1, as well as the hysteretic or conditioning effect of inward current, which passes off after large flows, as shown by the small final response to outward currents.

Sensitivity about 12 mv. per division, zero and 100 mv. being indicated on each record (+ and - calibration on a). Residual current (r) about 0.15 μA per cm.$^2$ of cell surface. Time marks 1 second apart.
being elicited down to 10 or even 5 µa/cm² in some cases. The lowest densities produce, however, proportionally less effect than the higher ones, and the response tends to die away, either on continued flow, or on successive passages of current. Some of these effects are shown in Fig. 3; a lowering of the threshold for later current flows is also to be seen in Figs. 5, 8, and 13.

Another type of record bearing on the non-proportionality of response in the delayed state is shown in Fig. 5, where step-wise increments or decrements of continued inward flow are employed. The rather large changes at the threshold density compared with the much smaller ones above this are clearly shown, as well as some of the "hysteretic" effects by which polarizations are maintained, after larger flows, by densities originally unable to produce them. The gradual dying away of this condition during the continued flow of inward current (10 µa) is also shown, as well as a progressive fall of threshold from 60 µa down through 50 (Record d) to 30 (Record f) and even to 20 µa (Record g). 10 µa, however, is never able to maintain positivity for long.

One might say that the passage of the threshold polarizing current has conditioned the protoplasm to a regularly polarizing state, for the curves shown in Fig. 5, and perhaps even better in Fig. 8 for 5 µa increments or decrements in the mid-range of densities (e.g. 10 to 25 µa total flow) are extremely regular, reproducible, and symmetrical,

Fig. 3. Characteristics of fully delayed polarization in *V. macrophysa* (marks as in f, Fig. 1). The cell (impaled about 3 weeks) is extremely non-reactive to lower current densities, displaying practically no counter E.M.F. to currents up to 40 µa/cm² in either direction. Only at a threshold of 45 µa does counter E.M.F. develop, and here very abruptly to 200 mv. positive P.D. The conditioning effect of such flow is, however, shown by the succeeding records, taken after an increase to 50µa, with progressively smaller inward currents. Good polarizations are elicited down to 15 µa, but much weaker at 10 and practically none at 5 µa. Increases again through 15 are not very effective, the threshold being 20 µa where a sigmoid reversal curve results. The sigmoid character is again nearly lost at 25 µa.

Sensitivity about 11 mv. per division, with values indicated on each record and calibrations on b. Residual current (r) about 0.05 µa/cm². Time marks 1 second apart.
both for charge and discharge (increments and decrements) and for successive current flows (equal changes). This distinctly resembles the state of affairs in regular polarization except that here the inward conditioning current must continue to flow, with small changes made in its value.

Indeed, the conditioning sometimes lasts long enough for fairly good polarizations to be produced to actual outward flows. This is a third effect of one current flow upon succeeding ones. In Figs. 2, 6b, and 7 are shown such temporary polarizations to outward currents following closely on inward ones. But in every case, there is only a cusp, with a quick recession and successively smaller polarizations to later flows. This may be due to the spontaneous loss of polarizability, which occurs even during the continued flow of small inward currents (e.g. Fig. 5). But the process is speeded up, as evidenced on records not here shown, by the flow of outward current itself. The "deconditioning" effect of outward current is likewise shown by the subsequent behavior of inward currents, for which the threshold may again be somewhat raised, although usually only the time of response is increased (Figs. 6 and 7), a more elongated sigmoid curve resulting.

**Transitional States**

Fig. 2 showed a transitional state of a cell which had formerly been regularly polarizing, but later displayed some of the characteristics of delayed polarization such as a threshold where inward current gave rise to suddenly increased positive P.D. in a sigmoid curve. As cells

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**Fig. 4.** Records showing the increasingly rapid response obtained with successive inward currents in *Valonia macrophysa*, impaled over 2 weeks (marks as in f, Fig. 1). The threshold in this cell is 20 μA/cm² but the response is extremely slow at this density. A sigmoid curve is still obtained when the density is increased to 30 μA, becoming less and less pronounced on succeeding flows in Record d, and practically smoothed away in e. The speed of depolarization also depends upon the length of time the current had flowed, being fastest after short flows, slowest after long ones. (1 second intervened at the spot marked on Record e, shortened to place it on the page.)

