BIOELECTRIC PROPERTIES OF PLANTS AND POLAR TRANSPORT OF THE PLANT HORMONE, AUXIN

Thesis by

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Footnotes:

1. Published in part elsewhere (Clark (1937a))
2. Published in part elsewhere (Clark (1935))
3. Published in part elsewhere (Clark (1937b))

Introduction

The polar basal transport of the growth substances (auxins, growth hormones) in plants is a well known phenomenon, demonstrated first by Went (1928), and studied in detail by van der Weij (1932, 1934). These investigators used the Avena (oat) coleoptile.[1] That the phenomenon is more or less general is indicated by the polar transport of auxin in roots (Cholodny,1934), (Nagao,1936); in hypocotyls of Raphanus (van Overbeek,1933), Pisum (Skoog,1936); in leaves (Avery,1935); in the Avena coleoptile (Went, 1928), (Laibach and Kornmann,1933), (van der Weij, 1932,1934), and (Skoog,1936); in corn coleoptiles (van Overbeek,1936); in Elaeagnus (woody cutting) (van der Weij, 1933); in stems of Coleus, Vicia, and Phaseolus; and in hypocotyls of Vicia, Phaseolus, and Lupinus (Mai,1934).

Other workers have reported non-polar transport of auxin in plants. Hitchcock and Zimmerman (1935) and Zimmerman and Wilcoxon (1935) have shown an apical transport of heteroauxin (indole-3-acetic acid) and several other active compounds in stems of Helianthus tuberosus, Nicotiana tabacum, and in Lycopersicum esculentum, as indicated by induction of adventitious roots and by epinastic response of leaves. Loehwing and Bauguess (1936) have shown that heteroauxin could by absorbed by the root system of potted seedlings of Matthiola incana and be transported apically to increase the stem elongation over that of the controls. Both

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1. Coleoptiles are leaf-sheaths which envelop growing points and first foliage leaves of grass seedlings.

the Boyce-Thompson workers and Loehwing and Bauguess have merely shown that auxin applied in high concentrations can be carried in the transpiration stream. This, of course, will not give polar transport. Higher concentrations of auxin may have effects which are not normally encountered. (e.g. high concentrations applied at the base of Pisum cuttings induce roots there, whereas in the lower, more physiological concentrations roots may be induced at the bases only by applying auxin at the morphological tips. Went and Thimann (1937)).

Laibach and Fischnich (1936) have shown that the transport of heteroauxin was not strictly polar in leaves of Coleus and in cotyledons of Cucumis sativa, but that transport could occur apically. The apical transport was much smaller than basal transport, however. Avery (1935), as mentioned above, found only basal transport. Avery determined this by diffusion of the auxin occurring naturally in the leaves, whereas Laibach and Fischnich applied heteroauxin in a concentration of 0.5 percent in lanolin, an altogether unphysiological concentration.

Jost and Reisz (1936) demonstrated apical transport of high concentrations of heteroauxin in Avena coleoptiles. This was seen from the growth of sections with their basal ends immersed in the auxin solutions, and from actual transport experiments in which auxin was collected in agar blocks at the apical ends of sections supplied with auxin in agar at the basal ends. The apical transport was, however, much less pronounced than the normal basal transport, and hence semi-polarity still exists. The concentrations

of auxin used in the transport experiments was 1:200,000, the length of the sections 10 mm., and the time of transport overnight. As to its effect on growth, the apical transport may have been effected by capillarity in the hollow coleoptiles. Regardless of these findings, Went, van der Weij and others always observed strictly basal transport in short sections (1 to 4 or 5 mm.) when shorter periods of time (1 to 3 hrs.) were used. Moreover, as van der Weij (1932) mentioned, when higher concentrations of auxin are used (1:200,000), the auxin may be transported by capillarity in films of water condensed on the surfaces of the sections. At any rate, although strict polarity is always difficult to observe when high concentrations of auxin are used, the polarity still dominates apical transport. The concentrations ordinarily used are of the order of $1:10^6$ or less (Went and Thimann (1937)).

As mentioned, Cholodny (1934) and Nagao (1936) demonstrated a polar basal transport in roots. Gorter (1932), de Haan (1936), and others claim otherwise. These controversial statements will not be discussed at this point, since they have no bearing on the polar basal transport in Avena.

With regard to the various reports in the literature in which it is claimed that there is no strictly polar transport, the discussion of Went (1937) becomes pertinent. Went studied the transport of indole-3-acetic acid in the Avena coleoptile sections as a function of concentration of the applied auxin. He found that apical transport from base to apex (reversed transport) occurred if the applied basal concentration of auxin was at least one hundred times in excess of the applied apical concentration; in other words, that at each concentration of applied auxin, a certain amount in excess of auxin is transported

normally than is transported in the reverse direction. This certain amount in excess is independent of the concentration gradient and is therefore similar in every respect with ion accumulation in certain plant cells. (Hoagland and Davis, 1922-1929, see Hoagland and Broyer, 1936; Steward, 1936). In these plant cells there is also a certain quantity of ions accumulated in excess over ions lost; this process being independent of the external concentration. As mentioned above, Went and Thimann (1937) showed that higher concentrations of auxin applied basally will induce roots in pea cuttings. The amount of auxin necessarily applied basally exceeds that necessarily applied apically approximately a hundredfold, thus illustrating a second example of the analogy to ion accumulation. (Nitella, Valonia, potato tuber, barley roots).

