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PROEFSCHRIFT

TER VERKRIJGING VAN DEN GRAAD VAN DOCTOR IN DE WIS- EN NATUURKUNDE AAN DE RIJKS-UNIVERSITEIT TE UTRECHT, OP GEZAG VAN DEN RECTOR-MAGNIFICUS Dr. TH. M. VAN LEEUWEN, HOOGLEERAAR IN DE FACULTEIT DER GENEESKUNDE, VOLGENS BESLUIT VAN DEN SENAAT DER UNIVERSITEIT TEGEN DE BEDENKINGEN VAN DE FACULTEIT DER WIS- EN NATUURKUNDE TE VERDEDIGEN OP MAANDAG 20 MAART 1939, DES NAMIDDAGS TE 4 UUR

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ELECTRIC CONTROL OF POLARITY IN PLANTS

by

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CONTENTS

INTRODUCTION.

The aim of this research was to investigate, to which extent the problem of polarity can be elucidated by studying the variations of bio-electric potentials during regeneration processes.

To that purpose we had in the first place to try to get clear on the source or sources of bio-electric P.D.'s in plants. Opinions concerning their origin are rather divergent. In literature these potentials are indicated as redox-potentials as well as concentration-potentials while other, less defined possibilities have been discussed too. This divergency of views may be caused partly by contradictory experimental results, partly by the use of different electrode systems.

The first part of this paper only will deal with the nature of the potentials. Not until their principles are known it will be possible to valuate the importance of the bio-electricity with regard to plant physiology.

The next mootpoint is, whether the P.D. is cause or effect of the vital processes concerned. With this we touch the question of electrophoresis. Are the P.D.'s large enough to cause a migration of electrolytes? In that case the possibility of their formative influence might not be ruled out. If, however, the potentials only are by-products of the vital processes, they merely can be considered as indicators of functions and conditions of the cells.

We know two kinds of potentials: action currents (better action potentials) and rest potentials. The potentials studied here are rest potentials.

The problem of polarity has been studied in several manners. So has been controlled: the influence of gravity, centrifugal force, light, humidity, assimilation, transport of assimilates, auxin and so on. It seems interesting to look after a possible relation between the regeneration processes and variations of the P.D. If this connection exists it might be possible to reveal something more about the internal alterations of the cells during the period of regeneration.

So we can state the problem as follows:

1. Which are the sources of the P.D. in plants?
2. Does a correlation exist between regeneration and P.D.? If so, is the P.D. cause or effect?
3. What can be concluded on the processes determining polarity?

PART I.

REVIEW OF LITERATURE ON PLANT ELECTRICITY.

The problem of bio-electric phenomena was studied already in the end of the past century. In confining ourselves to the most important papers of this period, we cite first Kunkel's research (1878). This author was of opinion that the water transport must be considered as the cause of plant electricity. Haake (1892), however, showed that alterations in transpiration are not correlated with variations of the electric currents. By changing the intensity of transpiration, fluctuations in the current occurred indeed, but no conformity could be obtained as to the sense or as to the magnitude of the variations of both phenomena. Rejecting Kunkel's view on plant-electricity as capillar-electricity Haake considered respiration and assimilation the source of the currents. Increase of the O_2-pressure caused increase of the currents *independently of its direction.* In 100 % N_2 the amperage did not decrease down to zero. This was explained as a result of intramolecular respiration. Influence of CO_2-pressure could not be detected satisfactorily, but by darkening the objects, the amperage was diminished. So Haake concluded that assimilation might play a part as well as respiration. It should be mentioned here that Haake was measuring currents. His instruments did not enable him to measure potentials.

More than 30 years passed until a more detailed research was set up. Waller (1925, 1929) studied the effect of light and CO_2. From the fact that illumination of a chlorophyll containing part (leaf) causes a photoelectric reaction Waller considered this reaction quantitatively related to the CO_2-percentage of the surrounding air, and concluded that the currents were a product of the oxidation and reduction of a hypothetical acid. It is not clear why the effect was only short-lasting. As a rule exposure to light caused a positive wave, darkening a negative one but the reverse occurred also. So it seems to be a more complicated phenomenon.

The relation between metabolism and bio-electric currents has been studied profoundly by Lund and his co-workers. In a series of five papers (Lund and Kenyon 1927, Lund 1928a and b, Marsh 1928, Lund 1928c) it was stated that the electric polarity shows a quantitative relation to the metabolism

of the cells. The parts in the roots of *Allium Cepa* which show the highest P.D.'s also are the parts of the most intensive methylen blue reduction and discoloration of phenol red. KCN and ether reduce the respiration and with it the potential differences.

According to Lund, the bio-electric potentials are due to "the flux equilibrium of the oxidation-reduction systems". With flux equilibrium is meant the dynamic balance between oxidation and reduction and so the P.D. is fluctuating continuously.

The P.D. of a tissue is considered as the algebraic sum of the P.D.'s of the individual cells, which are connected to each other partly in series, partly parallel. This is stated once more by Lund and Bush (1930) and by Miss Rosene (1935).

Rosene and Lund (1935) suggest that the electric, functional and morphological polarities are coupled. In the roots of *Allium Cepa* the maxima of P.D., O_2-consumption, methylen blue reduction, concentration of sulphydrilcomponents, CO_2-production and growth (undifferentiated tissue) are found to coincide. The authors emphasize that the metabolism is not the only factor to which potentials are due in cells, "but it (the flux equilibrium) is the only type thus far considered in the literature of electro-physiology which can mantain in a direct manner a continuous output of electric energy".

The theory of Lund is supported by many experiments by Marsh. According to Marsh (1930a) the recovery curves after an applied current are due to a redox system. Ether and KCN reduce the potential of *Valonia.* In a discussion Marsh (1930b) agrees with the idea of Lund (1927) that the recovery cannot be explained clearly by assuming that the permeability of the membranes, increased by a stimulus, should be restored to its normal value by metabolism. The hypothesis of alterations of permeability is based on changes found in the resistance of the membranes. It is possible, however, that the decrease of the resistance might be due to a counter-E.M.F. If this were true the resistance cannot be considered to be a measure for the permeability. So Marsh concludes once more to the flux equilibrium theory of Lund.

The formula of Lund (1931) expressing the electric polarity of a cell:

$$E_p = -\frac{RT}{2F} \ln \frac{Pc_1}{Pc_2},$$

Pc being a function of the O_2-pressure, is treated mathematically by Marsh (1935, 1937) and is shown to be in accordance with the experimental facts.

The objection has been made that Lund and his co-workers

did not use noble metal electrodes. So a real redox potential cannot be measured. Lund, however, accepts certain "structures (or interfaces)" in the protoplasm bearing the properties of noble metal.

Marsh also studied the effect of CO_2 (1935—'36) and of light (1936-'37) on the potentials of *Valonia*. These results match those of Lund.

Ponder and Macleod (1937), however, could inhibit the respiration of the frog skin by certain carbamates and lysins without changing its P.D. According to the authors, this needs not necessarily contradict the hypothesis of Lund.

Another argument for the bio-chemical character of the source of the P.D. is furnished by experiments on the influence of the temperature on it. Marsh (1934-'35, 1936) showed that the Q_{10} at low temperatures amounts to about 4 and at high temperatures to about 2. It seems difficult to me, however, to draw conclusions from the experiments, the results being rather divergent. As can be seen from his curves, in many cases no change at all occurred (the "undershooting" and "overshooting" effect excepted).

Umrath (1934) studied the effect of temperature on inherent (= rest) potentials and on action currents. The increasing phase of the latter lasted 2—3 times longer at a sinking of the temperature of 10° C. The Q_{10} of the inherent potential also is an indication that this potential too depends on a chemical process. Umrath (1933) interpretes the P.D. as follows: "Im Plasmalemma sind oberflächenaktive Molekeln orientiert eingelagert, welche durch eine elektrische Polarität das Plasmalemmapotential bedingen, beziehungsweise einen Beitrag zu demselben liefern". With reference to the effect of temperature Umrath (1934) specified: "Es liegt nahe, anzunehmen, dass die Menge der orientiert in das Plasmalemma eingelagerten Molekeln von Stoffwechselvorgängen abhängt, wodurch die beobachtete Temperaturabhängigkeit des Potentials verständlich würde".

The rate of conduction of action currents in *Mimosa* is increased also two to three times by rising the temperature 10° C. (Umrath 1937).

Quite a different hypothesis is formulated by Osterhout (1928a). The protoplasm would consist of three layers: an outer one (X), an inner one (Y) and between them the rest of the protoplasm (W). X and Y are semi-permeable membranes. X is in contact with the cell-wall, while Y is surrounding the vacuole. So this system may give rise to diffusion potentials, boundary potentials and Donnan-potentials. From a calculation Osterhout (1930a) concluded the diffusion potentials to predominate.

As in general a more concentrated solution is negative to a more diluted one, the conclusion seems justified that the membranes are impermeable for anions (Osterhout and Harris 1930). Umrath (1934a) described the formation of such a membrane on the surface of his electrodes after insertion in the protoplasm of *Spirogyra* and *Vaucheria*.

There are exceptions, however. Damon and Osterhout (1930)showed the more diluted solution to be negative in *Valonia*. Amberson and Klein (1928) found the sign of the concentration potentials across the skin of the frog depending on the p_H. At the alcaline side of the iso-electric point of the membrane the dilute solution is positive, at the acid side it is negative.

As to the nature of the protoplasmic surfaces Osterhout concluded (for a survey see Osterhout 1936):

They behave as a liquid. In plasmolysis and after removal of the cell-wall the protoplasm rounds up "like an oily liquid".

They are non-aqueous. This is concluded from the fact that electrolytes enter the protoplasm as molecules chiefly. (Jacques and Osterhout 1930, Osterhout 1933, Jacques 1936).

They are layers more than one or two molecules thick. Bases seem to combine chemically with certain constituents of the layers. (Osterhout 1933, 1937, Kraus 1934, Keller 1936). Furthermore it seems doubtful whether a thickness of one or two molecules only suffices to cause the very slow penetration of many substances (Jacques 1936-'37).

They are not homogeneous. The reversible loss of the potassium effect in distilled water is used as an argument. With potassium effect is meant the decrease of P.D. when NaCl is substituted by KCl. If, however, cells are placed in distilled water for some days this effect vanishes. This fact is explained by assuming that a certain organic compound (or compounds), R, which sensibilizes the layers for K^+, diffuses away. It is possible to restore the potassium effect by placing the cells in a concentrated solution of these diffusable substances (Osterhout and Hill 1933-'34, Hill and Osterhout 1938 and 1938a). So in normal circumstances the membranes would consist of at least two compounds.

The sign and the magnitude of the P.D. (when considered as a diffusion potential) would be determined by selective permeability. On its turn the selective permeability would be determined by diffusion constants and concentration gradients which depend upon paritition coefficients. As diffusion constants and partition coefficients vary with alterations of temperature, concentration, the presence of electrolytes, other substances and additional factors, the P.D. varies too.

The temperature coefficient for penetration of substances proves to be high in many cases. For dyes the Q_{10} amounts to 4 or more (Irwin 1925-'26, 1926-'27) while for bromide has been found $Q_{10} = 2 - 3$ (Hoagland, Hibbard and Davis 1926-27).

Organic substances can affect the P.D. to a great extent. This is shown, for example, for guaiacol (Osterhout 1936a, 1936-'37, 1938a). So it is possible that metabolites in the protoplasmic surface influence the P.D. The relation between the O_2 % and P.D. is not a direct one. As to this Osterhout (1936b) remarks: "Redox potentials do not enter into the picture since no metallic electrodes come in contact with the living cell (but oxidation and reduction may affect the P.D. by changing the organic composition of the protoplasmic surface or the concentration of ions in contact with it). Redox potentials would not change with change of KCl concentrations as does the living cell".

Blinks, Dersie Jr., and Skow (1938-'39) suppose the sensibilizing substance R of Osterhout to be a product of respiration. So O_2 influences the P.D. via the amount of this metabolite.

The membranes X and Y are unlike to each other. This appears when a cell is placed in vacuolar sap one electrode being applied outside while the other one is pierced into the vacuole. So we have a chain: sap | X | W | Y | sap. The fact that a P.D. results indicates that the chain is not symmetric (Osterhout and Harris 1928, Osterhout, Damon and Jacques 1928). Damon (1930) too concluded on a dissimilarity of the membranes from the shape of the action current in *Valonia*.

The P.D.'s in X and Y can vary independently of each other (Osterhout and Hill 1935).

Osterhout and co-workers used single cells in their experiments. The objects were *Valonia*, *Halicystis* and *Nitella*. Of course it is of great interest to study the bio-electric phenomena in large single cells and not only in more complicated tissues. Since this paper, however, deals with higher plants, only the results which seem to be of general significance are quoted here.

Blinks studied the nature of the membranes by measuring the resistance of single cells. The living protoplasm offers a very high resistance (in *Nitella* up to $3\frac{1}{2}$ megohm). An electric stimulus reduces it temporarily (Blinks 1930). This is supposed to be due to alteration of the permeability of the membranes. Gicklhorn and Dedjar (1931), however, became negative results on *Spirogyra*. Suolathi (1937) also could not find any influence of an electric current on the permeability of *Chara*.

Blinks (1935-'36a, b, c) showed that the resistance largely is due to the development of a counter-E.M.F. caused by the flow of the applied current. The ohmic resistance only plays a subordinate role. This "polarization" is found at *Valonia*, *Halicystis* and *Nitella*. So Blinks concluded that the protoplasmic surfaces may exist of ions (probably of fatty acids) which can be transported electrophoretically.

The capacity of the protoplasm might be based on two principles. It may be caused by a (lipoid) film with none or little (in the latter case an equal) permeability to anions and cations. Secondly it may be caused by one or more boundaries through which one kind of ions can pass faster than the other. Probably both exist at the same time; the second case, however, is dominant (Blinks 1936).

Brauner (1927, 1928) described the geo-electric effect. The lower side of a membrane always is positive to the upper side. It is the result of the migration of the cations under influence of gravity, while the anions are adsorbed to the membrane to a high degree. This effect, however, has nothing to do with vital processes.

Recapitulating one can state that the bio-electric potentials are influenced by oxygen pressure, KCN, ether and chloroform and furthermore by the differences in concentration of electrolytes and certain organic substances at the inner and outer surface of the protoplasm.

According to Lund the P.D.'s are redox potentials, according to Osterhout diffusion potentials.

The arguments of Lund are the relation between P.D. and respiration, the decrease of the potentials by KCN, ether and chloroform. The Q_{10} indicates the chemical nature of the cause of the P.D.

The arguments of Osterhout are based on the influence of salts and some other substances on the potentials. A calculation points out that the potentials can be considered as diffusion potentials. Moreover it is possible to explain the shape of action currents as the result of diffusion through the membranes X and Y separately. The temperature coefficient is high, but this does not necessarily indicate a chemical reaction, though this seems to occur in some cases. The influence of the oxygen tension does not indicate a redox potential because no noble metal electrodes were used and because of the salt effects. The fluctuations are due to the quantitative or (and) qualitative variations of metabolites in the protoplasm, by which the diffusion constants and the partition coefficients of the electrolytes can be affected.

METHODS.

The potentials are measured with the aid of a potentiometer with a valve amplifier (P h i l i p s 4060). The diagram is given in fig. 1. The apparatus was built according to E l e m a (1932) as far as the plate circuit is concerned. The grid circuit was arranged as described by K o r d a t z k y (1934 p. 126). In this way the advantages of an easy and exact control of the plate current on the one side and a minimal risk of leakage currents on the other side were obtained.

As one can see in the diagram the measurements were done according to the compensation method. The unknown potential of

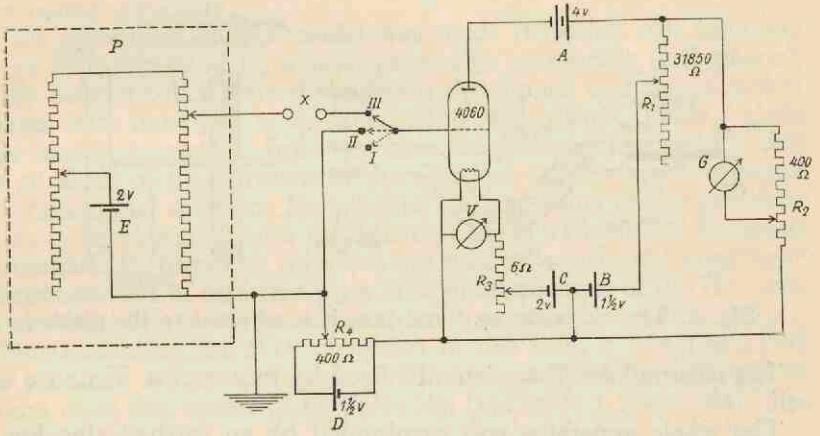


Fig. 1. Diagram of the potentiometer. For explanation see text.

the object (X) was taken up into the grid circuit. This caused a deflection of the galvanometer (H.). Then a counter-E.M.F. to the potential of X was supplied until the galvanometer pointed to zero again. The value of the counter-E.M.F. could be read from the scale of a Cambridge A potentiometer (P, indicated by the part surrounded by the dotted line).