Sensitivity about 11 mv. per division, zero and 100 mv. positive being marked on each record, derived from the calibration on a. Time marks 1 second apart. Residual current (r) about 0.1 μA/cm².
Fig. 5. Further characteristics of delayed polarization in *V. macrophysa* (cell impaled 2 weeks). Polarization is first elicited on increasing the inward current from 30 up to 60 $\mu$A/cm$^2$. $10 \mu$A decrements then produce nearly equal decrease of P.D. down to about 20 $\mu$A, where recession begins, and becomes very marked at 10 $\mu$A, this density being unable to maintain positive potentials, as shown on several of the records. On the other hand 20 $\mu$A becomes increasingly capable of maintaining positivity as a result of the conditioning effect, the threshold first falling to 30, then to 20, and eventually (in h) to 10 $\mu$A, although a brief interruption here is followed by recession. Small polarizations to 10 $\mu$A outward flow conclude the record.

Sensitivity about 8 mv. per division in Records a to e inclusive; reduced to 12 mv. per division in f to h. Residual current (r) about 0.1 $\mu$A/cm$^2$. 100 mv. calibrations on e and h. Time marks 1 second apart. Marks as in f and g, Fig. 1. Upward arrows signify inward, downward arrows, outward currents.
FIG. 6. Further characteristics of delayed polarization in *V. macrophysa* (same cell as Fig. 5), especially in relation to the time factors. (Marks as in f, Fig. 1.) The threshold for inward current polarization is 30 $\mu$A/cm.$^2$, the sigmoid inflection occurring only after about 7 seconds continuous flow. A second passage of this current, however, produces rapid effects, the inflection being almost smoothed away, although still perceptible in $b$. Following this, 30 $\mu$A outward current produces an appreciable counter e.m.f., which rapidly recedes, and on second exposure remains low. After this deconditioning by outward current, the time course to inward current is somewhat lengthened ($b$) to become again shortened on subsequent flows in Record $c$ which also shows the effects of adjusting the bridge balance from the true ohmic value of the capillary (here 20,700 ohms) to the effective resistance of the cell plus capillary, totaling 23,100 ohms. Two steps of 1000 and 4 of 100 ohms each are shown, bringing the galvanometer to zero. The current is then interrupted, the typical "break" deflection being produced, much as in Fig. 1. It is seen to have exactly the same shape as the depolarization curves preceding and succeeding it with the same current density. (Ohmic balance was restored before the second half of $c$.)

Following on these 30 $\mu$A flows, the current was built up ($d$) through 5 equal increments of 5 $\mu$A, to 25 $\mu$A, at which density a response was obtained, although reaching a steady value extremely slowly ($e$). This current was then interrupted for varying lengths of time: 2.5 and 4 seconds on $f$, and 8 seconds between $f$ and $g$; the decreasing speed of response is noticeable. Finally, after 11.3 seconds interruption, 20 $\mu$A is seen to evoke very slight response.

Sensitivity about 11 mv. per division, with zero and 100 mv. positive being marked on each record. Calibrations on $a$ and $c$. Time marks 1 second apart. Residual current ($r$) about 0.1 $\mu$A/cm.$^2$. Upward arrow signifies inward, downward arrow, outward current.
stand quietly in sea water after impalement, they tend spontaneously to pass over through such transitional stages to regular polarization again. One of the invariable signs of this transition is the production of a more or less permanent positive P.D. as the result of current flow. We have already seen many examples of a tendency to linger at positive values, the more pronounced after long current flows. Fig. 9 shows this tendency exaggerated to an eventual flattening out of the P.D. to a temporary positive value, after increasing inflections. When this has occurred, positivity is often regained even after outward flows of high density, as shown in Fig. 9 c and d. Upon this positive P.D. as a base, very characteristic polarizations are now produced, small currents in either direction producing immediate and very regular counter E.M.F.'s, which become more and more cusped with increasing density. Another characteristic is the tendency for polarizations with outward currents to become even larger than the corresponding ones to inward currents (Fig. 9 f) so that the resistance is greater to outward than to inward currents, quite contrary to the usual situation. Eventually, the positive P.D. is seen to disappear, and with it these characteristics of polarization.