1. The Mechanism of Polar Transport

The mechanism of polar transport (or of ion accumulation) is as yet little understood. Went (1928) showed that the transport was always in a basal direction in the Avena coleoptile when physiological concentrations were used; that in his special case, the velocity of this transport was about two hundred times greater than that of ordinary diffusion (being 10 mm. per hour); and that its polarity was unaffected by gravity. van der Weij (1932, 1934) confirmed these findings and, in addition, showed that the transport would occur against a considerable concentration gradient, suffering no appreciable change. He also found that the velocity of the transport was reversibly lowered to that of diffusion when the temperature was lowered to 0°C. At this temperature, however, the polarity of the transport persisted. Polarity, on the other hand, was reversibly abolished by ether narcosis(van der

Weij (1932,1934)). Bonner (1934,1936) indicated that transport was dependent upon the presence of oxygen. With regard to the independence of transport and gravity, Pfaeltzer (1934) found that 14.5 x gravity, produced by a centrifugal field, had no effect on polar transport in the Avena coleoptile.

a. Protoplasmic Streaming as the Mechanism

van der Weij (1932) concluded that polar transport was a "vital process" but that protoplasmic streaming had nothing to do with it, since the velocity of transport was independent of temperature down to very low values, e.g. 0°C., while the velocity of protoplasmic streaming depended upon temperature within wide limits, citing Lambers (1926). This view is supported by the observation of Schumacher (1936) that fluorescein shows polar diffusion in the plasma of stem hairs of Cucurbita pepo, the rate and direction of this transport being constant, while the rate and direction of protoplasmic streaming varied. Bottelier (1934) favoured some correlation between protoplasmic streaming and transport, finding the velocity of streaming (3 cm. per hour) to be constant between 17° and 35°C., while the amount of protoplasm in actual rotation (streaming intensity) increased with temperature, just as transport intensity increases. (van der Weij (1932)). Furthermore the velocity of transport (one cm. per hour) is too great to be explained by a diffusion process unless it is of the nature of the model described by van den Honert (cf. below). Bottelier (1935) also found that oxygen limited protoplasmic streaming, as it presumably does transport.

From this discussion it is probable that protoplasmic streaming has nothing to do with the polarity of transport, but may be a factor in its velocity. This theory is also supported by observations described in this paper in which transport may be abolished while protoplasmic streaming continues.

b. Activated Diffusion as the Mechanism

A possible mechanism for transport is that suggested by several workers (cf. Brinkman and Szent-Györgyi (1923); van den Honert (1932); Söllner (1933)), which demonstrates the transport of surface-active substances at interfaces whose interfacial tension has been lowered at one end by the addition of this substance ("spreading"). The velocity of the transport of KOH in van den Honert's model was in one case 68,000 times greater than that of ordinary diffusion of KOH.

Mason and Maskell (1928) found that the diffusion of sugar in the cortex of the cotton plant complies with the rules of concentration gradients and directional flow for diffusion, but that the velocity was between 20,000 and 40,000 times greater than the ordinary diffusion of sugar would be expected to exhibit. Mason and Phillis (1936) found that oxygen was necessary for such transport in the cotton plant, and state: "It is suggested that the mechanism activating diffusion consists in some special organization in the cytoplasm, maintained by metabolic energy, whereby the resistance to solute movement is so reduced that materials diffuse in the sieve-tube at rates comparable with those in a gas". Phillis and Mason (1933), moreover, have shown that sucrose is transported against a concentration gradient in the leaf of the cotton plant. This recalls the similar transport of auxin

against a concentration gradient in the Avena coleoptile, but is different in that the sugar transport is not as polar. The "organization" spoken of by Mason and Phillis is as yet unknown, although the model of van den Honert is suggestive.

Hence "activated diffusion" may be a factor in determining the velocity of auxin transport in plants, particularly since auxin is surface active (; Kögl, Erxleben, and Haagen-Smit (1934)), but it is difficult to see how such a mechanism could explain polarity.

c. Electrical Polarity

Brauner (1927) found that the underside of horizontally placed plants became electropositive to the upper side. He also found that the shaded side of illuminated seedlings developed an electropositivity with respect to the illuminated side. Later Brauner and Bünning (1930) correlated the geo-electric effect with electrotropisms. Cholodny (1927) had already developed the theory that the plant growth hormone is electrically transported in the plant, accumulating more on one side than on the other, thus causing differential growth and a tropism. Dolk (1930, 1936) assumed that the growth hormone was an acid, and suggested that the dissociated anion would be transported to the geo- or photo-induced positive pole. For a review of this literature, see Went (1932).

From a survey of older literature on the subject of electrical polarity in living organisms, Went (1932) formulated in his "Botanische Polaritätstheorie" the idea that the dissociated anion of auxin is transported longitudinally in the plant as a

result of the inherent electrical polarity of the organ in question. By this time it was known that auxin was a weak acid (Kögl and Haagen-Smit (1931)). Applying his theory to seedlings, Went suggested that the apical end of a seedling was electro-negative to the basal end, and that auxin anions were electrically and polarly transported basalward. He supported this theory with experiments demonstrating that Impatiens cuttings exhibited a bipolar staining in acid and basic dyes. (cf. experimental part of this paper).

Previous indirect evidence has seemed to favour a causal relation between lateral transport of auxin and bioelectric potentials in plants.

Brauner and Bünning (1930) found that the ventral side of horizontally placed stems and roots became electropositive with respect to the dorsal side. They found that positively geotropic organs (roots) bend toward the negative pole when placed between oppositely charged metal plates in air. (Field 640 volts/cm.). Negatively geotropic organs (coleoptiles) bend to the positively charged plate.

Amlong (1933) confirmed the geoelectric measurements of Brauner(1927) that the ventral side of horizontally placed shoots and roots become electropositive to the dorsal side. He also succeeded in inducing curvatures in such organs by application of electrolyte solutions. These solutions were applied either on one or both sides of the organ in question. Amlong believed that the resulting "chemotropisms" could be explained by the concentration potential-differences (P.D.) resulting by the unequal electrolyte

concentrations. Shoots bend away from, and roots toward the more dilute solutions, thus the positive pole of a P.D. between cell sap and exterior.