The instrument was adjusted as follows. After connection of the batteries the grid was connected to I. The plate current, regulated by the "free grid" potential caused a deflection of the galvanometer (G). Then this deflection was compensated by regulating the resistance R_1. Now the grid was connected to II. So it loosed the charge when R_4 is short-circuited. The deflection of G caused by this

manipulation was compensated by adjusting R_4. In this way the applied grid potential was exactly the same as the "free grid" potential. So the danger of leakage currents was minimized. Finally the grid was connected to III. The galvanometer deflected again, affected by the E.M.F. of the object. The resistance of the potentiometer (P), which provides the counter-E.M.F. to the potential of the object, was regulated till G pointed to zero again. (The zero-point of G was controlled before each reading). One division on the scale of the potentiometer P indicated 0,2 mv.

The Zernike galvanometer (G) was made aperiodic by shunting it with a resistance of 150 ohm (not shown in the figure). The deflection caused by 1 μA amounted to 12 cm at a distance from scale to mirror of 40 cm.

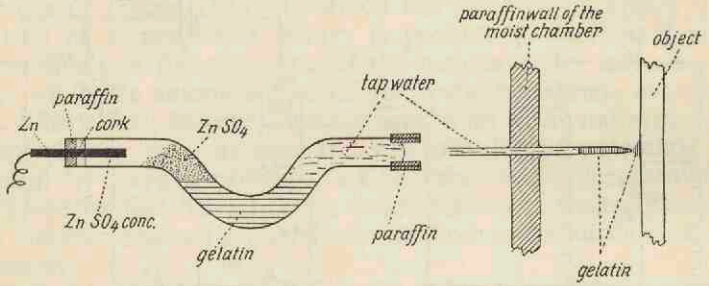


Fig. 2. The electrode used and how it is adjusted to the plant.

The accumulator E was standardized by means of a Weston-cell.

The whole apparatus was surrounded by an earthed zinc box.

The electrodes used have been described already in a previous paper (De Groot Jr. and Thomas 1938). So a brief description will suffice here. The U-shaped part of a glass tube (fig. 2) was filled with a solution of 30 % pure gelatin in tap water. An amalgamated zinc rod was immersed in saturated $ZnSO_4$ solution in redistilled water in one of the horizontal limbs, which was closed by means of paraffin-soaked cork. The other limb was left open and filled with tap water. Its end was paraffined to prevent the water from flowing out. By placing the open end of the electrode over the contact-tube, the contact with the plant is made. The contact-tube existed of a narrow glass tube which was filled with tap water and closed at one end by means of gelatin. This end was fixed to the plant by a drop of gelatin (15 %). By adjusting the contact-tubes to all spots required before the beginning of the

experiment, it was possible to measure the P.D.'s of different points by placing the open end of the electrode over the successive tubes without stimulating the plant.

The objects were placed in moist chambers. As, however, different types of chambers are used they will be described with the experiments on which they refer. If, in some cases, the plants were not placed in the dark, they were illuminated by the light of a 100-watt bulb at a distance of 1 m.

The temperature of the room varied from 20 to 22° C.

It is important to know whether the potential measured with these electrodes is due to the diffusion of all kinds of ions or that the influence of particular ions is predominating. In this respect H^+-ions are meant especially.

To investigate this, a model was made in which two solutions were kept separated by a semi-permeable membrane (cellophane). Two contact-tubes were dipped into the liquids and the measurements were done just as in experiments with plants. Table 1 gives the results. As appears from the table the potential measured is due to all kinds of ions present in the solution. Dilutions as compared to the original solutions are positive and the value of the potential is as to be expected from the equation of N e r n s t for diffusion potentials. If, however, solutions are used of nearly the same concentration but of different p_H a little predominance of the H^+-ions is shown, but it is not permitted to draw any conclusion as to the p_H. Thus measuring the P.D. on plants in this way, it is not justified to conclude on the diffusion of H^+-ions particularly although in some cases this seems to be probable. (see table 1, page 384).

No mean errors were calculated. In this respect I refer to a preceding paper (D e G r o o t J r . and T h o m a s 1938). It has been pointed out there, why it is difficult to determine them: the P.D. is fluctuating continously. However, the fluctuations of the potentials of the objects used here were much less than in *Phaseolus*. We confine ourselves to show this in the next way. Seven contact-tubes (numbered 1 up to 7 including) were fixed along a stem of *Bryophyllum calycinum*. At first was measured the P.D. of 1 (the apical contact-tube) to 7 (the basal one). Then the electrode in contact with 1 was placed over 2, the other electrode resting over 7. Going on in this way the P.D.'s are measured as indicated in the upper row of table II. Furthermore the P.D. at two contact-tubes in succession to each other was determined by moving both electrodes (table II, middle row). Finally the electrode placed over 1 was kept

TABLE I. Diffusion potentials caused by various ions passing though cellophane, measured with $Zn\text{-}ZnSO_4$ electrodes.

Solutions measured to each other.	mv.
tap water to tap water	1,43
HCl 0,1 n to HCl 0,01 n	— 55,20
Phosphate buffer pH = 6,00 to same solution diluted 5×.	— 27,93
Phosphate buffer pH = 6,24 to same solution pH = 7,38	— 8,32
Glycocoll + HCl pH = 1,2 to Glycocoll + NaOH pH = 12,9	— 24,88

at its place, the other one was moved and as a final control 6 to 7 was measured again (lower row).

One reading was made pro minute while the measurements of the different combinations followed each other immediately. So the whole experiment lasted 84 minutes.

It appears from table II that the P.D. of 1 to 7 increased 0,48 mv during 70 minutes, while that of 6 to 7 decreased 0,13 mv during 50 minutes. The data from this table enable to calculate the P.D. between every pair of contact tubes desired and to compare this value with the measured one. Table III shows the accordance between the P.D.'s measured and calculated.

As one sees, the maximal divergence between the computed values and the experimental ones amounts to 1,12 mv (with the contact-tubes 3 and 4). Taking into account, however, the slow natural variations of the potentials the results are most satisfactory.

TABLE II. Control of exactness.

Contact-tubes	1 to 7	2 to 7	3 to 7	4 to 7	5 to 7	6 to 7
Readings in mv.	— 5,30	— 8,18	— 1,95	— 4,53	— 2,51	—20,98
	— 5,29	— 7,77	— 1,88	— 4,59	— 2,32	—21,14
	— 5,03	— 7,98	— 1,96	— 4,56	— 2,42	—21,02
	— 5,17	— 8,06	— 1,95	— 4,60	— 2,41	—21,06
	— 5,30	— 7,92	— 1,88	— 4,53	— 2,47	—20,98
Mean	— 5,22	— 7,98	— 1,92	— 4,56	— 2,43	—21,04

Contact-tubes	5 to 6	4 to 5	3 to 4	2 to 3	1 to 2
Readings in mv.	+ 18,07	— 2,88	+ 1,52	— 7,46	+ 3,26
	+ 18,03	— 2,71	+ 1,34	— 7,34	+ 3,20
	+ 17,93	— 2,90	+ 1,46	— 7,48	+ 3,12
	+ 18,08	— 2,88	+ 1,66	— 7,32	+ 3,04
	+ 18,00	— 2,91	+ 1,64	— 7,40	+ 3,20
Mean	+ 18,02	— 2,86	+ 1,52	— 7,40	+ 3,16

Contact-tubes	1 to 3	1 to 4	1 to 5	1 to 6	1 to 7	6 to 7
Readings in mv.	— 4,04	— 1,62	— 4,14	+ 14,42	— 5,72	—20,91
	— 4,03	— 1,62	— 4,09	+ 14,43	— 5,69	—20,92
	— 4,00	— 1,61	— 4,14	+ 14,43	— 5,69	—20,89
	— 4,12	— 1,60	— 4,18	+ 14,44	— 5,69	—20,87
	— 4,13	— 1,57	— 4,10	+ 14,43	— 5,73	—20,94
Mean	— 4,06	— 1,60	— 4,13	+ 14,43	— 5,70	—20,91

TABLE III. P.D.'s measured and calculated compared to each other. The measured values are data from table II.

Contact-tubes	P. D. figured out in mv.		P.D. measured in mv.
1 to 2	(1 to 7) — (2 to 7)	+ 2,76	+ 3,16
	(1 to 3) — (2 to 3)	+ 3,34	
2 to 3	(2 to 7) — (3 to 7)	— 6,06	— 7,40
	(1 to 3) — (1 to 2)	— 7,22	
3 to 4	(3 to 7) — (4 to 7)	+ 2,64	+ 1,52
	(1 to 4) — (1 to 3)	+ 2,46	
4 to 5	(4 to 7) — (5 to 7)	— 2,13	— 2,86
	(1 to 5) — (1 to 4)	— 2,53	
5 to 6	(5 to 7) — (6 to 7)	+ 18,61	+ 18,02
	(1 to 6) — (1 to 5)	+ 18,56	
6 to 7	(5 to 7) — (5 to 6)	— 20,45	— 21,04 and — 20,91
	(1 to 7) — (1 to 6)	— 20,13	

MATERIAL.

Vicia Faba. The beans were soaked during 24 hours in tap water. Then they were planted in moist saw dust and cultivated at 23° C in a dark room. The roots were used when they had reached a length of 4 to 7 cm.

Coleus hybrida var. Bertha Grosze was cultivated in the greenhouse. In most cases plants were used with a stem of 7 to 10 nodes.

Of *Bryophyllum calycinum* grown likewise in the greenhouse specimens were used with a stem of 10 to 14 nodes.

The isolation, defoliation and adjustment of the objects occurred at least 3 hours before the beginning of the experiment. In long lasting regeneration experiments the gelatin bridges between the contact-tubes and the plant were renewed each 3 days. This was necessary because of the moulding of the gelatin.

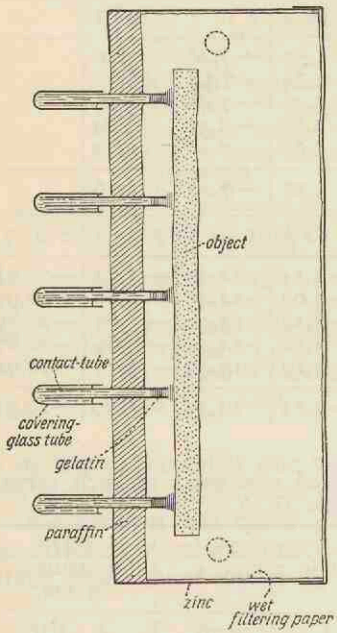


Fig. 3.
Moist chamber used for long-lasting experiments. The dotted circles indicate the ventilation tubes in the front and in the back wall respectively. When not in use, glass tubes, filled with tap water, are placed over the contact-tubes.

EXPERIMENTS.

As was cited in the review of literature L u n d and co-workers consider the bio-electric potentials as redox potentials, while O s t e r h o u t and his school are regarding them as concentration phenomena. So, in the first place, it seemed of interest to repeat the experiments on the influence of narcotica on the P.D.

Influence of ether.

To this purpose pieces of the stem of *Bryophyllum calycinum* and *Coleus hybrida* were defoliated and placed in a moist chamber as shown in fig. 3. The contact-tubes to which the plant is adjusted pierced through the paraffin wall. The other walls were covered with wet filtering paper. A current of air,

saturated with moisture, was led in through the bottom. If required, the air was also saturated with ether.

In total 10 experiments were done; 7 of them on *Bryophyllum* and 3 on *Coleus*. Moreover 4 controls were made. To this purpose

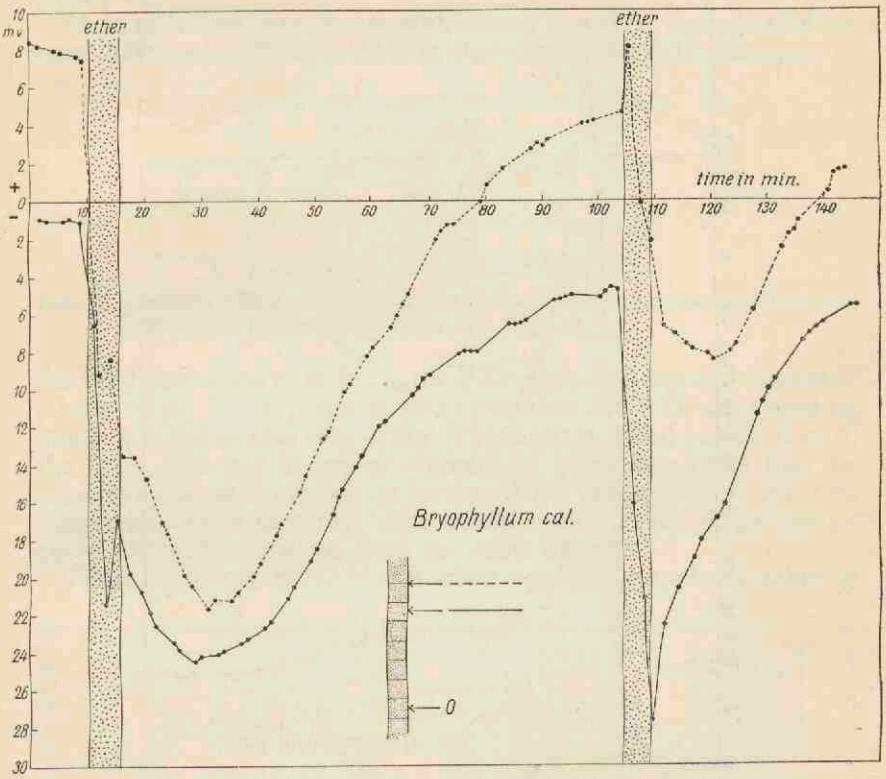


Fig. 4. Influence of ether supply upon the P.D. Duration of the exposure: 2 ∿ 5 minutes. Exp. 3f.

the stems (2 of each species) were killed by dipping them into boiling water for 15 minutes.

Fig. 4 shows the results of one experiment on *Bryophyllum*. In each case the basal electrode was placed on an internode, while the apical electrode was connected to a node or an internode alternatively. In the diagrams the position of the contact tubes is indicated by arrows. So the potentials of both apical spots refer to that of the basal one. The nodes are represented by horizontal lines.

Considering fig. 4 one can see the effect of air saturated with ether. Immediately a negativation at the apical contact tubes resulted. After 5 minutes the ether supply was stopped and fresh air was allowed to enter. It then lasted 3 minutes until the ether was

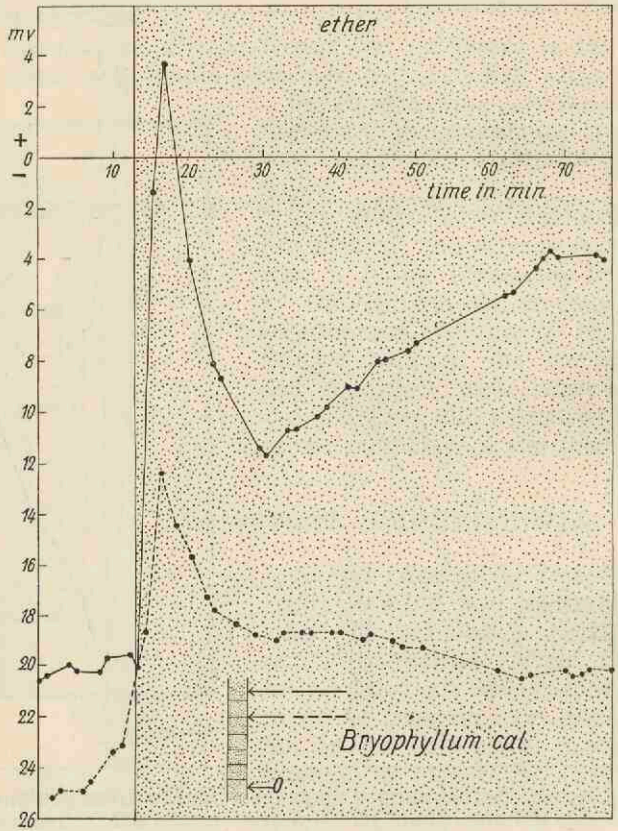


Fig. 5. Influence of prolonged exposure to ether upon the P.D. Exp. 3g.

removed completely. The P.D. began to restore itself 15 minutes afterwards. The same procedure was repeated after 90 minutes.

In fig. 5 an experiment is shown in which the plant was staying in ether vapour for more than one hour.

From the experiment of fig. 4 one may conclude that the ether acted as a "stimulus" only, time being too short to allow a thorough penetration. In that case the respiration would not have decreased

considerably but the membranes of the peripherical cell layers would have been injured to such an extent that an action current resulted. If this is true, still attention must be paid to the fact that after removal of the ether the recovery begins not until 20 minutes passed on.

In long lasting treatments with ether, however, the metabolism must have been affected. Also in these cases the P.D. changed; in

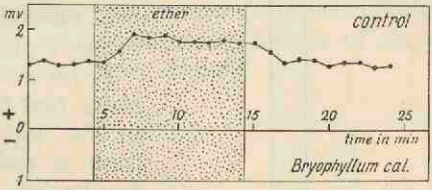


Fig. 6. Control: killed stem piece. No influence of ether can be observed. Sign refers to the apical electrode. Exp. 3k.

the fully drawn curve of fig. 5 the P.D. decreased but did not reach a zero value. In only one more experiment the P.D. decreased as much as it did in that case of fig. 5. As even in killed objects (fig. 6) the P.D. does not disappear completely, these two cases may be considered as an argument in favour of the redox-theory. But this cannot be said of the other experiments and even not of the dotted curve of fig. 5, obtained from the same object.