**Fig. 7.** Characteristics of the delayed polarization state in *Valonia macrophysa.* There is only a slight counter E.M.F. developed with outward currents up to 25 μa per cm.² of cell surface, and very little more with inward currents up to 15 μa. At 20 μa, however, strong polarization develops, with a sigmoid course. At break there is an abrupt drop of positive P.D., followed by a slower, nearly linear approach to zero and the original negative P.D. The same is true for larger inward currents except that the speed is increased, the sigmoid curve becoming smoothed out. After 25 μa inward flow, the current was reversed; the temporary polarization to outward current, which is rapidly decreased and not recovered in succeeding flows, is characteristic. So also is the somewhat slower time curve, with sigmoid course, produced by 25 μa inward flow following on these outward flows. It may be noted as a further characteristic that the higher inward current densities (40-50 μa) produce little if any greater permanent effect, the first increase being succeeded by a recession to a nearly constant level (here at about 200 mv. positive P.D.). Finally there is a temporary polarization with 50 μa outward current, followed by a rapid recession, and still less response on a second flow.

Sensitivity about 16 mv. per division, with zero and 100 mv. + and 200 mv. values shown on each record, derived from the calibrations on a. Time marks 1 second apart. Residual current (r) 0.05 μa/cm.² inward, or 1 per cent of the lowest experimental current density here used. Marks as in f and g, Fig. 1.
Further Characteristics of Regular Polarization

When the regular state is eventually regained, some of the transitional characteristics remain, especially the tendency for positivity to become more or less permanent following on current flow in either direction. This is seen in Fig. 10, and becomes exaggerated when the cell is placed in acidified sea water (Fig. 10 e, f). Here also the polarizations become much greater to outward than to inward currents, contrary to the usual condition. It is as if the p.d., being already somewhat positive, can only be driven a small distance farther by inward current, while outward current has more leeway, being able to carry it to zero and some distance negative in addition. Otherwise, the regularity and symmetry of curves should be noted, with a good proportionality which falls off with increasing densities due to recessions from a higher value, at the cusp.

One further characteristic of polarizations in the regular state is shown in Fig. 11, in which an attempt was made to determine whether the resistance in series with the cell had any influence upon the time course. Various extra resistances were introduced to bring the resistance in series with the cell from 40,000 ohms (capillary plus its equivalent balancing resistance) up to 100,000 and 200,000 ohms. Little if any change in the time curves results for the same current densities (produced of course by proportionally higher applied potentials).

Fig. 8. Records illustrating characteristics of the “variable” state in V. macrophysa, with delayed polarization and a threshold for polarization which is lower for decreasing currents than for increasing ones (“hysteresis”). Polarization was at first slight for 5 and 10 μa/cm.², on Record a, but 15 μa passing inward produced polarization with sigmoid course. Larger currents produce this more rapidly and add the characteristic cusp and recession, which may be followed by a slower rise again. Decreasing currents maintain polarization down to 10 μa and even slightly at 5 μa. Record b shows a step-wise increase and decrease of current without interruption of flow. Very regular increments and decrements of response obtain over part of the range. At 5 μa the positive p.d. begins to recede, although 10 μa brings it back, and after 4 seconds interruption during which only the residual current r passes, 5 μa produce increasingly less effect. Records c and d show more of these steps, the last especially showing the slow disappearance of polarization on successive flows of 5 μa, until it becomes at last negligible. Currents are entirely inward across the protoplasm.

Sensitivity about 12.5 mv. per division, with values as indicated on each record and calibration on d. Time marks 1 second apart. Marks as in f and g, Fig. 1.
Fig. 9. The production of lasting positive P.D. by current flow in *V. macrophysa*, and the further effects of current flow during positivity. The cell was an extremely hardy one which had lived impaled for 5 months, with frequent measurements (it finally died 7 weeks later). During much of this time it was in a transitional state between delayed and regular polarization, displaying small counter E.M.F.'s to small currents in either direction, but also, superimposed on these, the characteristic sigmoid reversal curve, as shown in Record a. An increasing tendency is evident to linger at positive values after each inward flow, with an inflection around 60 mv. positive, and a long slow approach to negativity. Finally, at 30 μA/cm.² the P.D. remains reversed (Record c) and returns to positivity even after an outward flow, although only after a lag at negative values (Record d). With the positive P.D. as a base, small inward currents are now passed (e), giving good polarizations, increasingly cusped at higher current densities. With outward currents (f), the polarizations are seen to be larger than with inward currents. Finally, after longer and longer lags at negative values (f) the P.D. remains again negative after outward flows (g).