He claimed an essential identity between such concentration potentials and geoelectric potentials. Lastly he confirmed Brauner and Bünning in the electrotropisms induced by a static electric field. (Field strength approximately 2000 volts/cm.). Roots bent toward the negative pole, shootstoward the positive. He claimed that the latter tropisms are explained by the fact that in a static field, an opposite charge is induced on the side of the organ facing the pole, thus behaving essentially as a condenser. The time relations and direction of bending during geotropisms "Chemotropisms" and electrotropisms were all similar. These findings were interpreted as proving that the P.D.'s induced either by gravity or applied electromotive force (E.M.F.) actually determine a lateral redistribution of auxin, subsequent differential growth of the two sides of the shoot, and hence a tropism. The negatively charged auxin anion is transported to the positively charged side of the organ, increasing growth there, and hence a curvature away from that flank. In roots, since auxin inhibits growth, the negatively charged dorsal side of a horizontally placed organ will grow more than the positive side where auxin is accumulated and where it inhibits growth. Koch (1934) found that roots will bend toward a positive pole when between two charged platinum electrodes in water. No opposite charge is induced since the roots were immersed and therefore were in a conducting medium.

Avena coleoptiles bend toward the positive pole when under water. Koch claims the coleoptile cuticle acts as an insulating dielectric and hence an opposing E.M.F. is induced on that side, thus, the side toward the negative pole becomes electropositive. No measurements were made to confirm this hypothesis.

Koch believed, as did Amlong (1933), Went (1932), Boysen-Jensen, (1936), and in general, most plant hormone workers, that auxin is laterally transported to a positively induced pole (photo-, geo-, electro-tropisms) where it increases growth over and above that of the other side and hence causes a curvature. He claimed that auxin is carried to a positive pole in actual diffusion experiments of auxin-a in agar. Auxin-a (cf. Went and Thimann (1937); Kögl (1934)) was collected from Avena coleoptile tips by diffusion into agar blocks, at the same time an electric current was passed for 1 to 3 hours between two platinum electrodes inserted in the agar. The source of E.M.F. was a 4.4 volt dry cell. The agar block was then cut into zones and each zone analyzed by the Avena technique (Went and Thimann (1937); see also below). Two Avena test plants were used for each determination. The tests showed that auxin was transported toward the positive pole. The experiments are to be criticized in that only two test plants were used. The percent error in the Avena technique is about 10%, when all conditions are ideal. Usually twelve plants are used for each determination. It is dangerous to draw conclusions from only two test plants.

Koch then essayed to prove that a tropism may be induced in Avena coleoptiles by lateral transport of the inherent auxin to an applied positive pole. Platinum electrodes were inserted in

the coleoptiles and a small current passed. Traumatropisms obscured any effects, so that Avenas were no longer studied. Helianthus and Lupinus seedlings were then treated similarly, the positive electrode being inserted just below the cotyledons, and the negative electrode in the opposite side two centimeters below the first. Current was passed from a 4.4 volt dry cell for 30 minutes. 90 minutes later a curvature away from the positive electrode occurred. Controls were treated similarly but no current was passed. No curvatures occurred. If curvatures resulted from such injuries they would have been toward the site of greatest injury. (Cholodny (1931), Tendeloo (1927), Stark (1916)).

Koch then claimed that he could counteract or facilitate the tropisms induced by light and gravity, by means of applied E.M.F.'s. Koch found that the minimum P.D. necessary to cause a tropism in upright seedlings was about 10 millivolts; and that 10 mv. would also inhibit geotropisms if the electrodes were oriented such that the positive pole was in the dorsal surface of the horizontally placed plant.

Katunskij (1936) confirmed Brauner and Bünning (1930), Amlong (1933), and Koch (1934) by showing that coleoptiles bend toward the positive pole in an electrostatic field; roots toward the negative pole. To prove that auxin is transported laterally to a positively induced pole in plant organs he split Avena coleoptile tips (2-4mm.) and placed the coleoptiles in a vertical direction in a transverse electric field for 20-30 minutes so that the slit was (1) parallel and (2) perpendicular to the field. After about four hours he observed curvatures of 60-80° toward the positive pole when the slit was parallel to the lines of force, but no curvature if the slit was perpendicular to the lines of force.

This indicates that auxin is actually transported laterally to the side next to the negative pole. If the organ has an induced positivity on this side, as is claimed, the evidence would seem clear. In this as in all electrotropism work, however, no measurements have been made to prove that this side does actually become positive. Moreover, a cut surface such as described above might prevent lateral migration of auxin across its face because of (1) injury potentials or (2) enzymatic destruction.

Katunskij also observed that auxin diffusion from coleoptile tips into agar is facilitated if the agar block is made a positive pole; and the diffusion is inhibited if it is made a negative pole. This supports Kögl (1936) (see later section on transport) who found that auxin diffuses more easily from agar blocks into coleoptiles when the latter is made a positive pole.

Thus the evidence extant favours a causal relationship between auxin transport and bioelectric polarity. The evidence seems fairly clear-cut in tropisms, but is not convincing in longitudinal polar transport. This point will be discussed in a later section. With regard to lateral transport in tropisms, it may well be that light and gravity induce differential changes in permeability of cell membranes to auxin, so that it is more easily transported laterally across the stem axis, than longitudinally.(Brauner (1923), Small (1918)). This, however, is just another way of saying that the bioelectric polarity is related to lateral transport. A differential change in permeability of the two sides of a horizontally-placed organ will no doubt cause unequal diffusion potentials between these two sides. The other assumption is that the effect of

gravity is due to the geoelectric potentials studied by Brauner (1927,1928), Brauner and Bünning (1930), and Brauner and Amlong (1933).

Brauner and Amlong (loc. cit.) have shown that the effect is probably due to an effect of gravity on ion mobilities, and therefore on diffusion potentials, rather than on effect of gravity on electrokinetic and therefore streaming potentials.

The photo-induced potential differences theoretically causing lateral transport of auxin and therefore phototropisms, is not well understood. This will be discussed later. The differences between lateral and longitudinal transport will also be discussed later.