From fig. 7 can be seen that when repeatedly applying ether at

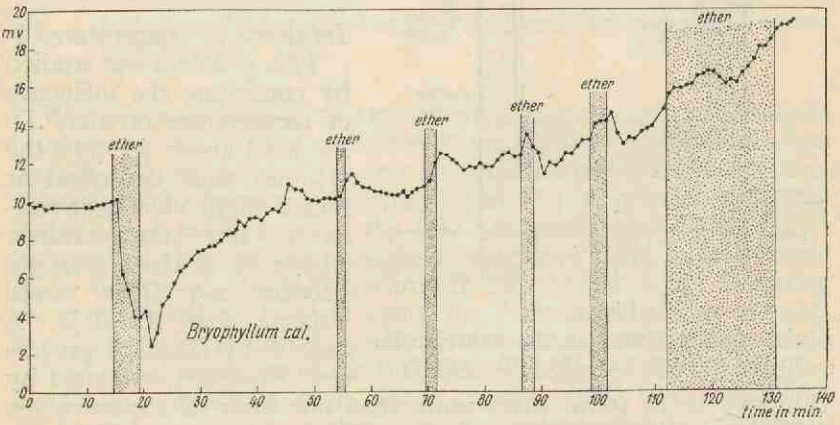


Fig. 7. Repeated exposure to ether within short intervals. Sign refers to the apical electrode. Exp. 3a.

short time intervals only the first exposure causes an effect. In the meantime the P.D. increases so that the apical part of the plant becomes more positive.

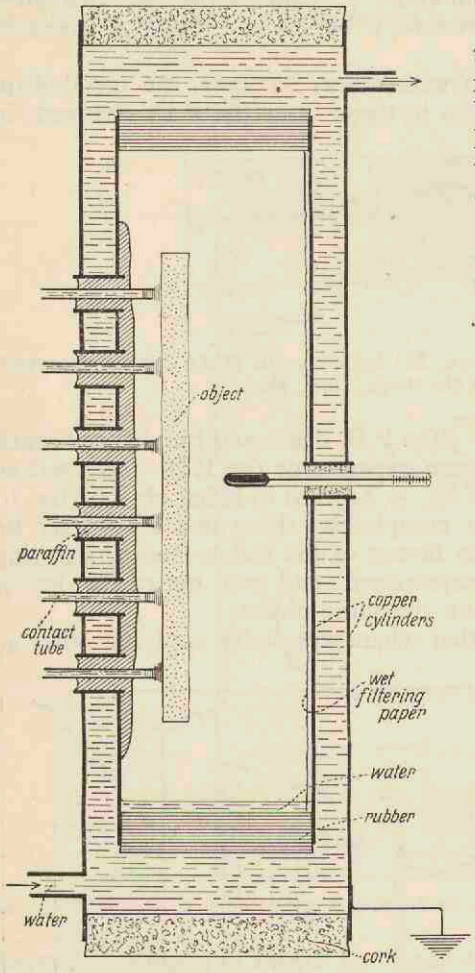


Fig. 8.
Moist chamber, used in the experiments on the influence of the temperature.

The other experiments of this kind showed similar results. It cannot generally be predicted whether the P.D. at the apical electrode will become more negative or more positive.

From all this we may conclude that the P.D. measured in this way is not resulting from a redox system. The results of experiments of others which seem to affirm the flux equilibrium theory perhaps can be explained in another way. This will be discussed once more in the end of part I.

The next question is whether the source of the bio-electric potentials concerned is of a chemical nature at all.

Influence of temperature.

This problem was studied by controling the influence of temperature on the P.D. As cited above (p. 377) the Q_{10} has been described as rather high, chiefly at relatively low temperatures. Above 18° C the Q_{10} ranges between 2-3. This would indicate that the P.D. is caused by chemical processes. However, as stated by U m r a t h, in some cases more than one hour of exposure to a different temperature is required until an effect occurs. Moreover the experiments of M a r s h seem to diverge to some extent.

So it seemed useful to study the influence of temperature once more. To this purpose a defoliated stem piece of *Coleus* was placed in a moist chamber as indicated in fig. 8. This chamber consists of two copper cylinders telescoped into each other. The ends of the inner one are closed by rubber- and those of the outer one by cork stoppers. A flow of water of the temperature required is led between the two cylinders. Both cylinders are perforated by brass tubes coated with paraffin to enable the adjustment of the contact-tubes. Also a thermometer is fit into the inner cylinder. The temperature of the water is regulated by means of an apparatus after Hille Ris Lambers (1926).

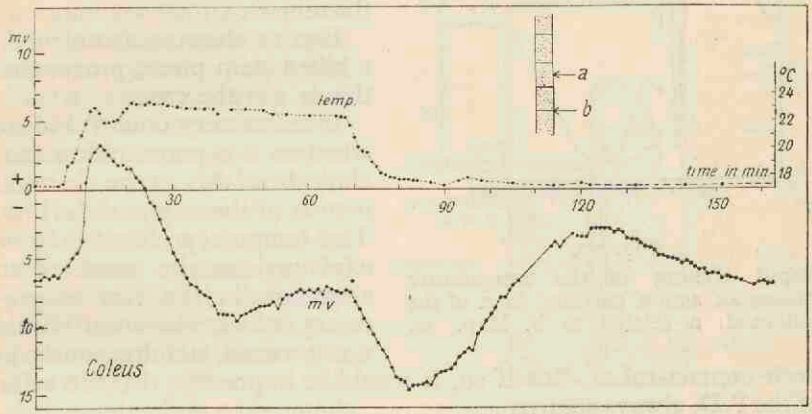


Fig. 9. Influence of temperature on the P.D. Sign of the potential: b related to a. Exp. 4j.

This series consists of 10 experiments. As the results are uniform in all cases, we confine ourselves to the discussion of one of them (fig. 9). The situation of the contact-tubes is shown in the diagram. A rising of the temperature from 17,3 to 22,3° C is followed immediately by a positivation of the P.D. (b related to a). This effect, however, is short-lasting and, the temperature being kept constant at its new level, the P.D. returns to its original value. Lowering the temperature to 17,3° C again, the P.D. curve follows at once, but also here for a short time only. It then returns to its former level.

The initial alteration of the P.D. after a change in the temperature cannot be considered as an action current. This is demonstrated by fig. 10, showing an action current superimposed on the phenomenon described above. In this experiment the temperature was

lowered 10° C. As this was done very abruptly, the cooling acted as a "stimulus" (perhaps injuring the membranes). It will be clear that this effect is not identical with the slow variations and therefore cannot be due to the same process.

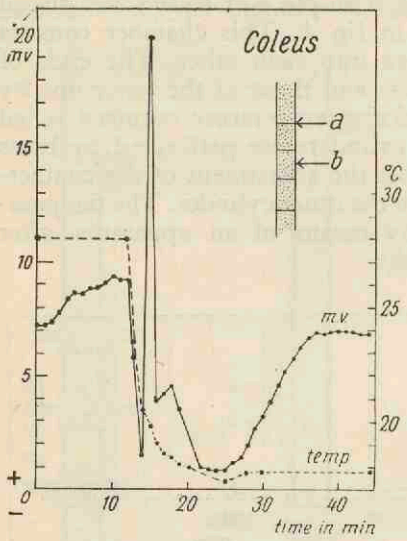


Fig. 10.
Rapid sinking of the temperature causes an action current. Sign of the potential: a related to b. Exp. 4c.

It might be possible, however, that the slow variations are due to unequal heating or cooling of the electrodes and thus have nothing to do with the object.

Fig. 11 showing a control on a killed stem piece, proves that this is not the case.

It seems very doubtful to me whether it is permitted to conclude from this on the chemical nature of the origin of the P.D. The temporary effect could be explained as the result of an alteration in the rate of processes which alteration would not proceed simultaneously at both contact-tubes. But if so, it would be impossible that the value of the P.D. always returns to its original level after 20 to 30 minutes.

According to U m r a t h the influence of the temperature on the P.D. of *Nitella* is only noticeable a much longer time after a change in temperature. A small object like *Nitella* undoubtedly accepts the temperature of its medium in less than 5 minutes. Also in this case it seems questionable to me whether one may conclude on the chemical

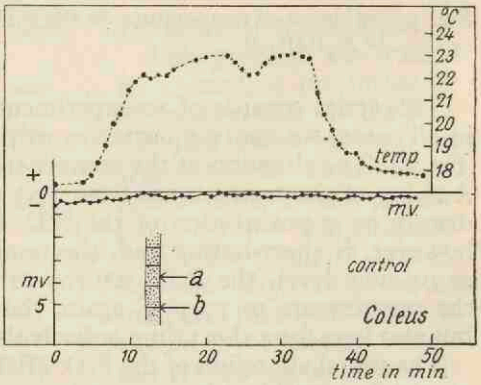


Fig. 11.
Control: killed stem piece. Sign of the potential: b related to a. Exp. 4k.

nature of the source of the P.D.

The discussion on the short lasting effect is deferred to the end of part I. We only state here that *it is not possible to conclude from these experiments on a P.D. caused by chemical processes.*

Influence of migration of water and ions.

So we must try to find another explanation of the problem. There are three obvious possibilities left. Firstly the bio-electric potentials may be diffusion potentials. Further they may be D o n n a n potentials. Finally there is a possibility that the P.D. is due to membrane potentials. Of course, it is not quite excluded that other phenomena, such as streaming-potentials (caused by the water-transport in the vascular system), play a part but it seems not very probable that they can be influenced in such a way as has been and will be shown in the next experiments.

O s t e r h o u t (1930a) demonstrated by mathematical reasoning that the influence of diffusion potentials is predominating. He admits that D o n n a n potentials as well as membrane potentials too may play a part, but, if so, they are of minor importance.

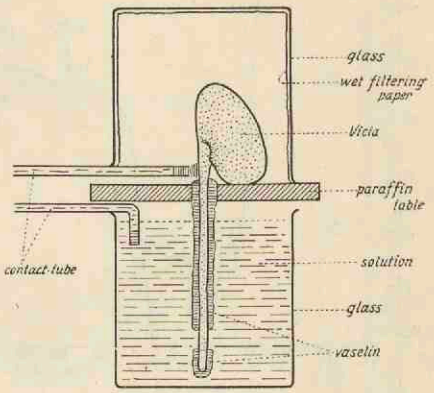


Fig. 12.
Method used in the experiments on concentration.

So the influence of varying concentrations of electrolytes was studied next. The root of *Vicia Faba* proved to be a suited object for this purpose (for its cultivation see p. 386 "Material"). The bean was adjusted on a paraffin table by means of gelatin (fig. 12), the root piercing downward through a hole. Over the bean a tumbler, coated with wet filtering paper, was placed. The root was covered previously with vaselin to prevent drying out. One and a half centimeter next to its tip was left uncovered. This end dipped into the test solution. Moreover the advantage of this procedure was that when changing the solution exactly the same part of the tissue was in contact with the liquid.

One contact-tube was adjusted to the basal part of the root, while the other one was put into the solution.

The method used required a most careful treatment. The P.D. immediately reacted on the slightest vibration of the table. So a little disturbance could not be avoided at the replacement of the solutions as can be seen in the graphs. Some practise could reduce this inconvenience to a minimum.

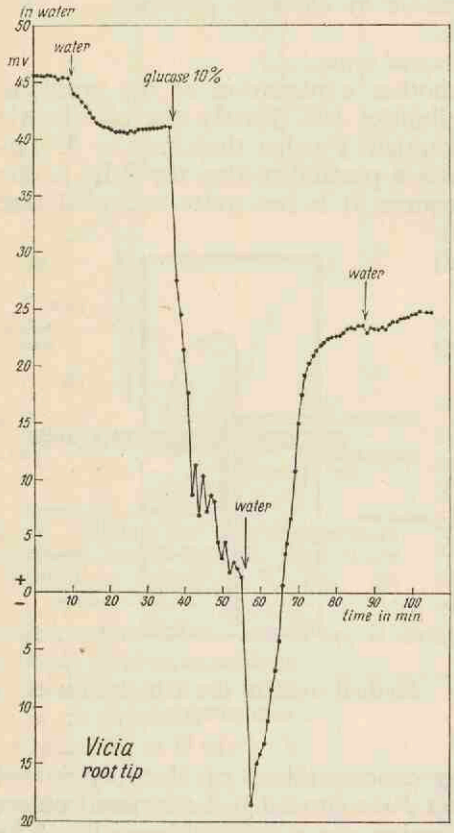


Fig. 13.
Influence of a 10 % glucose solution. Sign of the potential: root tip related to root base. Exp. 5b.

When studying concentration-effects, the concentration can be modified in two ways. Firstly the concentration of ions can be changed in the surrounding solution and secondly the vacuolar sap can be diluted or concentrated. As it had to be discriminated whether the P.D. is caused by the diffusion of ions, present in the plant, the second procedure was chosen and happened as follows.

The root tip was put in tap water first. Two to three hours later the P.D. had reached a constant level. In this state an equilibrium must be reached between the entrance of water into the vacuole and the turgor. In this phase the concentration of the electrolyte solution in the root is the lowest possible. By applying a solution of a non-electrolyte as medium, water will be withdrawn from the cells and the solution of electrolytes in the root will become more concentrated. In this way only concentration effects of the "natural" ions are studied.

As was cited above, the more concentrated solution is negative to the more diluted one as the anion-permeability of the protoplasmic membranes is relatively small. Consequently, it can be expected

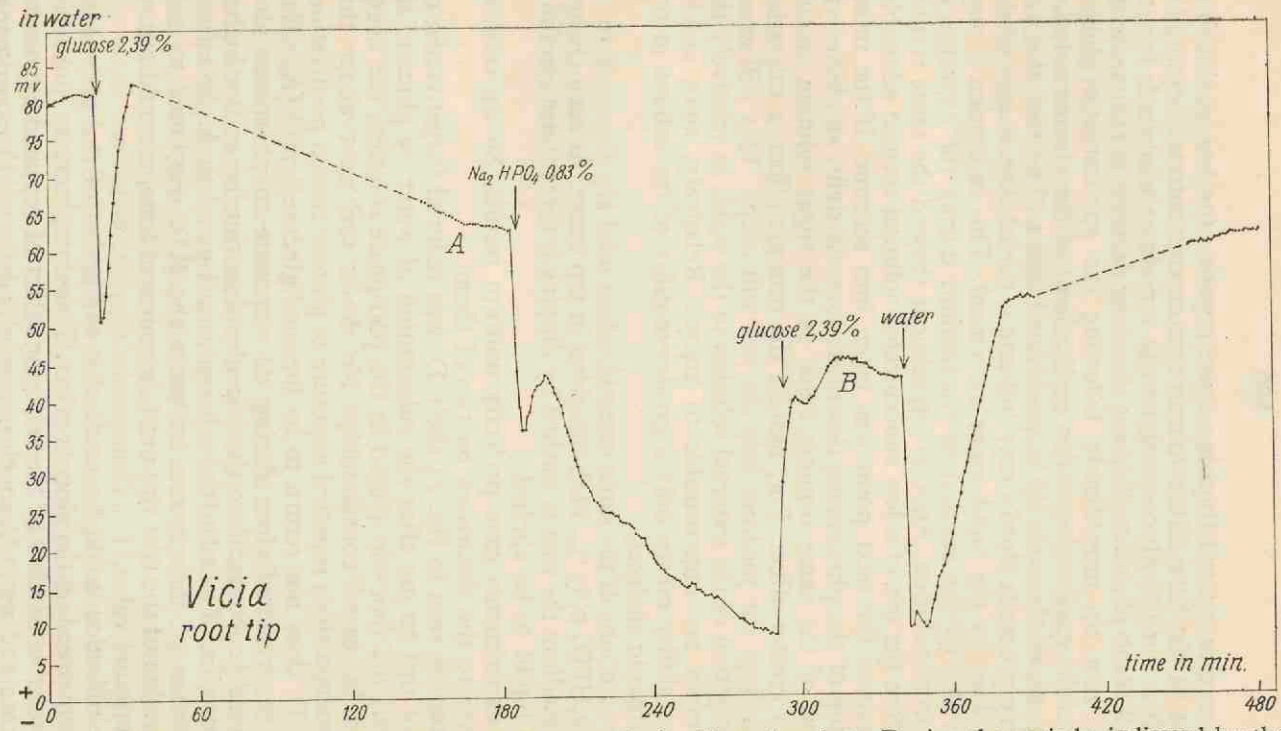


Fig. 14. Difference between the effects of glucose and of sodium phosphate. During the periods, indicated by the dotted lines, no observations were made. Sign of the potential: root tip related to root base. Exp. 5e.

that the root tip will become more negative (or less positive) in relation to the base of the root in a more concentrated solution.

At first a 10 % glucose solution in tap water was used; fig. 13 shows a result of one experiment. A strong decrease of the positivity of the root tip immediately following the application of glucose matches the expectation. After replacement of the glucose solution by tap water the root tip is positivated again. The fact that the recovery proceeds slowly may indicate a partial disturbance of the membranes by the rapid water movement. This indication is supported also by the continuous fluctuations during the exposure to the glucose solution. Another experiment showed the same results. Therefore the use of a less concentrated solution seemed advisable.