Sensitivity about 13 mv. per division, zero and 100 mv. + and − being marked on each record (derived from calibrations not shown). Time marks 1 second apart. Residual current (r) when P.D. is negative, about 0.1 μA/cm.² (inward flow); when P.D. is positive, about 0.5 μA/cm.² (outward flow). Marks as in f and g, Fig. 1.
The interpretation of this finding appears to be that a polarization capacity is responsible for the counter E.M.F.'s, since the charging time constant of a static capacity should be markedly increased by such increase of series resistance, while that of polarization capacities is independent of it.6

Experimental Production of Regular Polarization

It has been seen that regular polarization may be produced by (a) time, (b) the flow of sufficient inward current to condition the protoplasm. It remained to determine whether other treatments might be effective. A hint is given in Fig. 10, where acid sea water was found to exaggerate the normal regular characteristics to the extent of inducing greater positivity. Fig. 12 shows the effect of exposing a cell in the delayed state to sea water made slightly acid (pH 6.0) with HCl. As the record proceeds, the characteristics of delayed polarization pass over into those of regular polarization, the counter E.M.F.'s becoming larger and prompter for smaller currents, until finally the exaggerated positivity and reversed polarizability of Fig. 12 e result after an hour's exposure.

These effects continue for some time after restoration of the cells to normal sea water, or even at higher pH, which suggests that internal, not external acidity is responsible for the maintenance of regular polarization. An attempt was therefore made to produce regular polarization by the application of various weak acids, such as acetic, butyric, etc., which are well known to penetrate cells readily. These were, however, scarcely more effective than HCl itself at the same pH, which suggests that it is the slow penetration of H ion (or possibly of CO2 released from the carbonates of the sea water), which produces the effect. This point, however, merits further study, in view of the extraordinary effects of certain phenolic compounds (phenol, cresol, guaiacol), applied at Dr. Osterhout's suggestion. In low concentration (below 0.01 M) they promptly cause the P.D. to become positive, as in Fig. 13, and simultaneously induce a nearly regular polarization. These are, of course, extremely weak acids, and were found to reduce the pH of sea water and sap scarcely at all. Yet their effects persisted

Fig. 10

EFFECTS OF CURRENT FLO
on replacing the cells in sea water, even of pH 10.3, for considerable
periods, ammonia alone, as in Fig. 13, restoring delayed polarizability.
Some specific chemical alteration is suggested.

(In these concentrations p-cresol is scarcely toxic, and the effects
may be produced in the same cell on several successive days without
apparent injury.)

**Effects of Ammonia**

The application of ammonia was suggested by its very striking
effects upon the P.D. of Halicystis, both alone and in combination with
current flow. In effect, it turns that genus into a "Valonia" elec-
trically, reversing its normal positive P.D. to negative values. Con-
versely, therefore, it seemed likely that it would restore negativity
to Valonia when that organism had been made temporarily a "Hali-
cystis," with positive P.D. This was indeed found to be the case; it
promptly restored negativity, and with it delayed polarization, in cells

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1 Report in preparation.
which had been rendered regular by p-cresol (Fig. 13), by acid (not shown), or had attained that state spontaneously (Fig. 14). It also emphasizes the delayed state, reducing polarizations at sub-threshold densities, increasing the threshold value, and exaggerating the sudden, all or none rise of positive P.D., making the sigmoid curve almost rectangular in abruptness as shown in Fig. 14. Furthermore, there is practically no persistence of the conditioning effect after cessation of inward current, polarizations to outward currents being practically abolished. On the other hand, during the actual flow of inward current, regular polarizations to small increments or decrements of that current are found in the middle range of densities (Fig. 14) much as before; the inward current, during its flow, is able to counteract the ammonia effect.

The concentrations of ammonia which are effective in abolishing regular polarization, and emphasizing the characteristics of delayed polarization, vary somewhat from cell to cell, apparently depending upon internal factors governing the permanence and extent of establishment of the regular state. Usually, however, about 0.005 M NH₄Cl in sea water of pH 8.0 was necessary to counteract regular responses in cells of V. macrophysa which had thoroughly attained that

FIG. 11. The effect of series resistance upon the time relations of current flow. A cell of V. macrophysa in the constant state, displaying immediate and proportional counter E.M.F.’s, was measured first with the capillary resistance (20,000 ohms) just balanced in the adjacent bridge arm, then with additional resistances introduced into each arm, bringing the total resistances in series with the cell respectively to 40,000 ohms in Record a, 100,000 ohms in Records b and c, and to 200,000 ohms in d. The time course of the counter E.M.F., in speed of both rise and fall, is found to be scarcely altered. This indicates that a polarization capacity, known to have a time constant independent of series resistance, is involved rather than a static capacity, which should have its time constant increased 5-fold by the 5-fold increase of resistance.