As stated above, Went believed that auxin anions are "cataphoretically" basally transported to an inherent electropositive pole in the plant. Bonner (1934) has shown that auxin is active in its effects on cell elongation only if it is in the undissociated form. No experiments exist which conclusively prove that auxin anions resulting from dissociation of the monobasic acid alone comprise the transportable form of the hormone. Koch (loc. cit.) rightly states that if the negative ion of the hormone alone is transported, a cataphoretic concept is untenable. Electrolysis alone would account for such a migration. Koch further points out that the undissociated molecule may be adsorbed to a larger negatively charged particle and that this particle may then be cataphoretically transported across charged membranes to a positive pole. The possibility of auxin being carried in an electrosmotic current of solution is excluded, since such a current would travel toward a negative pole.

The question of the possible correlation between lateral or longitudinal transport of auxin, and bioelectric polarity is therefore an open one.

The experiments of van der Weij (1932) and Bottelier (1934) favour a causal connection between protoplasmic streaming and the velocity and intensity of longitudinal transport, but the mechanism of the polarity of transport remains experimentally unsolved, as does the mechanism of ion accumulation and polar secretion across many plant and animal membranes which perform concentration work.

The purpose of the following paper is to reexamine the postulated correlation between bioelectric polarity and polar transport of auxin in plants. The work will be divided into two sections, namely: (1) Electrical polarity, and (2) Electrical polarity as related to polar transport.

Part I

INHERENT ELECTRICAL POLARITY

1. Polar Dye-uptake

Inherent electrical polarity may be measured by the polar uptake of oppositely charged dyes. As mentioned in the introduction, Went (1932), to substantiate his electrical polarity theory, demonstrated a bi-polar uptake of dyes in Impatiens cuttings. Negatively charged (acid) dyes penetrated most at the cut apices, and positively charged (basic) dyes at the bases of immersed cuttings. Referring to his paper, it is seen that his acid dyes included light-green, acid green, quinolin yellow, and methyl orange; while the basic dyes included safranin, methyl violet, prune pure, neutral red, thionin, and gentian violet. de Haan (1936) investigated differential staining in geotropically bending Vicia roots and found that basic dyes accumulated most on the convex side, as would be expected from Brauner's (1927) finding that this side was electropositive to the concave side. de Haan classified light-green and methyl orange as "anode-colouring" (basic) which contradicts Went's classification.

With respect to such controversies, electrophoretic experiments were performed with all of the dyes used in the experiments about to be described. The dye solutions (0.5 %) were made up in distilled water at pH 6.0, and a current passed from zinc electrodes through U-tubes containing the dyes. The products of electrolysis at the electrodes were washed away by automatic siphons during the current passage. At the end of the experiments, the pH values at the electrodes were found to be unchanged. When such experiments were performed, all dyes classified as acids were found to be

cathodic and all basic dyes anodic. Keller has said, however, that dye particles reverse their charges in protoplasm due to the fact that colloids adsorb the particles and impart to them the charge of the colloidal particle. (Keller (1929, 1932); Gicklhorn and Keller (1932)). An exception exists, he says, when the dye is in such excess that the charge of the adsorbed dye particles neutralizes that of the colloidal particle. In this case the charge of the dye particle is not reversed. Lauer (1930) could not confirm Keller's claim that protoplasm reverses the dye particle charge. de Haan himself found a lack of agreement between Keller's tables and "test-object" Hedera helix, used by Keller in making up these tables. Due to such uncertainties, it was assumed that the present electrophoretic experiments, conducted at pH 6.0, (which is approximately that of the cell content in plants) gave the true charge of the dyes used in the following experiments with plant cuttings. It was likewise assumed that the plant did not alter the sign of the charge on the particles. Electrical measurements later bore out this last assumption.

Pringsheim (1933) criticized Went's dye-uptake experiments in that the distances penetrated (1 mm. at best) observed by Went could not allow conclusions to be drawn. According to this criticism, de Haan's observations, based on the number of cells stained in cross sections of Vicia roots, would be even less valid. From such considerations, it became of interest to repeat Went's experiments and to test these findings with electrical measurements. The following descriptions show that Went's observations can be clearly duplicated.

Etiolated Impatiens balsamina seedlings grown in sand in the

dark-room at constant temperature and humidity, were prepared by cutting away the cotyledons and the parts underground. Two to three cut hypocotyls were placed in upright test tubes filled with the dye solutions in 1 percent sucrose. The experiments were run in the dark 15 to 24 hours. After this time the hypocotyls were removed and examined in daylight, the amounts of penetration being noted and recorded as shown in table I. The concentrations of the dyes used were from 0.1% down to 0.001%. In the case of the basic dyes, the higher concentrations caused more rapid infiltration of the tissue. In the case of the acid dyes, the lower concentrations frequently showed little or no staining at the apices and bases of the hypocotyls. The results of one experiment are summarized in table I, sections showing indistinguishable staining or infiltration being discarded. Three other experiments, run at other times, showed essentially the same thing, so that the table represents a typical case, and a confirmation of Went's experiments. The numerals are represented on the same scale as in Went's tables for purposes of comparison.

Table I

Bipolar Dye Uptake in Impatiens Cuttings

Key:

0---------No staining
2---------Slight staining
4---------Up to 0.5 mm. penetration
6---------1 mm. penetration
8---------1.5 mm. "
10---------2.0 mm. "
14---------3.0 mm. "

Negative dyes

Dye	Penetration	
	Apex	Base
Trypan blue	2.0	3.0
Light-green	7.0	0.5
Methyl blue	5.0	2.0
Methyl orange	7.0	2.5
Congo red (colloidal)	4.5	6.5
Orange-G	10.0	1.0
Average ----	6.0	2.6

Positive dyes

Safranin	7.0	9.3
Methyl violet	5.0	11.0
Neutral red	3.5	11.0
Janus green	5.0	9.0
Bismark brown	4.0	9.0
Thionin	5.0	7.0
Methylene blue	3.5	12.5
Cresyl violet	4.0	8.5
Brilliant cresyl blue	6.0	11.0
Nile blue A	8.0	14.0
Average----	5.1	10.2

For purposes of comparison, Went's averages for negative dyes were: apex 2.5, base 1.1; for positive dyes: apex 2.4, base 3.7. It is noteworthy that the greatest penetration Went obtained was, at the most, one millimeter; while as much as three millimeters penetration was observed in the present work. Presumably sucrose in the solutions maintained oxidations and kept the tissues in a more normal condition during the time they were immersed.