Moreover the next point was taken into account. If the interpretation of the phenomena described above is right, an electrolyte solution of the same osmotic value as the sugar solution should cause a greater effect. For, besides the osmotic effect, a migration of ions from the medium into the cell will occur. This will cause a negativation of the external solution to the plant, as relatively the membranes are impermeable to anions. Relatively, since anion-impermeability means only a greater motility of the cations in the protoplasmic surfaces.

The solutions of the same osmotic values used are glucose 2,39 % and Na_2HPO_4 0,83 %, both dissolved in tap water. At each change of the medium the root is washed by dipping it three times carefully in the liquid to be applied.

Six experiments gave perfectly uniform results. So we confine ourselves to the discussion on two of them.

As can be seen in fig. 14 the P.D. was reduced (negativation of the root tip) 20 mv after the substitution of water by glucose. If, however, the root was placed in the phosphate solution, the negativation increased considerably. No doubt can exist about this phenomenon since repeated exposure to glucose caused positivation. The P.D. does not return to its former "glucose level" (A). This could be predicted since during the exposure to phosphate ions these ions penetrated into the vacuoles thus causing at B a higher concentration of salts in the cell sap as compared to A. At a final substitution of the glucose by water the P.D. continued to rise (positivation of the root tip) until, in course of time, it would return to its original value.

The objection could be made that the action of the phosphate is superimposed on the glucose-effect. This does, however, not seem very probable in view of the restoring effect of repeated exposures to glucose and water. A conclusive proof is delivered by experiments

in which the water was directly replaced by the phosphate, as shown in fig. 15. The root tip was negativated considerably. After application of the glucose solution the negativation decreased. The substitution of the glucose by water gradually restored the P.D. to its original value.

So we may conclude from these experiments that *the P.D. also in the roots of Vicia Faba is determined by the concentration of the ions in the vacuoles.*

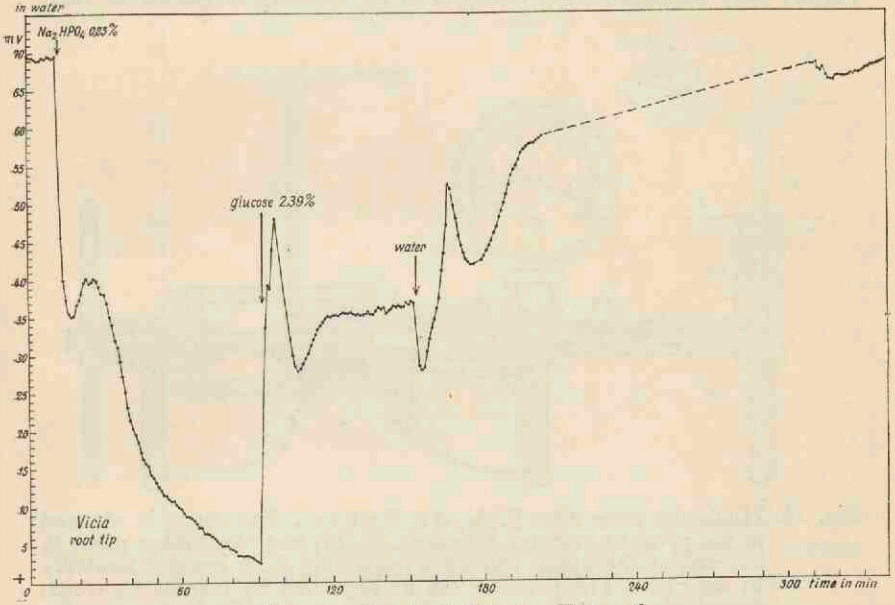


Fig. 15. See text fig. 14. Exp. 5f.

Influence of hydraulic pressure.

It must therefore generally be possible to influence the P.D. by concentrating or diluting the ions in the cell sap; in other words by moving water to or from the vacuole. It was still tried to cause this water movement by replacing the root pressure by an artificial hydraulic pressure. By varying the latter, it will be possible to alter the water content of the cells mechanically. The objects used were stems (the leaves were left attached to them) of *Bryophyllum calycinum*, in which the pericycle is well developed to a solid layer, which is necessary in these experiments. At the base the cortex is removed and the stem is placed on the hydraulic press as shown

in fig. 16. By turning A tighter, the rubber piece B is tightened up against the pericycle. The pressure can be regulated by C and is controlled by the manometer D. The hydraulic press has been devised and carried out by P. A. de Bouter, mechanist of the Botanical Laboratory, Utrecht.

Adjusting the plant on the press in this way, the water is sqeezed into the xylem only. So the natural pressure is imitated as well as possible. The results, however, are published with some restriction. Firstly they depend on the changes in the cell sap in the tissue next

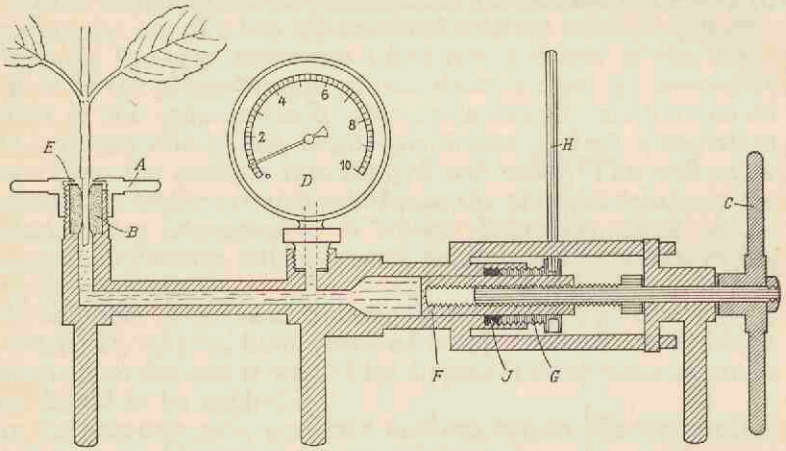


Fig. 16. Hydraulic press after P. A. de Bouter. The object is adjusted to the press by turning A tighter. In this way the rubber piece B, covered at the upper end by a brass ring E, is pressed alsidedly to the plant. The pressure can be regulated by turning C, which moves the piston F, and can be controlled at D. Leakage at the piston can be prevented by turning G tighter by means of the brass rod H. In this way the greased asbestus wool J is pressed to the piston.

to both contact-tubes. These changes are determined by the hydraulic pressure, the osmotic pressure and the turgor. If both the latter are equal in all parts of the tissue between the contact-tubes, one cannot expect a great effect. If, however, they are not, the question whether, by variation of the pressure, the tissue at one of the contact-tubes is positivated or negativated will depend on the values of the osmotic pressure and of the turgor as compared to those of the other part of the tissue. Further, in most cases, action currents could not be avoided as the pressure had to be

regulated constantly during the experiment. Finally, the vascular system sometimes being pinched, no effect occurred at all.

One of the positive results is shown in fig. 17. In this case the more apical tissue was positivated by applying an effective pressure of 1 atmosphere.

As is pointed out above, it is difficult to valuate these experiments. So we only state that it probably is possible to influence the P.D. by alteration of the pressure in the vascular system.

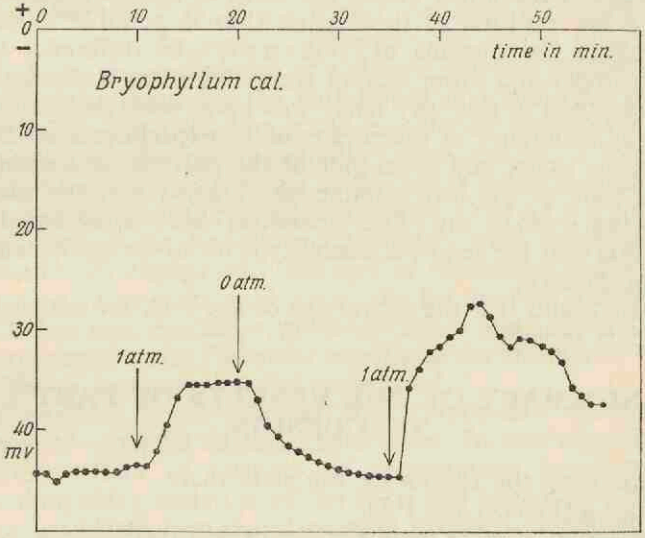


Fig. 17. Influence of hydraulic pressure. Sign of the potential: middle internode related to basal internode. Exp. 6f.

Finally an object was looked after, in which consistent alterations of concentration naturally occur. As the results of these experiments, made in collaboration with De Groot, have been published in a previous paper (De Groot and Thomas 1938), a short summary will do here.

Phaseolus multiflorus is capable of "variation movements" by means of pulvini. These movements are achieved by an alternating increase and decrease of the cell volumes of the motile tissues in the upper side or in the lower one of the pulvini. It has been made probable that the changes of volume are the result of alterations in osmotic pressure in the cells caused by a more or less intensive conversion of starch into glucose. The glucose constantly is removed by metabolism as well as by diffusion (De Groot 1938). So it

will depend on the rate of each of the both processes whether the osmotic value of the cells will increase (water intake, enlargement of the volume) or whether it will decrease (loss of water, decrease of the volume). So the concentration of the electrolytes in the vacuole will vary too. Assuming an ideal semi-permeability of the membranes, the electrolyte concentrations at different times are to be considered proportional to the reciprocal of the quotient of the volumes of the cells at the same moments. By measuring the volumes of the cells of the upper and lower sides of the pulvinus at a high and at a low position of the leaf, it thus is possible to calculate, according to the formula of Nernst, the difference in P.D. between upper and lower side of the pulvinus at both stages. The values obtained in this way fairly well agree with the experimental ones. The difference of the means of 11 experiments on the P.D. between the upper and lower side of the pulvinus at a sinking and a rising state of the leaf amounts to + 24,85 mv, the theoretical value being + 20,90 mv. The theoretical value being based on the assumption that the semi-permeability is ideal, the agreement seems to be satisfactory.

We also found that the alterations of the P.D. are coupled to the movements indeed.

SUMMARY OF THE RESULTS OF PART I. DISCUSSION.

Summarizing the results we can state that:

1. ether influences the P.D.
2. the action of ether results either in positivation or in negativation but not in a "loss" of potential (by using no lethal quantities).
3. the influence of temperature on the P.D. does not indicate a chemical process as source of the P.D.
4. the potential is showing a short-lasting increase when the temperature rises, while a short-lasting decrease is caused by sinking of the temperature.
5. by moving water to or from the vacuole it is possible to influence the P.D.

When assuming that the P.D. is the result of a flux equilibrium according to Lund we cannot explain why the potentials are not reduced to zero by ether as was the case in the experiments by his co-workers. Apparently a real temperature coefficient does not exist. In our objects a $Q_{10} = 2—4$ was not stated. Finally in the

experiments on the influence of the concentration of the cell sap on the P.D., the dipping of the root tip of *Vicia Faba* in a glucose solution caused a negativation. If glucose would act as an oxidizable substance and be a limiting factor in the respiration, the latter had to be increased and the P.D., if it were a redox potential (the tip being positive), had to be increased too instead of being decreased. If the glucose would not limit the oxidation process, no effect on the P.D. had to occur at all. The negativation found, however, cannot be explained in the terms of the redox theory of L u n d.

Considering the P.D. as a diffusion potential according to O s t e r h o u t one can readily interprete the stated effects. As is argued above, the application of a glucose solution instead of water negativates the tissue by the withdrawal of water thus increasing the electrolyte concentration in the vacuole. An electrolyte solution (phosphate) of the same osmotic value causes a much more pronounced effect, the removal of the water being attended with a diffusion of cations into the cells. Correctly O s t e r h o u t (1936b) remarks that, if the bio-electric potentials were redox potentials, they would not change with changes of electrolyte concentrations. O s t e r h o u t mentions this especially for the concentration effect of KCl, but this statement probably has a more general bearing.

In experiments with *Phaseolus multiflorus* the formula of N e r n st for diffusion potentials proved to be valid.

The question arises whether the temperature effect and the influence of ether fit in this explanation. In our experiments no permanent effect of changes of temperature has been found; only a temporary variation of the P.D. was stated. If a process of continuous diffusion in the plant were involved, one might expect, as permanent effect, a Q_{10} of about 1,2—1,3 as normally occurs in diffusion phenomena. Nothing of this kind could be stated; there is no question of a Q_{10}. For the present it is not possible to explain with certainty the temporary effect. The rate of the transport of cations and anions being different, I may suggest — as a preliminary attempt to explain this effect — that the rate of their migration in and through the membranes too is differently affected by changes in temperature. The latter difference, however, only could be ascribed to a reversible alteration of the membranes themselves caused by the change in temperature. Such an alteration might be due to changes in the adsorption-equilibrium and hydratation and consequently in the relative permeability to ions; factors, that could be responsible for temporary changes in the P.D., until a new stationary state is reached. The hypothesis given by M a r s h cannot explain our case. His "overshooting" and "undershooting"

effect (with sinking and rising of the temperature respectively) consists of a short lasting counter effect, which was not found by us.

The experiments with ether too do not conflict with our opinion. The permeability of the membranes is influenced by ether. It therefore will depend on the concentration of electrolytes in the vacuoles of a tissue whether it will be negativated or positivated in relation to another one.

The objection can be made that it is also possible to explain the effect of ether by assuming an unequal rate of penetration of the ether into the tissues at both contact-tubes. In this case the part in which the respiration is inhibited less would become positive. This, however, does not hold true since even at prolonged exposure the P.D. is not reduced in most cases.

So we conclude that, using our methods, no evidence is shown of protoplasmic interfaces acting like electrodes of noble metal according to L u n d. The theory on redox potentials fails to explain various results which can be understood by considering the bio-electric P.D. as a diffusion potential.

Of course it is not possible to draw more detailed conclusions from our experiments as to the nature of the membranes. It seems doubtful whether one, working with complicated tissues, can go further into details at all. To this purpose one would have to use single cells in which one of the electrodes could be pierced into the vacuole. In that case the results would not be troubled by the shunting effect of the cell walls.

Also in single cells, however, one has to realise that no absolute certainty as to the interpretation of the results can be obtained. In chemistry the data on membranes are derived from models in a dynamical balance. Selective permeability in vivo and in vitro are not absolutely the same, since in living cells the balance continuously is shifted by the metabolism.

The membranes are interfaces of the protoplasm. Since surfaces are, chemically spoken, the most reactive parts it seems probable that the metabolism chiefly occurs in or near the membranes. As further their structure immediately and profoundly changes at death, a close relation seems to exist between metabolism and membranes. We believe that *the structure of the protoplasmic interfaces is determined and regulated by the redox processes.*

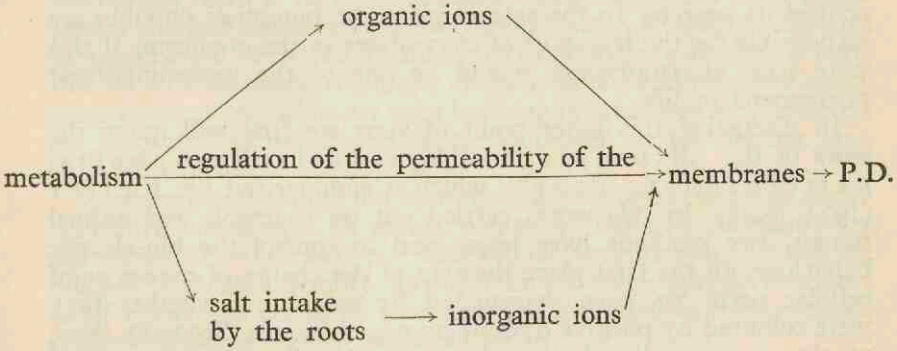
We will compare our point of view with that of O s t e r h o u t and that of U m r a t h. We can resume the hypothesis of the authors (see review of literature p. 377) shortly as follows:

Osterhout.	Umrath.
The O_2-effect on the P.D. may be due to a change in the organic composition of the protoplasmic surface or to changes in the ions (*e.g.* organic metabolites) in contact with it.	The O_2-effect on the P.D. may be due to a change of the number of P.D.-determining metabolites situated in a fixed arrangement in the membranes.

It seems questionable to me whether the dependency on the O_2-pressure can be explained satisfactorily by the hypothesis of Osterhout, whithout assuming that the structure of the membranes largely depends on the state of the metabolism.

On the other hand Umrath's explication is not quite satisfying for the understanding of concentration phenomena. Umrath does not deny absolutely the influence of selective cation-permeability on bio-electric potentials. His admittance, however, is not sufficient to explain quantitative relations between P.D. and electrolyte concentration. Moreover, as shown above, the absence of a Q_{10} in our experiments excludes a direct dependency of the P.D. on a chemical process.

We will summarize our opinion in the following survey:



Finally we want to add a note on the influence of glucose.

The results of the experiments described in this paper were explained by the movement of water causing a change in the electrolyte concentrations in the vacuolar sap and in the protoplasm.

Osterhout, however, considers the influence of non-electrolytes from quite a different point of view. According to this author (1938) the alterations in P.D., caused by organic molecules, may be due to a number of possibilities, such as: alterations of partition

coefficients, mechanical rupture of the membranes, production of organic ions in the protoplasm, changes in phase boundary potentials or in membrane potentials.

The effect of the movement of water has been studied by him by placing *Valonia* cells in diluted sea water. A negativation resulted as compared to the P.D. in normal sea water. The exposure to the solution in question lasted very short (about 5 minutes). However, these results cannot be compared with the initial alterations of the P.D. in *Vicia* roots after changing the solutions. The rapid changes of the P.D. occurring during the first minutes are not taken into account by me, as at this object they undoubtedly are the result of an inevitable irritation, caused by the replacement of the solutions.