The tendency for recessions to begin slightly earlier with outward than with inward currents is evident, as well as an increasing tendency for the P.D. to remain at positive values. Both are characteristic of the constant state.

Currents indicated in μA/cm², as in previous figures, upward arrows signifying inward currents, downward arrows outward currents. Sensitivity is about 15 mv. per division, zero and 100 mv. + and − being indicated on each record, with calibrations on each. Residual current (not marked by r in this figure) depending on the resistances employed, but not over 0.2 μA/cm². Time marks 1 second apart. Marks as in f and g, Fig. 1.
state, 0.001 M and 0.002 M being in several cases ineffective over exposures of an hour or longer. (This value becomes reduced with increased pH of sea water, the effect apparently being dependent, as in Halicystis, upon undissociated NH₃ or NH₄OH.)

This experiment was not tried with V. ventricosa, but based upon our observations a much smaller concentration would have been effective, if indeed the cells stood the treatment at all, since they usually break up into thousands of tiny cells (possibly this gives a hint as to the action of ammonia in incipient cases). V. macrophysa is, however, much more tolerant of ammonia, as witnessed by the long life and extremely healthy appearance of cells exposed for several months to 0.005 M NH₄Cl in sea water. These same cells, when tested electrically by us, displayed a high resistance of regular type, so that if the deconditioning had been produced by ammonia at all, it had evidently passed off again, and the cells had returned to regular polarizability. This is perhaps correlated with the fall of pH which occurred in their sap after a temporary rise due to entrance of ammonia and may be due to compensatory acid production by the cells (cf. Halicystis also).

**The Effects of Potassium**

Since KCl is present in high concentration (0.5 to 0.6 M) in the sap of Valonia, it was desirable to inquire whether the directional effects

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of current flow might be ascribed to the movement of potassium ions outward by outward flows, and inward by inward currents, enriching the protoplasm in the former case, and depleting it in the latter, of an ion assumed to have a high mobility therein.\textsuperscript{10} The resistance and polarization would be expected to fall in the former case and to rise in the latter; while the persistence of polarization after inward flows (conditioning) might reflect the temporary depletion of potassium ions in the protoplasm, to be later regained; slowly by diffusion, or rapidly by flow of current outward from the sap. Similarly, the appearance of delayed polarization after impalement might be due to an injury which liberated KCl into the protoplasm from the vacuole, to be depleted again only by sufficient inward current (at the "threshold") to overcome the outward diffusion. Spontaneous recovery of regular polarization would on the other hand represent the loss of potassium from the protoplasm, either by diffusion outward or by the normal accumulatory processes of the cell.

FIG. 13. The production of regular polarization by \textit{p}-cresol, and its inhibition by ammonia. Records \textit{a} and \textit{b} show typical, completely delayed polarization state in \textit{V. macrophysa}, impaled a few days. There is no counter E.M.F. developed with outward currents up to 50 $\mu$A/cm$^2$ and the threshold for inward currents is at 30 $\mu$A, above which larger currents again produce small additional effect, while smaller densities maintain polarization down to 20 $\mu$A, and even slightly at 10 $\mu$A, with a temporary response to 10 $\mu$A outward current. The cell was then placed in a dilute solution (0.01M) of \textit{p}-cresol in sea water. The P.D. immediately became about 50 mv. positive, as shown in Record \textit{c}, and polarizations began to appear to small currents in either direction, although still larger with inward than with outward currents. This condition persisted when the cell was replaced in ordinary sea water, although the P.D. again became slightly negative; Record \textit{d} was taken 10 minutes after restoration of sea water. Only when ammonia was added, as in Record \textit{e}, did typical delayed polarization reappear.

Currents are in $\mu$A/cm$^2$; outward currents being designated by downward arrows, inward currents by upward arrows, the duration of currents being indicated, where not made obvious by the counter E.M.F., by bars above the figures. Sensitivity about 12.5 mv. per division, zero and 100 and 200 mv. values being placed on each record, as derived from calibrations on \textit{a} and \textit{e}. Time marks 1 second apart. Residual current ($r$) about 0.1 $\mu$A/cm$^2$ (inward) when P.D. is negative, 0.5 $\mu$A/cm$^2$ (outward) when positive. Marks as in \textit{f} and \textit{g}, Fig. 1.