Ramshorn (1934) criticized Went's conclusions from such dye experiments, on the ground that actual electrical measurements of Impatiens cuttings showed electropositivity of the cut apices with respect to the cut bases. Upon repeating such measurements, measuring the dye uptake at the same time, quite the opposite was found. The experiment below is typical:

> Impatiens hypocotyls were cut and placed horizontally with each cut end in a cup of the dye solution in Shive's solution made up to 1% in sucrose. Agar-0.1 N KCl bridges from each cup led to a 0.1 N KCl solution in a cup in which the side-arm of a Zn, saturated $ZnSO_4$ half-cell could be placed. The potential differences between apex and base were measured with the Wulf string electrometer described later in this paper. Table II shows the potential differences (P.D.'s) expressed in millivolts (mv.), the polarity being expressed as the sign of the tip with respect to the base. The dye penetration is represented in millimeters, the recorded figures being the averages. Several (3 to 5) sections were used for each dye solution, hence the electrical measurements represent the average of the several cuttings in parallel circuit. The dye concentrations were 0.05%. All dye-charges were rechecked electrophoretically. The cuttings varied from 6 to 10 mm. in length.

In other experiments, the P.D.'s were read frequently over a period of several hours. It was revealed that the apex is at first positive[1] with respect to the base. In two hours after setting up

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1. In this paper the electrical polarity is expressed with respect to the external circuit.

Table II

Measured Electrical Polarity and Bipolar Dye Uptake in Impatiens Hypocotyls*

Negative dyes	Potentials 6:00 P.M. Mv.	Potentials 12 hrs. later Mv.	Dye Uptake Apex Mm.	Dye Uptake Base Mm.
Light-green	+11.0 (tip+)	-14 (tip-)	1.5	0.0
Methyl blue	+13.0	- 5.0	Trace	Trace
Methyl orange	+12.0	- 7.0	7.0	5.0
Orange-G	+15.0	- 5.0	1.5	0.0
Average:	+12.7	- 7.7	2.4	1.2
Positive dyes				
Safranin	+9.0	- 7.0	Trace	1.5
Methyl violet	+7.0	-12.0	1.0	2.0
Bismark brown	+3.0	- 3.5	Trace	Trace
Methylene blue	+1.0	- 1.5	0.5	1.5
Cresyl violet	+6.5	- 2.0	1.0	2.0
Brilliant cresyl blue	+9.0	- 9.0	Trace	Trace
Nile blue A	+6.0	- 9.5	1.2	2.5
Auramine	+9.0	- 6.0	5.0	7.0
Average:	+6.3	- 6.3	1.1	2.1
Controls				
Shive's solution	+8	-12		
Crone's solution	+7	-20		

*The hypocotyls were cut and placed in the cups at 5:00 P.M. (3 per dye).

the experiment it is electronegative, and remains so until the tissues appear abnormal (flaccid), after which the P.D. drops toward zero. This time-relation in establishing electrical polarities will be discussed in more detail under the section on P.D. gradients.

These electrical measurements confirm the dye uptake experiments, and thus the polarity first claimed by Went is real; that is, the apex of the Impatiens hypocotyl is electronegative to the base.[1]

Ramshorn's conflicting results may be explained by the time-relations in establishing the normal electrical polarity. It will be noticed that the apical negativity did not appear at once, but two hours or more elapsed before the tip became negative.

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1. The possibility remains that the electrical polarity revealed by dye uptake and the polarity revealed by measurements are alike by coincidence.

2. Measured Electrical Polarity

a. Brief review of the literature

Many attempts have been made to correlate morphological and physiological polarity with electrical polarity in living organisms. (cf. Ramshorn (1934); and Went (1932)). Child's school (Child and Hyman (1926); Hyman and Bellamy (1922)) claimed that the regions of highest metabolic rate in hydroids (apical regions) may be electronegative to other regions: Lund (1928,1931a),and Lund and Kenyon (1927), on the other hand, claimed that electrical polarity was dependent upon oxidation-reduction potentials (cf. discussion). Usually parts of polar tissues (apical end of hydroid stems, onion root tips) having the highest rates of oxidations were electropositive to other regions. Ramshorn (1934) stated that in seedlings of several different plants, electropositivity was directly linked with growth rate, such that potential distributions paralleled growth rate distributions. Barth (1934a)showed that in several different hydroids the electrical polarity varied, some hydroids exhibiting apical electronegativity while others showed positivity. It is difficult to make generalities from such conflicting statements.

In general, the cortex of a root apex is held to be normally electropositive to that of the base. (Lund and Kenyon (1927); Marsh (1928,1930); Ramshorn (1934)). In hypocotyls and coleoptiles of seedlings, the apical cortex is said to be electropositive to the basal. (Ramshorn (1934). In the Douglas fir, the apical cortex is as Ramshorn claimed to be the case in seedlings,i.e. electropositive with respect to basal; while the apical wood is electronegative to the basal. (Lund (1929, 1930, 1931b)). In

seedlings with internodes, in general, the nodal regions are electropositive to internodal zones; and the total polarity from apex (just below cotyledons) to the base of the stem shows an electronegativity of the tip to base, (Rehm (1936); Clark, this paper). Ramshorn (1934), on the other hand, claimed the opposite, i.e. that the tip was electropositive to the base. (cf. discussion).