PART II.

REVIEW OF LITERATURE.

Bio-electric potentials and polarity may be correlated in two different ways. In the first place the P.D. may be caused by various processes. In this case it only would be a by-product, useful to control its sources. In the second place the potentials possibly are responsible for the transport of electrolytes in the organism. If this were true, electrophoresis would be one of the most important phenomena in life.

In discussing this latter point of view we first will quote the work of the „Biologisch-Physikalische Arbeitsgemeinschaft Prag", which is summarized by Keller (1932, 1938). In this work, carried out on vegetable and animal tissues, two methods have been used to control the bio-electric behaviour. In the first place the sign of the charge of tissues or of cellular parts has been determined by examining whether they were coloured by positive dyes or by negative ones. Secondly these results are controlled by means of bio-electric measurements. It appeared that in various cases, in which organic and inorganic ions are moving against concentration gradients, the direction of this transport could be explained by electrophoresis. A number of substances, however, moved just in a way opposite as expected if caused by electric phenomena. Now it appeared that the direction in which those substances move depends on the presence of colloids. So e.g. some metals such as potassium, copper and magnesium are moving towards the anode in the presence of electro-negative

colloids at which they are adsorbed. Keller (1936) classifies the organic and inorganic substances into two groups. Biologically negative is the potassium-group, while the sodium-group is biologically positive. This classification includes undissociated molecules too. It can be considered as a supplement to the classification of antagonistic ions. The reported facts are very suggestive. When a negative compound enters the cell, a positive one leaves it. They never are transported in the same direction. The potential of the cells of diseased animal tissues changes of sign as related to the surrounding liquids. In such cases the direction of transport of *both* groups is reversed (Keller 1938).

As to the question of the source of E. M. F.'s Keller (1932) remarked: ". . . , dass im lebenden Gewebe in der Regel auf beiden Seiten einer Membran eine für das betreffende Gewebe und für seinen Zustand charakteristische Ladung fortwährend aufrechterhalten wird durch einen chemischen oder elektrischen Mechanismus, dessen Wesen bis jetzt unaufgeklärt bleibt und der, wie Lund vermutet, mit der Atmung der lebenden Zellen im Zusammenhang ist und mit ihr steigt und fällt".

We have pointed out in the preceding part, that in our opinion the bio-electric potentials are not directly related to respiration. We believe them to be concentration potentials. If only mere diffusion of charged complexes (or ions) would occur, it would be difficult to understand in which manner electrophoresis can play a part. But we will not absolutely deny electrophoresis and its significance since redox potentials must exist in living matter, they are, however, not measured in our experiments. Moreover we are dealing here only with plants.

Furthermore it should not be forgotten, that one is working under controlled conditions. In nature plants are irritated constantly by the action of wind, rain etc. and action currents will disturb the inherent P.D. considerably. So under natural conditions the transport of all substances would be very irregular if not temporarily reversed, if electrophoresis were responsible for it.

On the other hand the influence of electrolytes on the P.D. and chiefly the quantitative relation between their concentration and the variation of the potentials are facts indicating diffusion effects. As to this, Keller (1932) points to the fact, that molecules can penetrate the protoplasm more easily than ions. Osterhout (1936) assumed the membranes to be an oily liquid in which dissociation is minimal. Kraus (1934), however, showed that formation of charged complexes occurred in media of low dielectric constants.

In our opinion, the results obtained up to the present affirm the hypothesis on diffusion potentials while others are not in contradiction to it.

The idea, that polarity in plants might be due to electric phenomena, has been suggested by F. W. Went (1932). This author supposed the polar transport of the negatively charged auxin-ion to be caused by the potential differences between top (—) and base (+). Experiments with acid and basic dyes seemed to affirm this hypothesis. Things became more complicated as Ramshorn (1934) discovered that plants are electrically tripolar. Shoot tip and root tip both are positive while their base is negative. Growing tissues appear to be positive in relation to resting ones. Ramshorn therefore concluded that auxin cannot be transported electrically.

The theory of Went has been rejected by various authors. Their objections are based partly on the different sign found for the charge in the tip and the base of the plant. An enumeration of these contradictory data is given by Hellinga (1937). This author also discarded Went's hypothesis. Hellinga did experiments with stem pieces of *Coleus*, placed in water partly in a normal position, partly in an inversed one. Always the end pointing upward was found to be negative. Unfortunately his method shows an error as the electrodes are not of the same nature. An Ag-AgCl electrode was inserted into the upper end, while the contact with the other pole is performed by dipping a platinum wire into the water in which the stem piece had been placed. So the negativity of the upper part is not due to geo-electric phenomena but to the method used.

We will not cite all literature concerning the question whether the auxin transport is directed by bio-electric potentials. We will confine ourselves to cite Clark, a student of F. W. Went. This author (1937a) measured the P.D. on *Avena* and other objects. The shoot tip was found to be negative. This agrees with Went's theory on auxin transport. Clark (1937b), however, came to the conclusion that it is impossible for the present to show a direct relation between the electric polarity and the auxin transport. Artificially the potentials of *Avena* were changed by shunting, by counter-E.M.F.'s and by varying the influence of the geo-electric effect. From these experiments negative results were obtained. Moreover, the auxin transport could be stopped by applying sodium-glycocholate (1/100.000) which had not any influence on the electric polarity, respiration and semipermeability (Clark 1938). So, according to Clark, a discrimination on Went's theory is

yet impossible.

As was cited Clark showed the tip of *Avena* to be negative. According to Ramshorn (1937) on the contrary it is positive. As was found by the latter, this discrepancy is due to the fact, that Clark's objects were kept in the dark. So the mesocotyl will grow out more rapidly as compared to the tip. This will cause a positivity of the base of the coleoptile. But, if the seedlings are cultivated in light, the development of the mesocotyl is suppressed and the tip is found to be positive. The positivity of growing tissues was not found by Ramshorn (1934) only, but in this respect the data as a rule are not contradictory. Lund and Kenyon (1927), Marsh (1928), Miss Rosene and Lund (1935), Drawert (1937) and others stated this too.

It was tried to show the correlation between electric and morphologic polarity by applying currents. Lund (1921) succeeded to reverse the polarity of *Obelia commissuralis* in this way. It has been demonstrated to be possible with the eggs of *Fucus* too (Lund 1923). The plain of the first division could be affected by the direction of the current flow. Furthermore a quantitative relation appeared to exist between current density and orientation of the axis of symmetry of *Obelia* (Lund 1924). For this object Lund (1925) demonstrated that the threshold-potential for inhibiting growth is of the same value as that of the inherent P.D. So, for this object, Lund (1931) drew up the hypothesis that "the oriented process of cell oxidation which is associated with this electric polarity should be subject to control or modification by an appropriate application of an E.M.F. of external origin". In this way correlation is explained without intervention of hormonal processes or of the nervous system.

Also, according to Schechter (1934), the polarity of the red alga *Griffithsia bornetiana* can be reversed. The rhizoids always develop at the anodal side.

Neilson Jones (1925), however, could not obtain positive results on seakale roots (*Salicornia maritima*).

Also the P.D. in plant cells can be regulated by an E.M.F. of external origin. In this respect I mention the way in which an action current is propagated. Osterhout and Hill (1930) showed this clearly by connecting two cells of *Nitella* by means of salt bridges. An action current of one of the cells passing through the salt bridges caused stimulation of the other cell.

In higher plants the P.D. can also be influenced by an E.M.F. applied externally, as was shown by Miss Rosene (1937). In the Douglas Fir the inherent P.D. was increased by applying a

potential in the same sense while it was decreased by an opposite one. It further appeared that the longitudinal electric polarity of the xylem and the cortex are opposite to each other.

The influence of electric potentials on the auxin transport has been studied by Brauner and Bünning (1930) by placing *Avena* coleoptiles between two plates in an electric field. The tip of the coleoptile curved towards the negatively charged plate, while the root tip showed a curvature to the positive plate.

Katunskij (1936) succeeded in affecting the growth of the same objects by placing them in an electric field in such a way, that the condensor-plates were adjusted perpendicularly to the longitudinal axis of the coleoptile.

In this respect we will consider the possibility of electric transport of auxin once more.

Experiments were reported by Kögl (1933) in which an E.M.F. was applied to *Avena* coleoptiles. It was concluded, that the growth could be influenced by accelerating or retarding the auxin transport by means of electricity. According to Cholodny and Sankewitsch (1937) this effect would be temporary only. At prolonged current flow in both senses retardation of growth resulted. A current, flowing from base to tip of the *Avena* coleoptile, initially caused an acceleration of the growth but a short time afterwards growth was retarded. When the current went from tip to base in most cases a retardation of the growth occurred. At breaking of the current, however, the growth was retarded once more. So the authors believe that, by applying a current, the translocation of auxin is not a direct consequence (electrophoresis) but an indirect one, via the complex system of the living protoplasm.

Du Buy and Olson (1938a) showed the influence of applied currents on the protoplasmic streaming in the coleoptiles of *Triticum* and *Avena*. According to these authors the auxin transport will be influenced too as it largely depends on the protoplasmic streaming. So a relation may exist between growth and electric polarity via this streaming. The same authors (Du Buy and Olson 1938b) stated the auxin transport to be regulated by three causes. In the first place the protoplasmic streaming, further electrophoresis and finally diffusion through the membranes between neighbouring cells. The first process is retarded by an applied current. It is indifferent in which direction the current flows. The next one, of course, is dependent of this direction, while the last one (Clark 1938), the charge of the membranes being responsible for the rate of the auxin transport through the protoplasmic surfaces, is dependent on the direction too. So, according to Du Buy and Olson,

the auxin transport depends on a complicated system. This agrees with the results of Cholodny and Sankewitsch (1937).

Clark (1938), however, succeeded in inhibiting the protoplasmic streaming in *Avena* coleoptiles without influencing the auxin transport by applying saponine in a concentration of 0,05 saturated. On the contrary sodium-glycocholate (1 : 5000) entirely stopped the auxin transport without affecting the protoplasmic streaming.

In the literature cited above, auxin has only been considered in its function of *growth* regulator. Olson and Du Buy (1937) have shown, however, that growth substance can play a role in the *polarity* of *Fucus*. If a solution of hetero-auxin (500 mgr/L) is applied to one side of the eggs, the rhizoids always develop on this side. The egg-cells and the spermatozoids appear to contain auxin in a high concentration (Du Buy and Olson 1937). According to Du Buy and Olson, the group effect (Miss Hurd 1920) and the influence of p_H (Whitaker 1937, 1938, Whitaker and Lowrance 1937) on the rhizoid formation of the same object is due to growth substance.

Rehm (1938) studied the relation between electric polarities and bud regeneration in *Phaseolus multiflorus*. At decapitation the node below the cut surface became strongly negative during the first hour. Then it positivated and remained positive during the next days. The temporary negativity of the base of the regenerating buds seems to be an effect of wounding. The positivity following afterwards and lasting for some days, however, probably is an indication of starting growth after decapitation. As has been cited already, growing tissue, as a rule, is positive in relation to resting cells. The base of a regenerating bud is positive to that of a resting sister bud; the tip is negative to that of the resting bud. Applied currents do not inhibit their growth. Only a hyponasty towards the negative electrode and an epinasty towards the positive one is the result.

As to regeneration phenomena in general an extensive review of literature is given by Van der Lek (1925), to which I refer.

Resuming, we state that the data on correlation in plants caused by bio-electric potentials are rather contradictory. Polarity cannot be considered to be based on the direct electrophoretic transport of growth substance. As to the question whether the P.D. acts indirectly in polarity phenomena, the opinions do not match each other.

As was mentioned above, we believe that the P.D. is due to a great extent to concentration differences. In that case the correlation

between P.D. and regeneration, if existing, must consist in either alterations in concentration of the vacuolar sap either alteration of the membranes, or in both.

EXPERIMENTS.

Before studying the problem of regeneration by means of bioelectric measurements it is necessary to show their mutual correlation. As cited above, a correlation between *growth* and *P.D.* exists. This could be stated by me too. Roots of *Vicia Faba* were adjusted in the moist chamber as shown in fig. 3. The contact-tubes were connected to the roots at a distance of 0,6 cm apart. The experiments were started one hour after the adjustment of the objects. The values obtained are the means of 5 readings (one reading pro minute). An interval of one minute was taken between each set of measurements at successive points. One electrode was connected to the contact-tube at the base of the root, while the other one was placed over the successive tubes. Five roots were tested in this way. The objects were aged 3 to 4 days. One of the results is represented in fig. 18. In all cases the extreme end of the root tip was strongly negative as compared to the other tissue. The growing zone always proved to be the most positive part of the root.

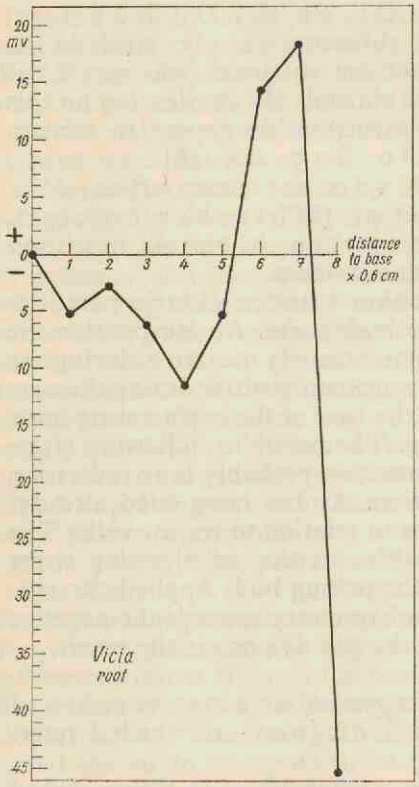


Fig. 18.
"Potential distribution" along a root of *Vicia Faba*. The sign of the potential refers to the base of the root. Exp. 7d.

Unipolar regeneration.

It is of interest to know first whether *regeneration* can be traced

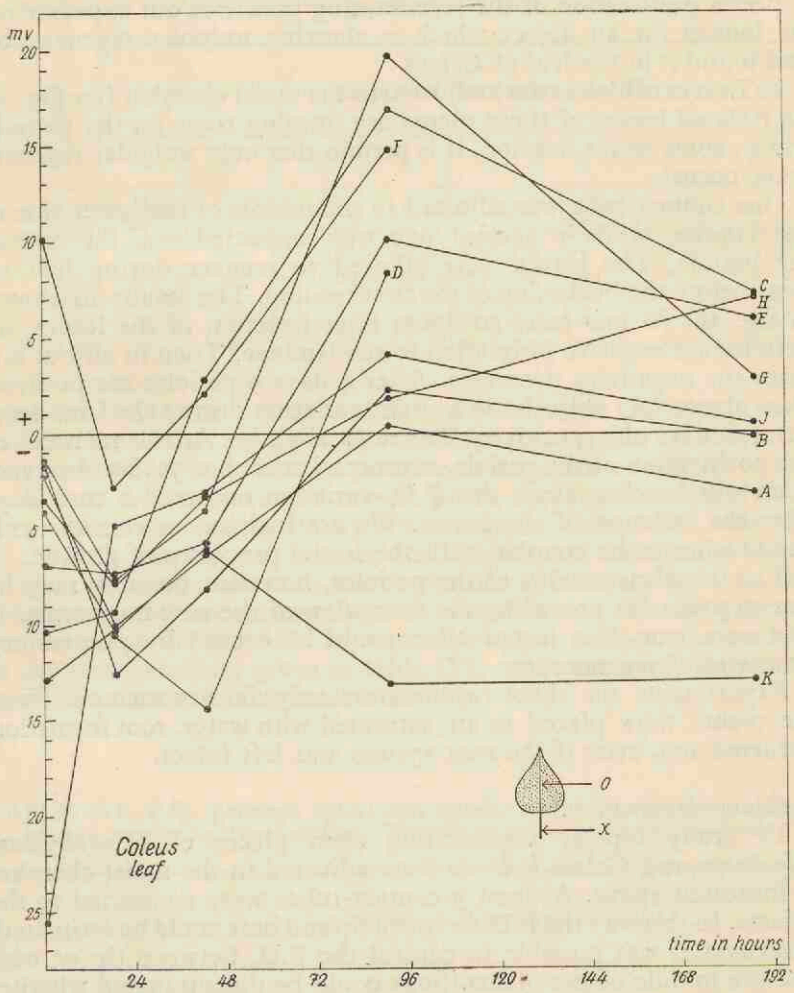


Fig. 19. Variation of the P.D. during unipolar regeneration of *Coleus* leaves. Sign of the potential: x related to o. Macroscopically visible roots are not formed. Exp. 7.

in the P.D. As in regenerating tissue growth is started, one might expect a positivation of the regenerating part. For our experiments we looked for an object which is showing unipolar regeneration and found it in the leaf of *Coleus*.

10 Leaves of *Coleus* were adjusted in the moist chamber (see fig. 3). As isolated leaves of these plants are forming roots on the petioles while shoots never develop, it is certain that only unipolar regeneration occurs.