\textsuperscript{10} Damon, E. B., \textit{J. Gen. Physiol.}, 1932–33, \textbf{16}, 375.
This hypothesis has been tentatively suggested in previous papers,\textsuperscript{1,2} and has been made by Osterhout the basis for a theory of the action current in \textit{Nitella}.\textsuperscript{11}

There appears, however, to be little basis for it in \textit{Valonia}. Not only may the polarizability be controlled by quite other factors than potassium, \textit{e.g.} ammonia, acids, \textit{p}-cresol but the experimental modification of potassium concentrations seems to have little effect upon the polarizability, striking though the effects upon E.M.F. may be. Unfortunately it has not yet proved possible to perfuse the vacuole of \textit{Valonia} with new solutions,\textsuperscript{12} which might replace the normal KCl of the sap. But modification of the external solution is readily accomplished, the cells living well for some time in "potassium sea water" (van’t Hoff artificial sea water with KCl entirely substituted for NaCl). The results are shown in Fig. 15, where the polarizability of a cell in

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\textbf{FIG. 14.} Inhibition of regular polarization and production of delayed polarization in \textit{V. macrophysa} by ammonia. A cell in the constant state, displaying very regular and proportional counter E.M.F.'s for low currents in either direction is shown in Records \textit{a} and \textit{b}. It was then placed for a few minutes in sea water of pH 8.0 containing 0.005 M NH\textsubscript{4}Cl. Record \textit{c} ensued; there was very little polarization to inward currents up to 20 \textmu{A}/cm.\textsuperscript{2} at which time a very abrupt sigmoid reversal curve was produced; 25 \textmu{A} (and higher currents) produced only temporarily higher effects, the positive \textit{E}.M.F. receding to about 180 mv. after a cusp. Outward currents produce practically no counter E.M.F., as shown in \textit{c}, even though following closely on inward flows; the conditioning effect is evidently short-lived as shown also by the very sudden depolarization curves. In \textit{d} the threshold to inward flow has fallen to 15 \textmu{A}, but with polarization occurring only after a very long lag. In \textit{e} the effects of equal 5 \textmu{A} increments from 10 to 25 \textmu{A}, and then 5 \textmu{A} decrements down to residual flow (\textit{r}) are shown.

The cell was then replaced in ordinary sea water without ammonia, and Records \textit{f} and \textit{g} were taken after 10 minutes; the effects of ammonia are still present, there being little or no polarization to small currents, and a threshold for inward flow at first 25 \textmu{A}, later 15, just as during ammonia treatment. Only after several hours did regular polarizability return. (It can be restored much more rapidly by acidified sea water.)

Sensitivity about 12 mv. per division, with 100 and 200 mv. values being indicated on each record (calibration on \textit{f}). Time marks 1 second apart. Residual current (\textit{r}) about 0.2 \textmu{A}/cm.\textsuperscript{2} (inward). Marks as in \textit{f} and \textit{g}, Fig. 1.


the transition state is scarcely distinguishable from that in ordinary sea water, despite the fact that potassium ions must now be moved from the outer solution into the protoplasm by inward currents as rapidly as they are moved from it into the vacuole. Depletion of these ions by inward flow can thus scarcely account for the polarizations produced.

Similar indifference of the protoplasm in its regular state is displayed to potassium sea water, polarizations being as large and as prompt as in normal sea water, showing that KCl does not immediately enter the protoplasm and produce the delayed state; and that the resistance to this salt remains about as high as to any other.

Practically the only difference sometimes observable is a rather more abrupt rise of positive p.d. at the threshold or above, somewhat evident in Fig. 15. This corresponds to the ammonia effect (Fig. 14), although it is much less pronounced. It would indeed be expected if potassium entered as KOH, corresponding to the entrance of NH₃ or NH₄OH. Its entrance in this fashion, however, is evidently so slow as to produce little effect upon the threshold, etc.

(These statements hold for exposures of the cells to potassium-rich solutions for several hours. Exposures of a day or longer are eventually effective in that the cells display greatly reduced polarization. While this might be regarded as evidence that potassium ions have at last begun to permeate the protoplasm, and to produce low polarization by their high mobility, the magnitude of times concerned in this effect is quite different from those met with in the polarization phe-

Fig. 15. Polarization in the presence of high potassium concentration. Shortly before the start of Record a, V. macrophysa had been placed in potassium sea water (artificial sea water according to van't Hoff, but with KCl substituted entirely for NaCl). The p.d. had become, as usual, more negative. Good polarizations persisted with currents in either direction, although somewhat larger with inward currents (Record b). After 20 minutes exposure to this solution, during which the p.d. fell somewhat, even larger polarizations were obtained, as shown on Records c and d. The very rapid rise and fall of polarization curves are characteristic of this condition.