The following descriptions concern themselves directly with the determination of the electrical polarity of the Avena seedling, and of a few other seedlings.[1]

Experimental

In determining the electric potential differences (P.D.'s) in Avena, various types of electrodes, contacts, and recording instruments were tried. A Dolezalek electrometer was used as the recording instrument at first, but was found to be difficult of manipulation and to have too long a period. A Compton electrometer is subject to the same criticism, although it is somewhat better than the Dolezalek instrument. Lindemann electrometers are too insensitive. A potentiometer is apt to draw some current from the living tissues.[2] Hence a Wulf string electrometer, constructed in the shops of this institute, was employed for the majority of the measurements. (cf. Wulf (1933)). Figure I represents the hook-up.

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1. Seedlings are preferred to mature, green plants because they have been more extensively studied, are more quickly obtained, and because they can be used in the dark. Light complicated the physiological behavior.

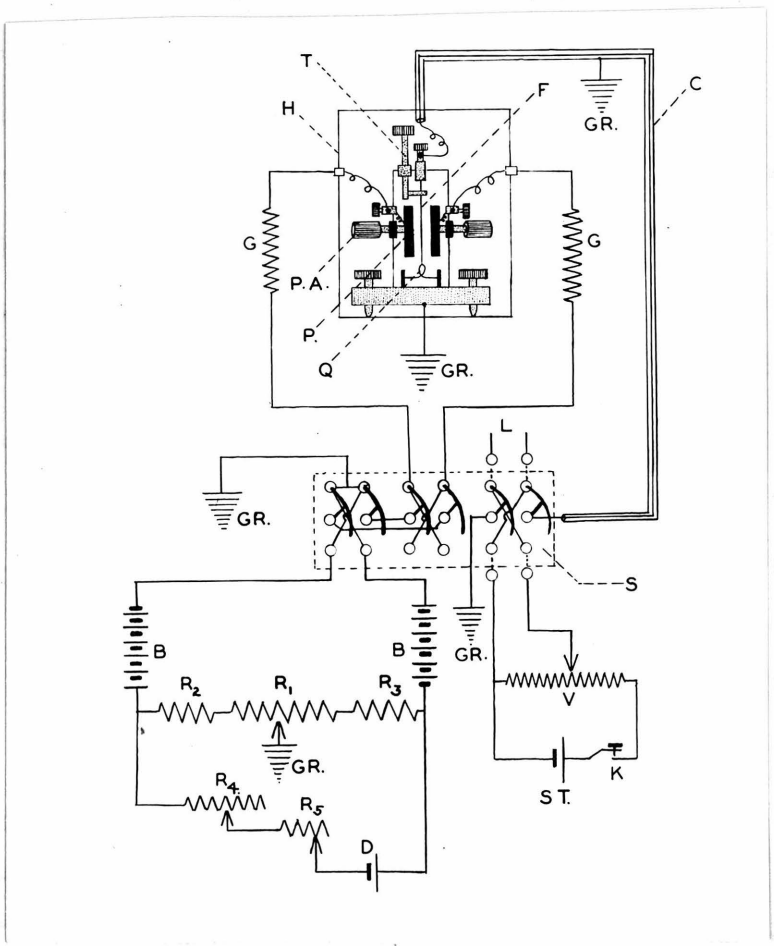
2. Fürth (1929) states that currents of 10^{-10} A. caused by shunting bioelectric P.D.'s will polarize cells or tissues, Marsh (1930) also demonstrates changes in inherent E.M.F. of onion roots when current is drawn.

Figure I

String Electrometer Circuit

Key

B	Six 45-volt heavy-duty Burgess B-batteries (long shelf-life)
C	Copper-tube shielding for fiber lead
D	1.5-volt dry cell battery
F	Platinum fiber (0.001 mm. diam.)
G	750,000-ohm wire-wound grid leaks
GR	Ground
H	Constant temperature housing
K	Key
L	Leads to unknown P.D.
P	Plates
P.A.	Plate adjustments
Q	Quartz fiber spring
R_1	10,000-ohm Yaxley wire-wound potentiometer
R_2, R_3	25,000-ohm Yaxley wire-wound potentiometers
R_4	750-ohm Yaxley wire-wound potentiometer
R_5	50-ohm Yaxley wire-wound potentiometer
S	Ceresin-covered mercury-in-paraffin switches
ST	Eppley standard cell
T	Tension adjustment
V	0.1-10,000-ohm plug-type Welch volt box



The sensitivity of the instrument in its final adjustment was more than one millimeter scale-deflection per millivolt with a scale-distance of one meter. Its period was about one second at lower sensitivities. The sensitivity depended upon the diameter of the string (platinum fiber 0.001 mm. in diameter), its tension, the distance between the plates, and the voltage across the plates. At higher sensitivity, damping increased the period to about three seconds. (This could be avoided by housing the instrument in a vacuum). The calibration curves approximated a straight line and remained constant for weeks at a time when the instrument was kept dry and at constant temperature. Because of the constant calibration and rapidity of motion of the string, readings could be made rapidly and accurately.