One contact-tube was adjusted to the middle of the lower side of the lamina, while a second one was connected near the base of the petiole. The leaves were allowed to recover during half an hour before the beginning of the first reading. The results are shown in fig. 19. As one sees, 20 hours after isolation of the leaves, all petioles are negative in relation to the laminae. Then in all but one cases the negativity decreases. After 4 days 9 petioles are positive. One object (K) only shows a slight variation during the first days. After 8 days this positivity decreases slightly. At the moment of the positivation of the petiole, regeneration cannot yet be observed. This will be clear since this P.D.-variation occurred 2 to 4 days after the isolation of the leaves. We are inclined to consider this positivation to be coupled with the initial processes of growth.

The initial negativity of the petioles, however, possibly may be due to processes preceding the formation of the new meristems. If this were true, this negativation could be caused by preparatory processes of regeneration.

Experiments on shoot-regeneration only did not succeed. Since the plants were placed in air, saturated with water, root formation occurred too, even if the root system was left intact.

Bipolar regeneration.

To study bipolar regeneration stem pieces of *Bryophyllum calycinum* and *Coleus hybrida* were adjusted in the moist chamber as indicated above. At least 3 contact-tubes were connected to the objects. In this way the P.D. between tip and base could be estimated. Moreover it was possible to control the P.D. between tip or base and the middle of the plants. So it could be discriminated whether for instance a positivation of the tip to the base was due to a positivation of the apex or to a negativation of the base.

The results on the regeneration of *Bryophyllum*-stem pieces are shown in table IV*a*.

TABLE IV*a*. P.D. between apical and basal node in mv in *Bryophyllum calycinum* during regeneration. The signs refer to the apical node.

exp.	number of nodes	intact	number of days after isolation								
			1	2	3	4	5	6	7	8	9
8a	3	+18,77	+ 8,64	+ 9,98	— 1,04	+ 4,16	+ 8,51	— 5,97	—13,67	—	—
8b	5	—30,97	+14,78	+16,93	+14,98	+ 0,51	+14,50	+18,24	+17,75	+15,28	—
8c	3	+48,51	—	—	+ 5,05	+44,68	+55,18	—	—	— 2,77	—1,56
8d	2	+ 4,11	—27,13	—	— 1,69	—18,04	—30,22	—	—	— 5,08	—0,69
8e	3	+24,90	— 1,74	—	+ 1,18	—	—	—	—	+ 0,87	+3,37
8f	3	— 9,78	+ 1,73	—	+ 7,27	+ 4,84	+ 5,00	—	—	—11,18	—6,96
8g	3	—24,49	—	—	— 3,83	— 1,78	— 1,50	—	—	—10,30	—6,33

In this table the potential differences between the apical node and the basal item are given. In the first place it can be observed, that the apical node can be positive or negative as related to the basal one. Apical parts also do not prove always to be negative as they were supposed to be by some authors. As to the variation of the P.D. during 9 days after the isolation no uniformity seems to exist. It is of interest to know whether at least the basal or the apical nodes behave uniformly in an electric sence. To this purpose the variations of the P.D. between the apical node to the middle of the stem piece are given in table IV*b*, while table IV*c* shows the same in relation to the basal node.

TABLE IV*b*. P.D. between apical and middle node in mv in *Bryophyllum calycinum* during regeneration. The signs refer to the apical node.

exp.	number of nodes	intact	number of days after isolation								
			1	2	3	4	5	6	7	8	9
8a	3	+17,56	+7,42	— 0,66	—0,15	+10,28	+1,48	+4,57	—1,13	—	—
8b	5	—21,26	+9,35	+13,06	+9,66	+11,16	+9,77	+,32	+2,20	+4,94	—
8c	3	+23,57	—	—	—8,88	— 4,15	—3,62	—	—	+1,94	+4,18
8d	2	+31,62	+0,25	—	+6,88	+ 1,79	—6,01	—	—	—3,35	+1,46
8e	3	+25,59	—2,64	—	+2,80	—	—	—	—	—3,16	—2,41
8f	3	+ 4,93	—9,49	—	—9,41	—13,43	—8,40	—	—	—9,88	+1,99
8g	3	—30,25	—4,23	—	—5,89	— 5,12	—4,22	—	—	—1,90	+0,13

TABLE IVc. P.D. between basal and middle node in mv in *Bryophyllum calycium* during regeneration. The signs refer to the basal node.

exp.	number of nodes	intact	number of days after isolation								
			1	2	3	4	5	6	7	8	9
8a	3	— 1,21	— 1,22	—10,64	+ 0,11	+ 6,12	— 7,03	+10,54	+12,54	—	—
8b	5	— 3,79	— 5,53	— 3,51	— 5,32	+10,62	— 4,37	— 9,92	—15,55	—10,34	—
8c	3	—24,84	— 3,60	—	—13,93	—48,83	—58,80	—	—	+ 4,71	+5,74
8d	2	+27,51	+27,38	—	+ 8,57	+19,83	+24,21	—	—	+ 1,73	+2,05
8e	3	+ 0,69	— 0,88	—	+ 1,62	— 0,22	—	—	—	— 4,03	—2,60
8f	3	+14,71	—11,22	—	—16,68	—18,27	—13,40	—	—	+ 1,30	+8,95
8g	3	— 5,86	—	—	— 2,08	— 3,34	— 5,72	—	—	+ 8,40	+6,46

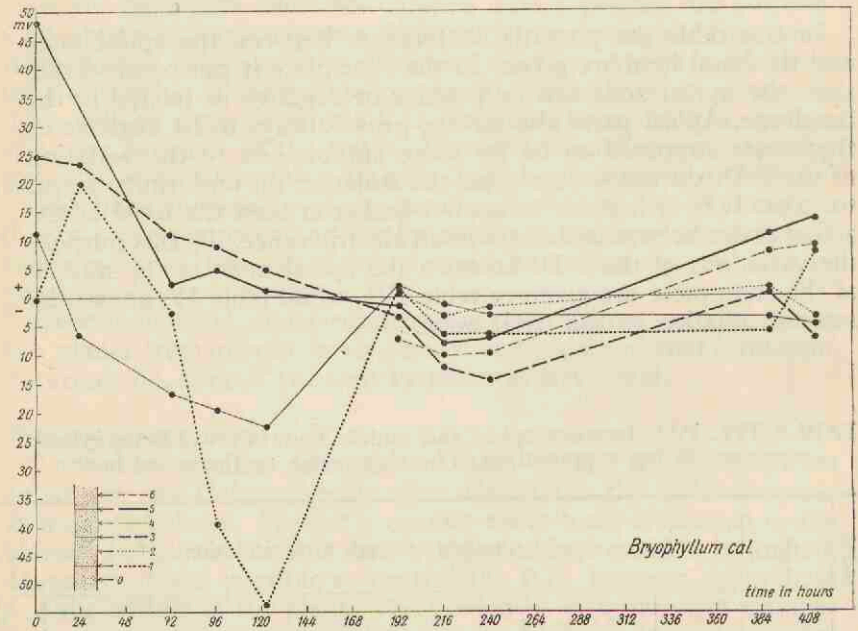


Fig. 20. Variation of the "potential distribution" along a stem piece of *Bryophyllum* during regeneration. All potentials refer to 0. The nodes are represented by the horizontal lines in the diagram. The measurements of 2 and 4 are lacking during the first 7 days. After 10 days macroscopically visible shoots are regenerated at 5, while roots are formed at 1 and 3. As in all experiments on regeneration, the isolation occurred after the determination of the points on the left ordinate. Exp. 8c.

It will be clear that no general conclusion can be derived from these data. The apical nodes seem to behave as irregularly as the basal ones do. It is of interest only, in connection with the next experiments, to state the negativity of the basal nodes on the day after the isolation of the cuttings. In this respect only exp. 8d is an exception.

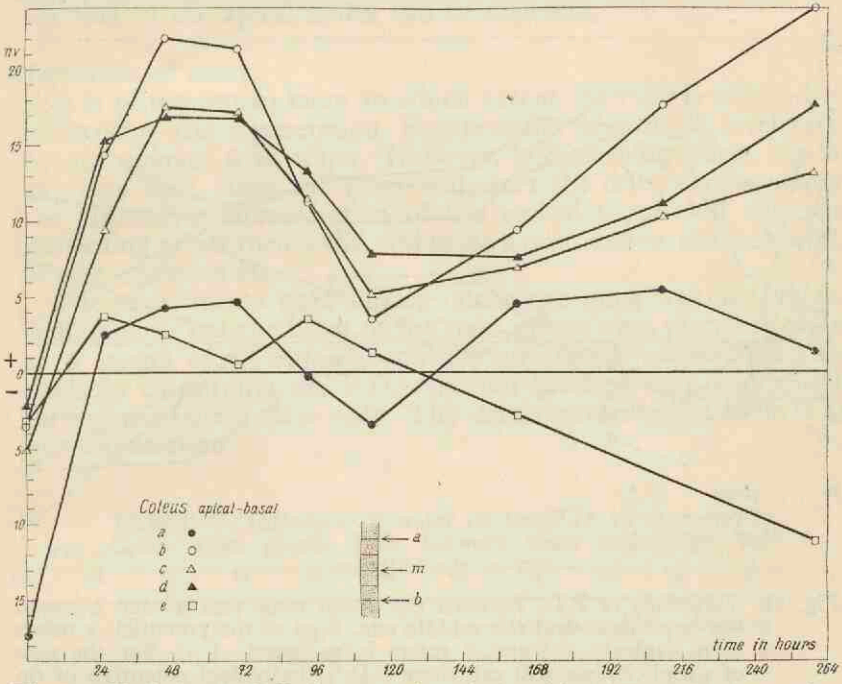


Fig. 21. Variations of P.D. between the apical and basal node of regenerating stem pieces of *Coleus*. Sign of the potential: a referred to b. The nodes are represented by the horizontal lines in the diagram. At the end of the experiment, callus was formed at all bases, while all apical nodes showed shoot regeneration. Exp. 10.

Fig. 20 shows the P.D.-variation of different points in one object (*Bryophyllum*). The situation of the contact-tubes is illustrated in the diagram. The nodes are indicated by the horizontal lines. The potentials refer to tube 0. Any other example, however, would be different. We only want to demonstrate, that the potential of the nodes is not principally different from that of the internodes. Clark stated (1937a) this only for young tissues. Only the basal

node temporarily negativates much more than the other ones do.

As *Bryophyllum* proved not to be a suitable object, the following experiments were carried out with *Coleus*.

Firstly 5 stem-pieces were treated in the same way as indicated above. The results are presented in fig. 21. The apical node in all cases proved to be positive as compared to the basal one during the first day after isolation. After 5 days the curves diverge. In studying

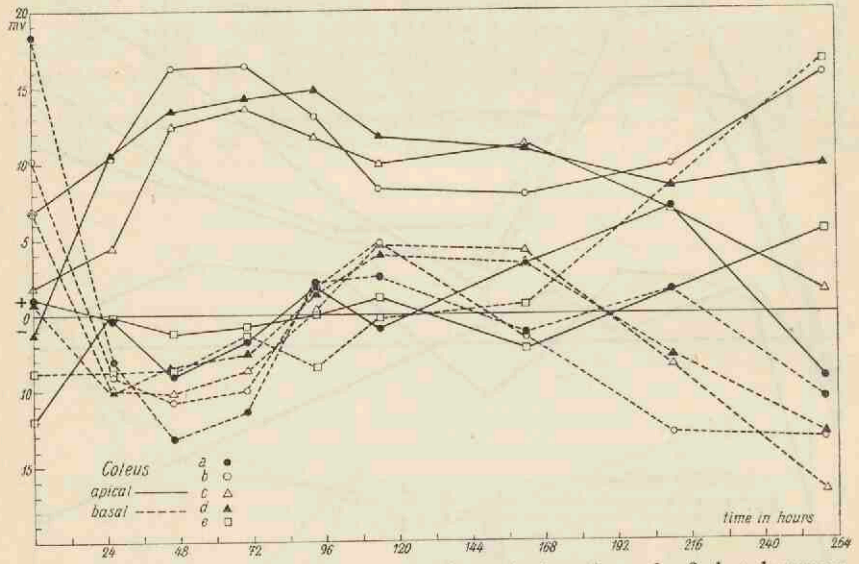


Fig. 22. Variations of P.D. between the apical node and of that between the basal node and the middle one. Sign of the potential: a refers to m (apical ——) and b refers to m (basal – – –). For the sake of simplicity we will call these P.D.'s: individual potentials of tip or base. The data are derived from exp. 10, see fig. 21.

the P.D.-variations of the same nodes as compared to the middle of the stem piece one finds (fig. 22) that the basal nodes during the first 3 days show a uniform electric behaviour by all growing negative to about the same extent. Then a slow positivation follows and afterwards the curves begin to diverge. These phenomena suggest that there is some correlation between regeneration and P.D. It is clear that we have not to deal with a reaction of the tissues in the neighbourhood of the cut surface since the apical nodes do not show a temporary negativation (*a* excepted) while they are situated near to a wound surface as well as the basal ones.

In considering the behaviour of the apical nodes no regularity can be observed. *b*, *c* and *d* show a shifting of the P.D. opposite to that of the corresponding basal nodes. This is very interesting indeed. However, exceptions are too frequent to base a conclusion on this phenomenon. So from this experiment we only will conclude that the initial positivation of the apical nodes to the basal ones (fig. 21) in general is due to a negativation of the basal nodes, while the reaction of the apical nodes can be different.

Influence of water.

It is of interest to know to which extent the P.D. is affected by influencing the regeneration. Regeneration of roots is accelerated by the contact with water. However, a disturbing effect can be expected here, since the water will enter the cells. Consequently, the electrolyte concentration of the soaked tissue will decrease. According to our theory this will cause a positivation, independently of the regeneration.

The experiments were carried out in the same way as the preceding ones, but the bases of the stem pieces were placed in water at a length of 0,5 cm. We confine ourselves by reproducing the readings concerning the P.D.-variation between base and middle during the first 2 days only. The data represented in table V fit our expectation.

TABLE V. Influence of water on the P.D. of regenerating *Coleus* stem pieces. P.D. between basal node (sign) and middle node in mv.

exp.	intact	number of days after isolation	
		1	2
9a	— 13,46	— 1,77	— 3,80
9b	+ 5,59	+ 8,30	+ 4,51
9c	— 6,01	+ 1,69	+ 12,59
9d	— 8,78	+ 10,43	+ 5,63
9e	—	+ 29,46	—
9f	—	+ 3,67	—

9a only remained negative, but in comparison to its P.D., when intact, a positivation occurred. The negativation which could be expected from the regeneration is superseded by a positivation due to the entrance of water into the basal tissue.

Influence of gravity.

According to V o e c h t i n g (1878) and others gravity influences the polarity to a certain extent. When cuttings of several plants are placed in an inversed position in moist air, polarity is not reversed, but the zone of the root formation is elongated much more in the apical direction. Therefore it will be interesting to control the P.D.

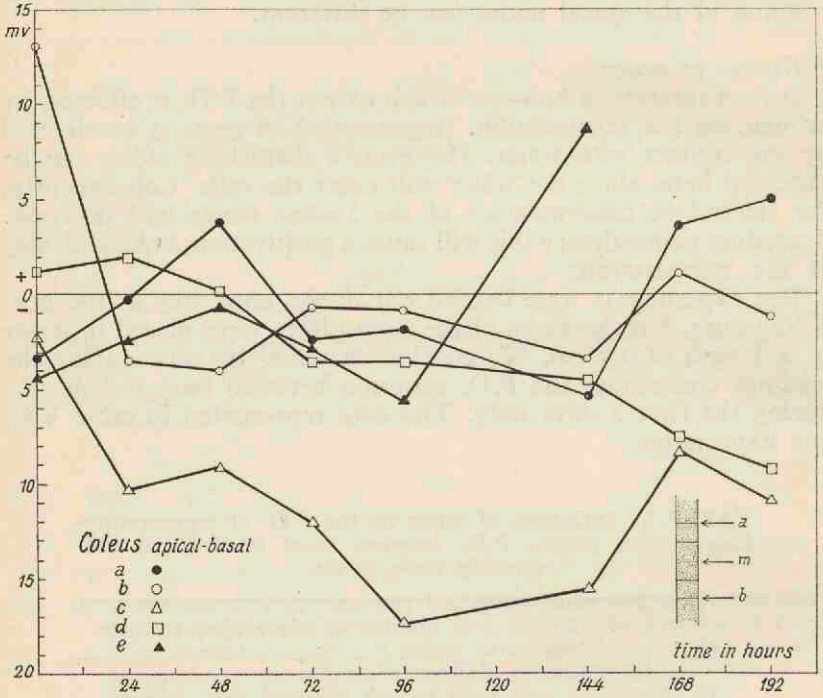


Fig. 23. Variations of P.D. between apex and base of inversely placed regenerating *Coleus* stem pieces. Sign of the potential: a referred to b. No macroscopic regeneration could be observed. Exp. 11.

alterations in regenerating, inversely placed stem pieces. 5 Experiments were taken in this way. The results are shown in fig. 23. As can be seen, the positivity of the apical nodes to the basal ones during the first period did not predominate any more. This does not mean that the apical nodes were negativated to about the same degree as the basal ones (fig. 24). The effect seems to be a disturbing one. The nodes of both, apex and base, did not behave according to any rule. From this we may conclude that the P.D.-reaction in

normally placed cuttings is influenced by gravity, but it is not caused by it. In the latter case the P.D. had to change its sign and that apparently does not happen. So, the phenomena described here are not due to a geo-electric effect. This effect, moreover, causes much weaker potentials.