Sensitivity about 16 mv. per division. Zero and 100 mv. + and — are marked on each record, with calibrations on a and b. Time marks 1 second apart. Currents, in or out, as marked, in μa/cm.² of cell surface. Residual current (r) about 0.2 μa/cm.² (inward) in Record a, falling to about 0.08 μa/cm.² in d.
nomena that it aims to explain, and it seems to be due rather to a profound injury\textsuperscript{13} from which there is no recovery on restoration to sea water.)

DISCUSSION

Since the movement of potassium ions cannot apparently account for the phenomena of delayed polarization in \textit{Valonia}, to what may the effects of inward current flow be attributed? It seems reasonable to assume that regular polarizations are due to the maintenance of an intact surface, across which ionic mobilities, partition coefficients, etc., govern the production of counter E.M.F. This surface may apparently be profoundly altered in some way, so that polarizations disappear, without killing or seriously injuring the cell, since it lives for some time in the state of delayed polarization. In this state there is apparently free ionic transfer across the surface, the effective resistance of the protoplasm being practically nil, and its P.D. low and negative. This remains true for large outward currents, but sufficient inward current suddenly restores polarizability, along with a strongly positive P.D. Since polarizability persists for a while thereafter to smaller currents, we may assume that the current flow has actually restored a surface of the protoplasm, or at least those of its properties governing polarization.

Furthermore, since this effect of inward current is duplicated by certain chemical treatments such as acidified sea water and various phenolic acids, which produce a positive P.D. and restore polarizability, the conclusion is suggested that inward current produces its "restorative" or conditioning effects by an increase of acidity, or an acid-like action, upon some structure of the protoplasm. Conversely since both outward currents and penetrating bases like ammonia tend to destroy polarizability and produce a negative P.D., we may postulate that they alter the surface (or its electrical properties) by an increase of alkalinity at some critical point. A possible mechanism would be a saponifying action upon lipoid constituents by bases or outward currents, counteracted by esterification and reformation of an oily or fatty acid film under the influence of acidic substances or of inward currents.

It is both theoretically possible (through the high mobility of H ion)

\textsuperscript{13} Osterhout, W. J. V., \textit{J. Gen. Physiol.}, 1924–25, 7, 561.
and experimentally demonstrated\textsuperscript{14} that current flow can produce such acidity changes (membrane electrolysis) at various interfaces such as gels and membranes. While the evidence upon which similar effects were postulated in living cells appears to have been erroneous,\textsuperscript{15} it is still quite conceivable that it occurs, even though not so visibly as claimed. We are strongly inclined to think that it must occur, on the basis of the \textit{Valonia} phenomena here described, and on even better evidence (additive effects of ammonia and current flow) in \textit{Halicystis}.

It will be discussed further elsewhere. However, the ascription of common causes to common effects is dangerous, and it may only be that various agents can act upon the same function (polarizability) in an all or none manner, both outward currents and weak bases destroying it; both inward currents and weak acids restoring it, but each acting through a different mechanism.

\textit{Comparison with Ag-AgCl Electrodes}

Although they have certainly nothing but a formal resemblance to the curves obtained with \textit{Valonia}, the accompanying string galvanometer records (Fig. 16) of current flow with a silver-silver chloride electrode of small area may be suggestive of what happens when a reversible ("non-polarizable") electrode becomes irreversible or polarizing by the passage of large currents (here reducing the AgCl to Ag). The slight polarization to small "inward" currents (electrode negative) followed by a delayed polarization with sigmoid curve at the threshold density; the relatively quicker polarizations with succeeding current flows and larger densities; and the restoration of non-polarizability by outward flow (electrode positive), are all reminiscent of the \textit{Valonia} records.

Grateful acknowledgment is made to the Carnegie Institution of Washington for opportunities to study at its Dry Tortugas Laboratory.