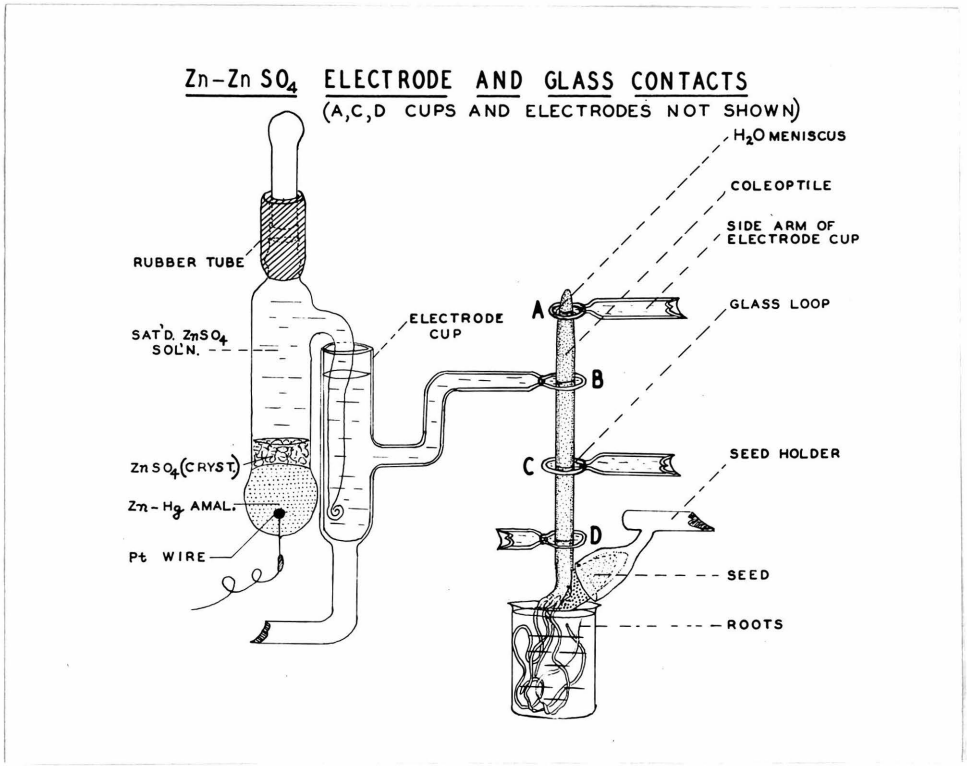
The distribution of potentials in the Avena seedling was first studied by moving contacts up and down the plant. Chambers's micromanipulators (40 pitch threading) were employed to move the electrode contacts.

Electrodes of various types were tried. It was found that bright or platinized platinum electrodes, however cleaned, gave non-reproducible readings, presumably because they were easily unpoised (cf. Gicklhorn, (1929); Umrath (1929); Dorfman (1936); and Fürth (1923)). Ag-AgCl wire loops or claws serving as contacts gave reproducible readings for a while, but demanded frequent re-plating. Quartz capillaries filled with fresh coagulated egg-white into which Ag-AgCl wire electrodes were set, gave reproducible readings. These were used for obtaining the internal distribution of P.D.'s. The gradients obtained with the Ag-AgCl

1. Metal electrodes in irreversible systems develop contact potentials which may be as large or larger than bioelectric potentials (cf. also Fürth (1929)).

loops and the quartz micro-electrodes, in general, gave similar results, these results being statistically comparable with those obtained with the more refined glass contacts and unpolarizable electrodes, although there was less constancy. The electrodes finally used for most of the measurements were Zn-saturated $ZnSO_4$ half cells (cf. figure 2). These remained iso-electric for months at a time. The types of contacts used in conjunction with these electrodes were varied. Usually the electrode was placed in a glass cup filled with the liquid used to make contact with the plant. A glass side-arm connected this electrode cup to the plant. At the point of contact, the side arm was fashioned into a small glass claw or loop, through which the plant led. The glass parts did not usually touch the plant, a meniscus of contact fluid performing this function. Tap water, KCl solutions, distilled water, and various nutrient solutions used in the glass contacts made no difference in the values obtained, if the same fluid was used in all contacts (cf. Rehm (1936a)). Amlong (1933) showed that geoelectric P.D.'s in plants depended upon the concentration of the contact fluids. The effects of concentration were not determined in the present work, but it was assumed that the P.D.'s measured were not a function of the ionic species of the solution, since any of the solutions gave the same polarity, and approximately the same magnitude of P.D. (cf. Rehm). The type of contact used for such measurements is seen in figure 2.

FIGURE 2

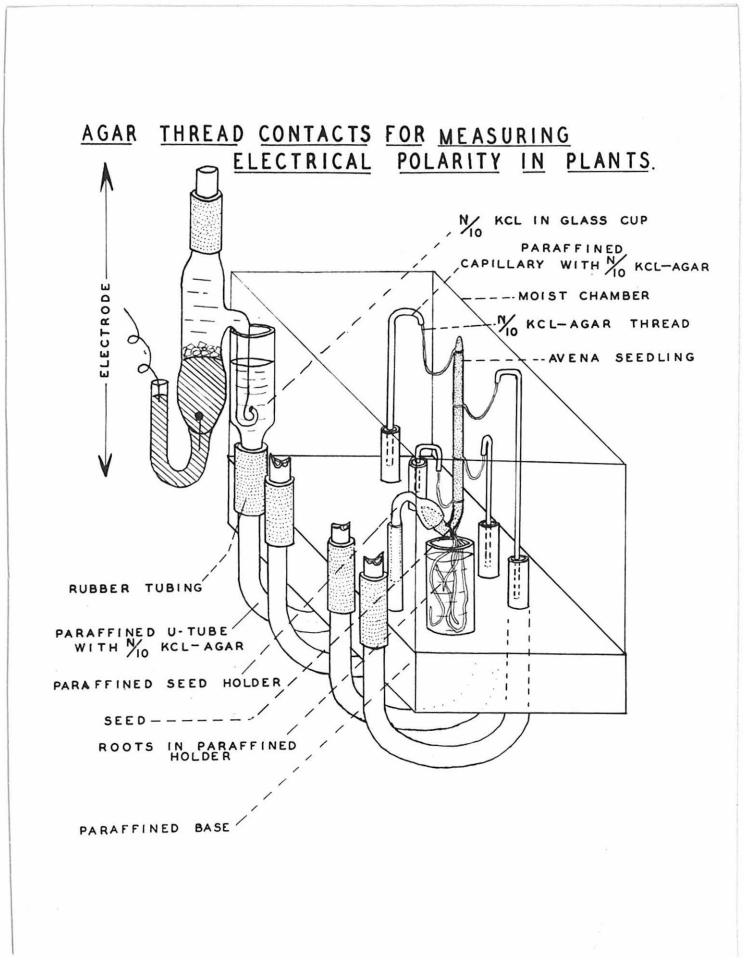

Zn-Zn SO4 ELECTRODE AND GLASS CONTACTS
(A,C,D CUPS AND ELECTRODES NOT SHOWN)
H2O MENISCUS
COLEOPTILE
SIDE ARM OF ELECTRODE CUP
RUBBER TUBE
ELECTRODE CUP
A
GLASS LOOP
SAT'D. ZnSO4 SOL'N.
B
ZnSO4 (CRYST.)
C
SEED HOLDER
Zn-Hg AMAL.
D
Pt WIRE
SEED
ROOTS

A variation of this type of contact consisted in the use of a cotton or linen thread which was made wet by being threaded through the side arm of the electrode cup to the fluid in the cup. This thread then could either be wrapped around the part of the plant being studied, or could merely touch it (Rehm (1936 a, 1936 b)). Such threads maintained an isoelectric condition satisfactorily if they were occasionally washed.