Another question arises in this respect. It might be possible,

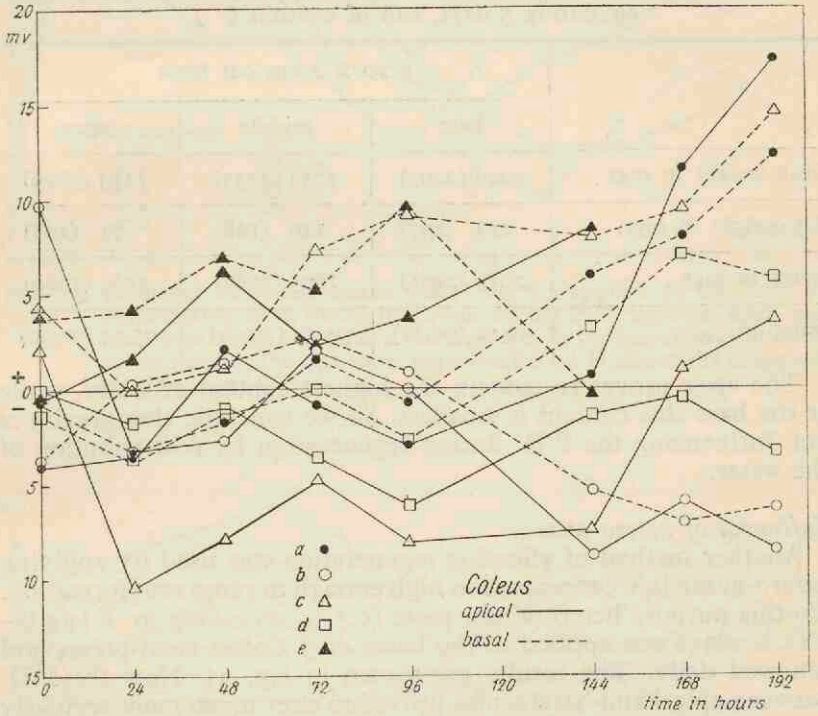


Fig. 24. Individual potential variations of the bases and tips of the objects of exp. 11. See fig. 23. Sign of the potential: see text fig. 22.

hat the movement of water in stem pieces is determined by gravity since the root-pressure and the transpiration have ceased. This seems not very probable since the initial positivation of the apex is due to a negativation of the base. If the water is moving downward a basal positivation could be expected. The experiment indeed proved that this transport does not occur.

To examine the water distribution, 10 *Coleus*-stem pieces were suspended in a moist chamber immediately after isolation and left

in it during 5 days. Then from apex, base and middle of each object a piece of 1 cm length was cut out. Of each 10 pieces together the fresh-weight and the dry-weight have been determined. The same has been done with controls of equal stem pieces. The results are shown in table VI.

TABLE VI. Water distribution in *Coleus* stem-pieces suspended in moist air during 5 days, and of controls ( ).

	10 x 1 cm cut from		
	base	middle	apex
fresh-weight in mgr.	2243 (2495)	1754 (2235)	1135 (1809)
dry-weight in mgr.	224 (227)	149 (168)	71 (128)
water in mgr.	2019 (2268)	1605 (2067)	1064 (1680)
water in %	90,01 (90,51)	91,51 (92,49)	93,74 (92,90)

The apex proves to contain the highest content of water, while at the base this content is smallest. So we conclude that gravity is not influencing the P.D. during regeneration by redistribution of the water.

Influence of hetero-auxin.

Another method of affecting regeneration was used by applying hetero-auxin in a concentration high enough to cause root formation. To this purpose hetero-auxin paste (0,5 % according to Fischnich 1935) was applied to the bases of 5 *Coleus*-stem pieces and renewed daily. The results are shown in fig. 25. Now the P.D. between apical and basal nodes proved to alter much more regularly in comparison to those of the untreated stem pieces (fig. 21). The mean resulting phenomenon, seems to be that *the maximal positivity is reached the day after the isolation instead of reaching it 2 to 3 days later on.*

Moreover, the curves are almost uniform even at prolonged regeneration. In all cases a minimum occurs in which the apex is negativated to the base. This minimum is then followed by some positivation. Only the synchronism is lost after the fifth day.

Considering fig. 26 showing "individual" P.D.-alterations of the apical and basal nodes, it is obvious that the basal reaction during the first day is the most striking phenomenon. Moreover, it appears

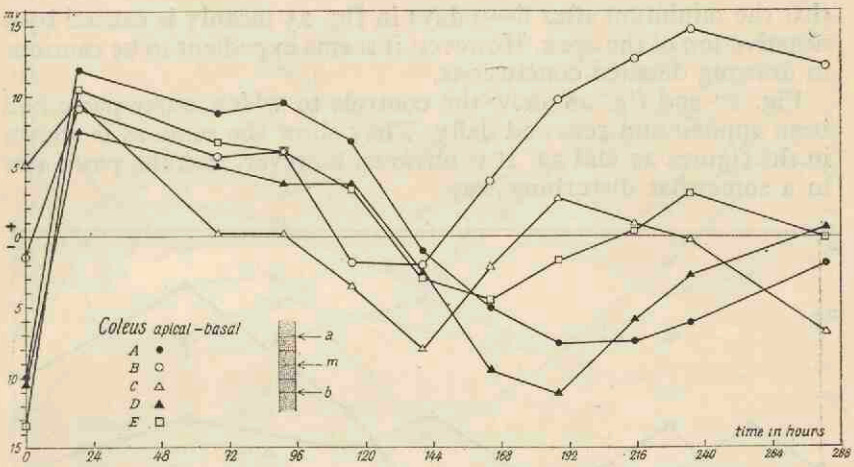


Fig. 25. Influence of hetero-auxin paste (0,5 %) applied to the base to regenerating stem pieces of *Coleus* on the P.D. between apex and base. Sign of the potential: a referred to b. At the bases root primordia are formed; at the tips shoot regeneration (at D also at the subapical node) occurred. Exp. 12.

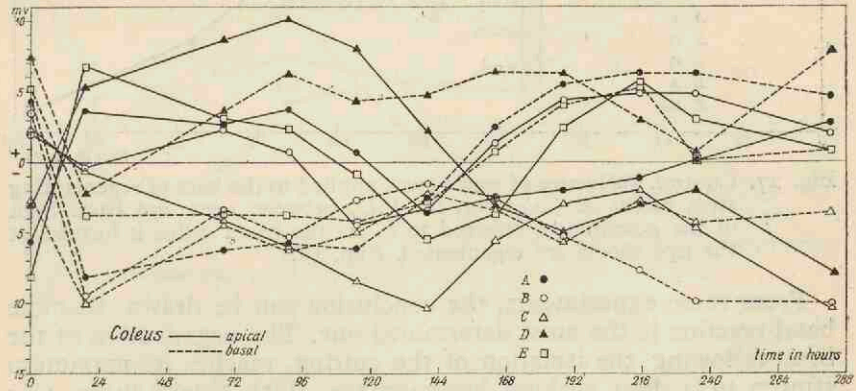


Fig. 26. Individual potential variations of the bases and tips of the objects of exp. 12. See fig. 25. Sign of the potential: see text fig. 22.

that the minimum after 6—9 days in fig. 25 meanly is caused by a negativation of the apex. However, it seems expedient to be cautious in drawing detailed conclusions.

Fig. 27 and fig. 28 show the controls to which water-paste had been applied and renewed daily. They show the same as is shown in the figures 21 and 22. It is obvious, however, that the paste acts in a somewhat disturbing way.

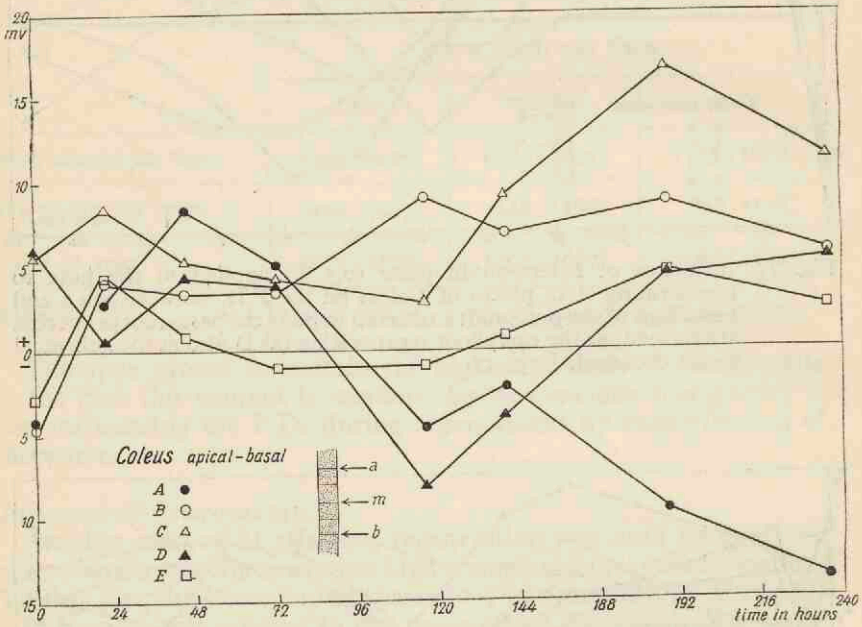


Fig. 27. Control. Influence of water paste applied to the base of regenerating stem pieces of *Coleus* on the P.D. between apex and base. Sign of the potential: a referred to b. At the bases callus is formed, at the tips shoots are regenerated. Exp. 13.

From these experiments, the conclusion can be drawn, that the basal reaction is the most determined one. The negativation of the base following the isolation of the cutting, reaches its maximum after 2 to 3 days without hetero-auxin. With hetero-auxin this maximum is reached in one day. The hetero-auxin seems to accelerate the process and to master it during the first time. The latter fact is derived from the initial synchronism. So a parallelism occurs between the influence of hetero-auxin on the P.D. and the regeneration of roots as far as its accelerating action is concerned.

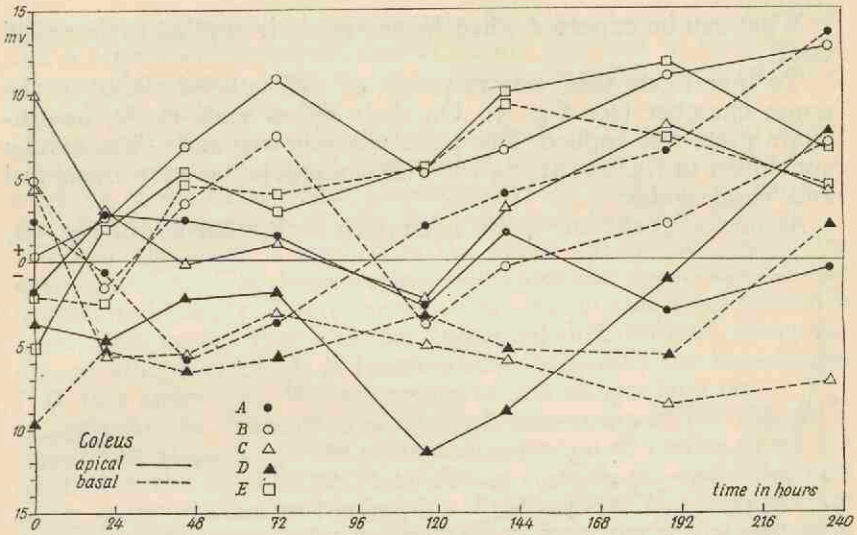


Fig. 28. Individual potential variations of the bases and tips of the objects of exp. 13. See fig. 27. Sign of the potential: see text fig. 22.

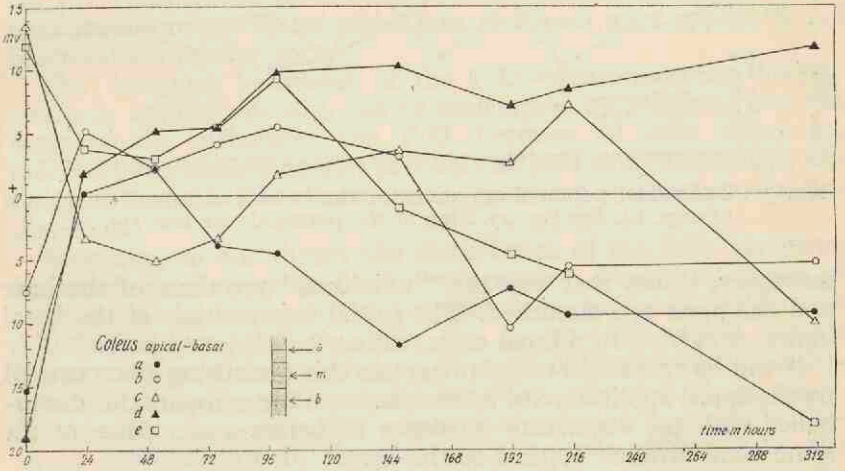


Fig. 29. Influence of hetero-auxin paste (0,5 %), applied to the apex of regenerating stem pieces of *Coleus*, on the P.D. between apex and base. Sign of the potential: a referred to b. A: root primordia at the basal node. B: no regeneration. C and D: shoot regeneration at the apical node, root primordia at the basal one. E: root primordia at the basal node. Exp. 14.

What can be expected when hetero-auxin is applied to the apical end?

To investigate this, 5 stem pieces of *Coleus* were placed in the moist chamber (see fig. 3). On their apical ends 0,5 % hetero-auxin paste was applied. The paste was renewed daily. The results are shown in fig. 29, giving the P.D.-variations between the apical and basal nodes.

As one sees, the curves do not match each other at all. Fig. 30,

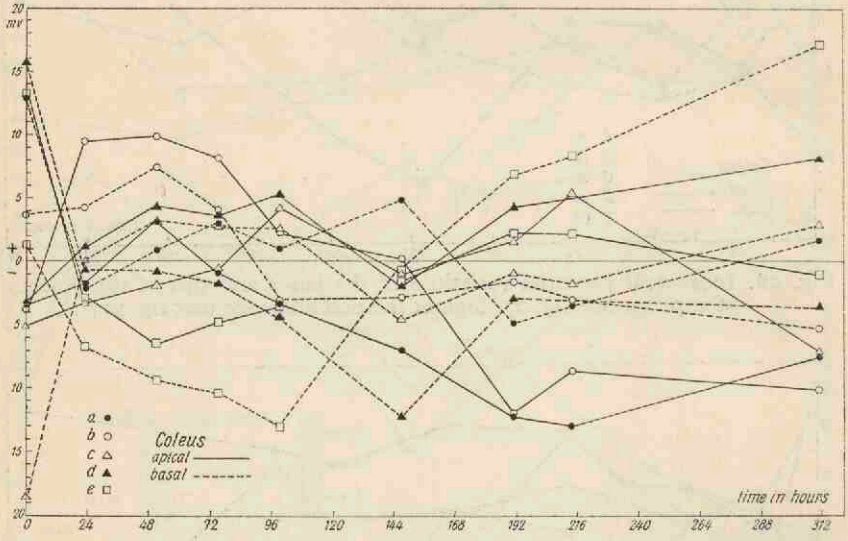


Fig. 30. Individual potential variations of the bases and tips of the objects of exp. 14. See fig. 27. Sign of the potential: see text fig. 22.

moreover, shows that also the "individual" reactions of the base and the apex are disturbed. The initial negativation of the basal nodes, occurring in normal cases, cannot be observed here.

It will be of interest to try to explain this disturbing effect caused by the apical application of hetero-auxin and to compare this disturbance with the regulating influence of hetero-auxin paste of the same concentration applied to the base.

In the first place we want to mention that growth substances are transported polarly. According to Van der Wey (1932) they move in the basal direction only. As to the polar transport of hetero-auxin, we further refer to Hellinga (1937). Theoretically hetero-auxin cannot move in the tissue when it is applied from the

base as happened in our experiments. Perhaps an uptake is possible but a significant transport cannot occur, even not in elements of the vascular system, since the water movement will be reduced considerably in isolated stems.

An intake of hetero-auxin by the basal tissue certainly did occur. Besides of all regeneration phenomena this intake was shown by a swelling of the basal tissue. Consequently we may expect that also the electric behaviour of only the basal part has been affected.

When the paste is applied to the apical cut surface things are quite different. The hetero-auxin will enter the tissue and will be transported basally. Therefore we cannot expect a localised influence here. This means that also the "potential-distribution" along the object will be affected, if hetero-auxin influences the bio-electric P.D. in a direct way. We will return to this subject later on.

Secondly the concentration of the hetero-auxin in the objects is important. If pastes of the same concentration of hetero-auxin are applied to the basal or to the apical cut surface, the concentrations in the plant will not be comparable. Probably the hetero-auxin will diffuse much more easily into the plant from the apical end than from the base. At basal application it will remain near to the base, while when applied apically it will be constantly removed from the tip. Considering these facts, it is not surprising that an initial negativation of the apical nodes fails to appear after application of hetero-auxin on the apex.

The irregular behaviour of the P.D.-variations when hetero-auxin is applied apically, can be understood by assuming that the hetero-auxin influences the P.D. between all cells, which are reached by this substance. As probably it flows along all cells, we can imagine that the P.D.-fluctuations, occurring in the controls, are disturbed.