SUMMARY

The effect of direct current flow upon the potential difference across the protoplasm of impaled \textit{Valonia} cells was studied. Current density


\textsuperscript{15} Blinks, L. R., \textit{Proc. Soc. Exp. Biol. and Med.}, 1932, 39, 1186. These qualitative observations of the author have been verified by Mr. R. D. Rhodes in the Stanford laboratories by objective photographic and spectrographic records which are being prepared for publication.
Fig. 16. Duplication of "delayed polarization" effects with electrode model. Two Ag-AgCl electrodes, one of small and one of large area were subjected to current flow in the bridge circuit used for Valonia. The large electrode had sufficient surface to remain non-polarizable with all current densities employed; the smaller was non-polarizable to lower current densities, but became polarized with a sigmoid curve of counter e.m.f. at a critical threshold density of inward current (electrode negative, attracting positive current). Long slow depolarizations follow cessation of current flow, with a tendency for the electrode to retain a positive charge. Passage of outward current, with the electrode made positive, then rapidly reduced this charge and made the electrode again non-polarizable (by deposition of AgCl), both to continued outward current, and again to inward current of low density. This resembles the conditioning and deconditioning in Valonia by inward and outward currents.

The actual sensitivities were not recorded, nor the current densities. The figures stand for applied potential in volts positive or negative, no current passing when 0 is indicated. Time marks 1 second apart.
and direction were controlled in a bridge which balanced the ohmic resistances, leaving the changes (increase, decrease, or reversal) of the small, normally negative, bioelectric potential to be recorded continuously, before, during, and after current flow, with a string galvanometer connected into a vacuum tube detector circuit.

Two chief states of response were distinguished:

State A.—Regular polarization, which begins to build up the instant current starts to flow, the counter E.M.F. increasing most rapidly at that moment, then more and more slowly, and finally reaching a constant value within 1 second or less. The magnitude of counter E.M.F. is proportional to the current density with small currents flowing in either direction across the protoplasm, but falls off at higher density, giving a cusp with recession to lower values; this recession occurs with slightly lower currents outward than inward. Otherwise the curves are much the same for inward and outward currents, for different densities, for charge and discharge, and for successive current flows. There is a slight tendency for the bioelectric potential to become temporarily positive following these current flows.

Records in the regular state (State A) show very little effect of increased series resistance on the time constant of counter E.M.F. This seems to indicate that a polarization rather than a static capacity is involved.

State B.—Delayed and non-proportional polarization, in which there is no counter E.M.F. developed with small currents in either direction across the protoplasm, nor with very large outward currents. But with inward currents a threshold density is reached at which a counter E.M.F. rather suddenly develops, with a sigmoid curve rising to high positive values (200 mV. or more). There is sometimes a cusp, after which the P.D. remains strongly positive as long as the current flows. It falls off again to negative values on cessation of current flow, more rapidly after short flows, more slowly after longer ones. The curves of charge are usually quite different in shape from those of discharge. Successive current flows of threshold density in rapid succession produce quicker and quicker polarizations, the inflection of the curve often becoming smoothed away. After long interruptions, however, the sigmoid curve reappears. Larger inward currents produce relatively little additional positive P.D.; smaller ones on the other hand,
if following soon after, have a greatly increased effectiveness, the threshold for polarization falling considerably. The effect dies away, however, with very small inward currents, even as they continue to flow. Over a medium range of densities, small increments or decrements of continuing inward current produce almost as regular polarizations as in State A.

Temporary polarization occurs with outward currents following soon after the threshold inward currents, but the very flow of outward current tends to destroy this, and to decondition the protoplasm, again raising the threshold, for succeeding inward flows.

State A is characteristic of a few freshly gathered cells and of most of those which have recovered from injuries of collecting, cleaning, and separating. It persists a short time after such cells are impaled, but usually changes over to State B for a considerable period thereafter.

Eventually there is a reappearance of regular polarization; in the transition there is a marked tendency for positive P.D. to be produced after current flow, and during this the polarizations to outward currents may become much larger than those to inward currents. In this it resembles the effects of acidified sea water, and of certain phenolic compounds, e.g. p-cresol, which produce State A in cells previously in State B. Ammonia on the other hand counteracts these effects, producing delayed polarization to an exaggerated extent.

Large polarizations persist when the cells are exposed to potassium-rich solutions, showing it is not the motion of potassium ions (e.g. from the sap) which accounts for the loss or restoration of polarization.

It is suggested that inward currents restore a protoplasmic surface responsible for polarization by increasing acidity, while outward currents alter it by increasing alkalinity. Possibly this is by esterification or saponification respectively of a fatty film.

For comparison, records of delayed polarization in silver-silver chloride electrodes are included.