According to our theory the disturbance of the P.D.-variations by hetero-auxin may be due to two reasons. Firstly migration of the ions of the hetero-auxin through the membranes could cause a diffusion potential. Secondly the hetero-auxin could change the properties of the membranes. It seems difficult to me to understand the disturbing influence by assuming a direct diffusion effect. For, in this case we might expect a correlation between hetero-auxin concentration in the plant and variation of the P.D.: the concentration in the apical part will be higher — at least in the beginning — than that in the middle part of the stem piece. So we might expect, during the first days, some uniformity of the curves representing the variations of the P.D. between apical node and middle one. This, however, does not hold true.

Considering as the next possibility the hetero-auxin as a modifier of the properties of the membranes, the P.D. can be supposed to vary with the varying degree of permeability of the protoplasmic surfaces. In this case the rate and the sense of the alteration of the P.D. depends partly on the concentration of the hetero-auxin too,

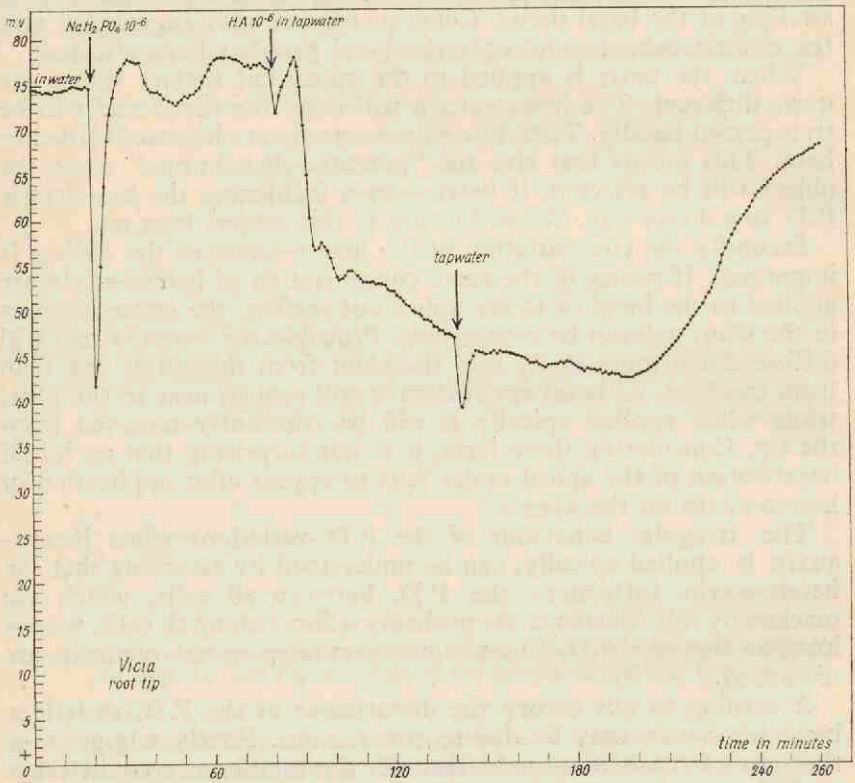


Fig. 31. Influence of hetero-auxin 1 in 10^6 of the potential of the root tip of *Vicia Faba*. Exp. 15f.

but chiefly on the concentration and the nature of the ions already present in the protoplasm and in the vacuolar sap.

Assuming such an effect on the membranes, the disturbance of the P.D.-variations by apical application of hetero-auxin can be understood by admitting that the diffusion potential of *all* cells is altered successively.

In this respect it was of interest to investigate whether hetero-

auxin can influence the P.D. by affecting the membranes. We studied this by applying hetero-auxin to the objects in such a low concentration that no diffusion potential of any importance could be expected. To this purpose the effect of hetero-auxin was compared to that of another electrolyte both in a concentration 1 in 10^6.

Roots of *Vicia Faba* proved to be suitable objects. They were adjusted as shown in fig. 12. Seven experiments were done. In all cases the results were equal. We therefore will confine ourselves to discuss the experiments shown by fig. 31 and 32. Before the beginning of the experiment the roots were placed in tap water and were allowed to recover for 2 to 3 hours. Then the water was replaced by a NaH_2PO_4-solution in a concentration of 1 in 10^6. This phosphate was used since it is eagerly absorbed by roots.

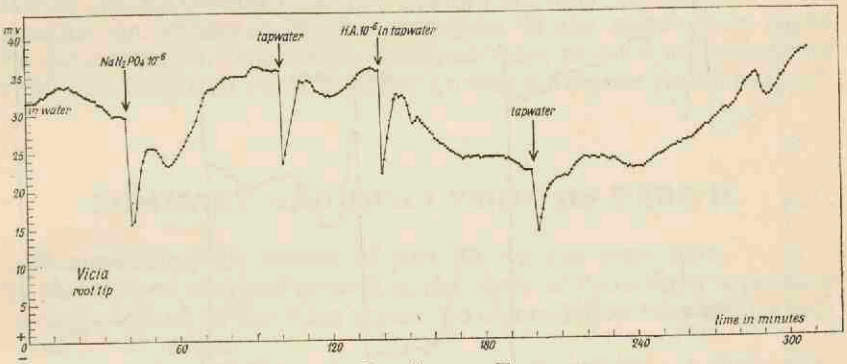


Fig. 32. See fig. 31. Exp. 15e.

The concentration used, however, is too low to cause any noticeable effect. The temporary variation is only due to stimulation. The P.D. having restored itself, the former level is attained. This proves that the effect of the transport of ions is too weak to cause a registrable P.D.-variation. If, however, hetero-auxin in the same concentration of 1 in 10^6 was applied a pronounced decrease of the positivity of the root tissue followed. Although the ion-concentration was less than in the applied phosphate solution, an effect was clearly recorded. We only can interprete this phenomenon by assuming that the membranes are altered. The permeability of the protoplasmic surfaces being changed, the diffusion potential caused by the electrolytes in the cells, will change too. There are two more facts affirming our hypothesis. Firstly the degree of the decrease of the potential depends on the value of the original P.D. (fig. 32). So it is not of an absolute magnitude. Secondly the P.D. is affected for

a long time. As can be seen in fig. 13 and 14, immediately after replacing an "effective" phosphate solution by an isosmotic glucose solution a restoration of the P.D. sets in. Hetero-auxin being applied, the recovery sets in only one hour and a half after substituting it by water. This only can be interpreted by assuming that the hetero-auxin is taken up in the membranes and causes a long-lasting alteration of the surface structure.

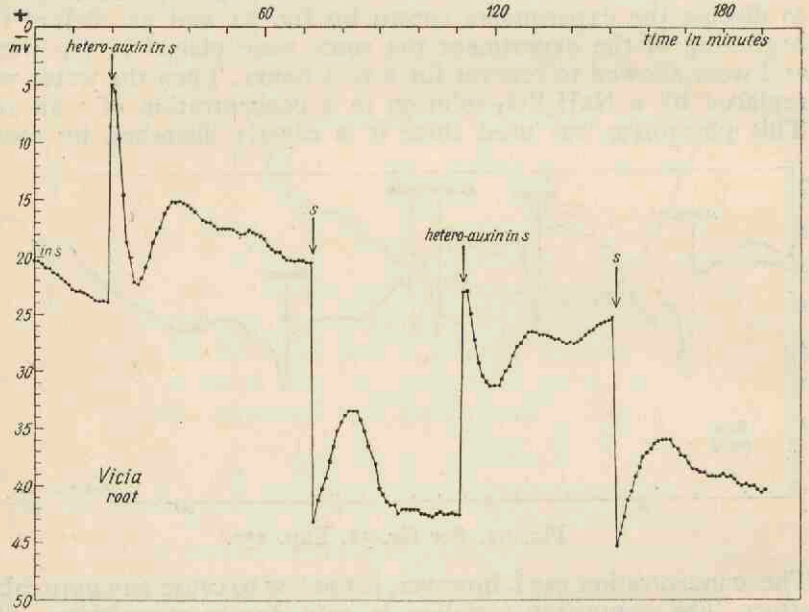


Fig. 33. Difference between the influence of hetero-auxin dissolved in the solution (s) according to Kögl and Haagen Smit and that of the blank solution, on the potential of the root tip of *Vicia Faba*. Exp. 15b.

It seems worth while mentioning that the after-effect of hetero-auxin does not occur when it is solved in a solution as indicated by Kögl and Haagen Smit (1931) and consisting of 0,2 cm^3 acetic acid and 150 mgr KCl in 1000 cm^3 water. This solution is used in experiments on growth since the hetero-auxin is much more active when dissolved in it. By this solution the measured P.D. is negativated. Fig. 33 demonstrates the immediate recovery of the potential after replacing hetero-auxin, dissolved in the mentioned solution, by the blank solution. The fact that in

this solution the P.D. is decreased by hetero-auxin (and not negativated as in fig. 31 and 32) also is a strong argument that the membranes are changed; i.e. their permeability is increased. We will not discuss this phenomenon further.

From these results we conclude that *hetero-auxin acts in the membranes*. This fact seems also important in relation to the effect of auxins on the plasticity of the cell walls.

It will be clear that many problems on the influence of hetero-auxin still remain unsolved. We mention in the first place the effect of various concentrations of it in the regeneration experiments as well as in those on *Vicia* roots. Further experiments on this subject must be left to the future. In our research it only was of interest to study whether hetero-auxin, which affects regeneration, affects the P.D.-variations too. Further we only wanted to control whether an influence on the properties of the membranes could be demonstrated. The results obtained seem to open an interesting field of research in relation to the growth substance problem itself.

SUMMARY AND DISCUSSION OF PART II.

Summarizing the results of part II, we can state that:

1. the zone of maximal growth in the roots of *Vicia Faba* is positive as compared to the other tissue. So a correlation between growth and P.D. exists.
2. the petioles of *Coleus* leaves are negative during 5 days after their isolation as compared to the middle of the under side of the lamina. Then they become positive. If we consider the positivity caused by the outgrowth of the root initials, the negative period seems to indicate the initial reactions of the cells preceding root formation. We are inclined to assume that this negativity is correlated with regeneration.
3. in isolated stem pieces of *Bryophyllum calycinum* the basal nodes are negative as compared to the middle ones during the first days after isolation. There is one exception in seven experiments. The apical nodes react very irregularly.
4. the potential-"distribution" along stem pieces of *Bryophyllum* and *Coleus* (the latter has not been reported here) does not show an essential difference in the behaviour of nodes and internodes during the first stage of regeneration.
5. during regeneration of *Coleus* stem pieces the basal node is negativated as compared to the apical one. This has been studied

by comparing the P.D. between apical and basal nodes as well as that between apical, resp. basal node to the middle one. The apical node yields either positivation or negativation.

6. gravity only partly influences the P.D.
7. the distribution of water in the stem pieces is not responsible for the value and the sign of the P.D.
8. hetero-auxin in a concentration causing root formation seems to accelerate the process, when applied to the base. The negativity of the base reaches its maximum after the first day after isolation. No influence could be observed on the apical node. When hetero-auxin is applied apically, the regularity in the electric behaviour disappears entirely.
9. in roots of *Vicia Faba* the P.D. is decreased by hetero-auxin in 1 in 10^6; it is made probable that the hetero-auxin acts in the membranes and increases their permeability to ions.

In discussing these data we have to start from our conception on the origin of the bio-electric potentials. Considering them as diffusion potentials according to Osterhout, we believe them to depend on two factors. Firstly the degree of the permeability of the membranes must be important. Secondly the presence of diffusible ions is necessary. Though other potentials may occur, we here will consider diffusion potentials only, since they appeared to prevail.

The positivity of growing tissue as compared to a resting one probably will be caused by both factors. Young membranes may be of a different composition as older ones, since the cell wall of the former consists of pectin, while at older stages cellulose or other substances are "excreted". Moreover, growing tissue is extremely rich in water. In this way the electrolyte solution may be different too.

The preceding period of temporary basal negativity seems not to be caused by alterations of the water content. For, if this were so, the water movement would be very complicated: we had to suppose that the water is transported apically during the first time after isolation of the stem piece and basally later on. The same, though in a reverse sense, could be said of the transport of the electrolytes. Such a transport, moreover, cannot be significant since the reasons of the movement of water and of salts in intact plants are absent in isolated stem pieces. Gravity proved not to affect the distribution of water. So we conclude that the P.D.-fluctuations during regeneration are not due to concentration effects.

The hypothesis on diffusion potentials being correct, the *P.D.*-

variations during regeneration must be caused by alterations of the properties of the membranes.

Since it was shown that growth is correlated with maximal positivity of the tissue, the initial negativity of the bases of regenerating stem pieces cannot be caused by this process. This phenomenon was found clearly too in experiments on root regeneration in the petioles of isolated *Coleus* leaves. The initial negativity of the petiole was followed by a pronounced positivity. The latter phenomenon was shown much more clearly in these objects probably since unipolar regeneration occurred here.

We consider the positivity correlated with the productivity of the formed meristem, while the negativity is attended with the initial processes of regeneration. We have shown, that the negativation in the regeneration experiments on stem pieces is not caused by the distribution of water in the plant. Moreover, it seems improbable that it is caused by transport of electrolytes since the root pressure and the transpiration are failing. We therefore concluded that the negativation is due to alteration of the membranes. This conclusion seems to be supported by the results of the experiments on the influence of hetero-auxin paste (0,5 %) when applied to the base. In this concentration hetero-auxin promotes root formation and at the same time it accelerates and regulates the basal negativation. This seems to indicate that the process occurring spontaneously is accelerated and, since we have shown that hetero-auxin influences the P.D. in *Vicia* roots by affecting the membranes, it is probable that this initial negativation is due to an alteration of the membranes.

We therefore conclude that *the polarity of regenerating stem pieces is correlated with typical changes of the membranes at the base, preceding the outgrowth of the formed meristems.*

With the method used such a specific reaction could not be detected in the new apex.

GENERAL SUMMARY.

1. The P.D. along stem pieces of *Bryophyllum calycinum* and of *Coleus hybrida var. Bertha Grosze* is influenced by ether.
2. If no lethal quantities of ether are applied, no "loss" of P.D. is stated. Either a positive or a negative variation of the inherent P.D. occurs. From this it is concluded that the bio-electric potentials, measured in our experiments, are not redox potentials.

3. No Q_{10} is found. Only a short-lasting influence of alterations of the temperature is observed. We therefore conclude that the potentials measured are not due to chemical processes.
4. It is shown that the P.D. can be influenced considerably by the motion of water into — or out from the cells.
5. From these results it is concluded that the potentials in our objects are diffusion potentials according to Osterhout.
6. In the roots of *Vicia Faba* the zone of maximal growth coincides with the most positive potential.
7. The regenerating petioles of *Coleus* leaves negativate during the first days after isolation. After 5 days they positivate.
8. As a rule the basal nodes of *Bryophyllum* stem pieces are negative during the first time after isolation. In *Coleus* this phenomenon is much clearer.
9. Gravity only partly influences the P.D.-variations during regeneration.
10. No redistribution of water in stem pieces could be stated; this factor is not responsible for the alterations of the P.D. in regenerating stems.
11. Hetero-auxin, applied basally in lanolin paste 0,5 %, accelerates and regulates the spontaneous variations of the P.D.'s in regenerating stem pieces of *Coleus*. When applied apically, however, the normal P.D.-alterations are disturbed.
12. It is concluded that in initial regeneration typical changes of the membranes at the base occur. In the new apex such a specific reaction could not be detected.

The investigations were carried out in the *Botanical Laboratory of the State University, Utrecht.* I owe much to Prof. Dr. V. J. Koningsberger for his interest in my work and his valuable criticism.

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STELLINGEN

I

Het is waarschijnlijk, dat de polariteit bij regenereerende plantendeelen berust op een specifieke basale reactie.

II

Hetero auxine beïnvloedt de ionenpermeabiliteit van de grenslagen van het protoplasma.

III

Een gelijktijdig apicaal transport van stikstof en basaal transport van koolhydraten in het phloeem is niet aangetoond.

Crafts, A. S. Plant Physiol. **13**, 791 (1938).

IV

De opvatting van F. W. Went, dat de semipermeabele membranen zich binnen het protoplasma bevinden, is in strijd met verschillende verschijnselen.

Went, F. W. Chron. Bot. **4**, 503 (1938).

V

Het verdient aanbeveling de *Marsiliaceae* onder te brengen bij de *Filicales*.

VI

Het buitengewoon speculatieve karakter van de evolutiegedachte maakt het ongewenscht een plantensysteem op deze leer te baseeren.

Nilson, H. Hereditas **24**, 377 (1938).

VII

Ten onrechte meenen Krueger en Northrop bewezen te hebben, dat de phaag productie een continu proces is.

Ellis, E. L. and M. Delbrück. J. gen. Physiol. **22**, 365 (1939).

VIII

De hypothese van Garrett, dat het parasitair vermogen van *Ophiobolus* in negatieven zin correleert met de activiteit en het aantal van de bodem organismen is niet afdoende bewezen.

Garrett, S. D. Ann. appl. Biol. **25**, 742 (1938).

IX

De opvatting, dat de haemolyseerende werking van lymphe te wijten is aan glycerine, is onwaarschijnlijk.

Johnson, V. and W. Freeman. Am. J. Physiol. **124**, 466 (1938).

X

De bij rekkingsproeven aan de voet van *Helix pomatia L.* verkregen rustkromme en volwaardige herhalingskrommen zijn vrij van verschijnselen, welke berusten op de dynamische component van de viscosoide tonus.