The most useful type of contact, shown in figure 3, consisted in the following: Long, thin agar threads, (made up of 2% agar in tap water, distilled water, 0.1 N KCl, Shive's, Crone's, or Hoagland's solution), 0.5 mm. or less in diameter, were made to hang from paraffined glass capillaries filled with the same agar. This was done by pushing an agar-filled capillary into more of the same agar, thus partly displacing the agar in the capillary as a thread. These capillaries were set in larger paraffined glass tubes filled with the same agar, which in turn were mounted in upright rows in a moist chamber. The glass tubes filled with agar led outside the chamber to paraffined electrode cups filled with the solution of which the agar was made. Zn-$ZnSO_4$ electrodes were placed in these cups. The agar threads hanging from the capillaries made contact to seedlings in the moist chamber, being held to the plant by a drop of 15% gelatin. Several such fixed contacts could be made to each plant; and several plants could be set up simultaneously. The threads were prevented from drying by maintaining the chamber at near saturation with water vapour from strips of moist filter paper on the sides of the chamber. This method has the advantage that the agar threads remain fixed to the plant in the same position, being carried by

upward growth without stimulating the plant. Seedlings, to which several glass contacts were fixed, frequently grew up through the more apical contacts, necessitating moving the apical contact back up to the tip. Such manipulations, however carefully made, usually stimulated the plant, thereby altering the P.D.'s.

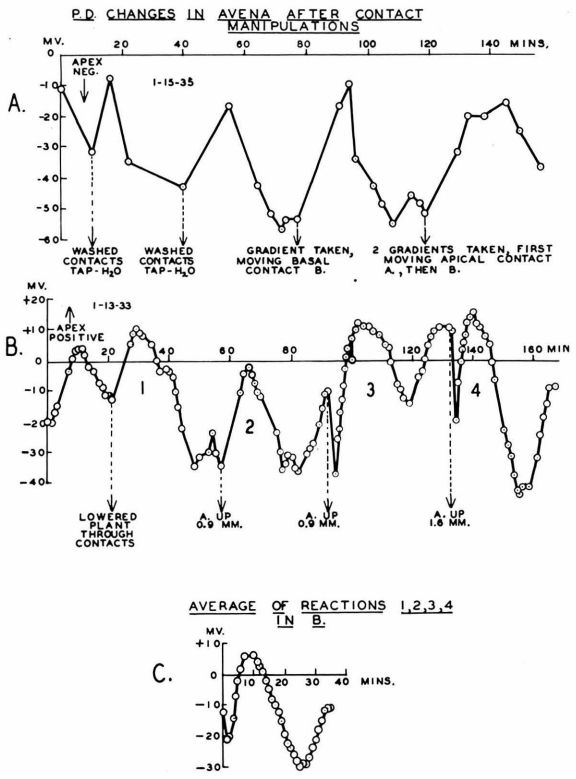
FIGURE 3.



a. Electrical Polarity and P.D. Distributions in the Avena coleoptile

As described in an earlier paper (Clark (1935); see section on photoelectric effects)) the P.D.'s in the Avena seedling, obtained by manipulation of two contacts up and down the plant, were not constant over any considerable period of time, since the manipulation always resulted in changes of P.D.'s. Such "handling reactions" are illustrated in figure 4. This was usually true

FIGURE 4



regardless of the care taken in making the manipulation, even if contact to the plant was made merely by a meniscus of water from the contacts. Moreover the orange light in the dark room proved to be a stimulus.[1] Plants left in complete darkness gave variable P.D.'s as soon as this light was again turned on (see section on photoelectric effect). Again, if two contacts were left on the plant in a fixed position, one at the tip and the other at the base of the coleoptile, and the plant left in complete darkness; constancy of the P.D. was established only after an average time of 110 minutes. This constancy was abolished occasionally by the appearance of rhythmical P.D. changes, due probably to growth of the coleoptile up through the contacts, and also due to nutations which cause rubbing against the glass of the contacts. Clean glass adheres rather strongly to the Avena coleoptile cuticle, and considerable stimulation is caused by such movements. This rhythmical effect was avoided by the use of glass contacts dipped into 15% gelatin, thereby rendering the contacts slippery to the cuticle, or by recourse to the agar thread technique described above.[2]

To obtain the normal P.D. distribution in the coleoptile, therefore, four fixed contacts were made to the plant, (cf. figure 2,A,B,C,D , and figure 3). The plants were left in complete darkness. After constancy of P.D.'s obtained (80 to 120 minutes),

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1. The light used to illuminate the dark room was filtered through a Corning filter no. 348, which cut out all wavelengths below 575 mu. No phototropisms occurred in this light.

2. Stimulation by contact is discussed by Pfeffer (1885).

the P.D. distribution could be easily and quickly determined. Figure 5 A represents the relative constancy of the P.D. between tip and base of Avena coleoptiles with fixed, gelatin-dipped glass electrodes; while figure 5 B represents the same type of experiment in which, however, the contacts were not gelatin-dipped.

Figure 5 C shows the much greater constancy obtained when the agar-thread contact method was used. The constancy lasts several hours. The figure has fewer points than either 5 A or 5 B, but that no changes occur between the points has been verified by many other determinations.

FIGURE 5

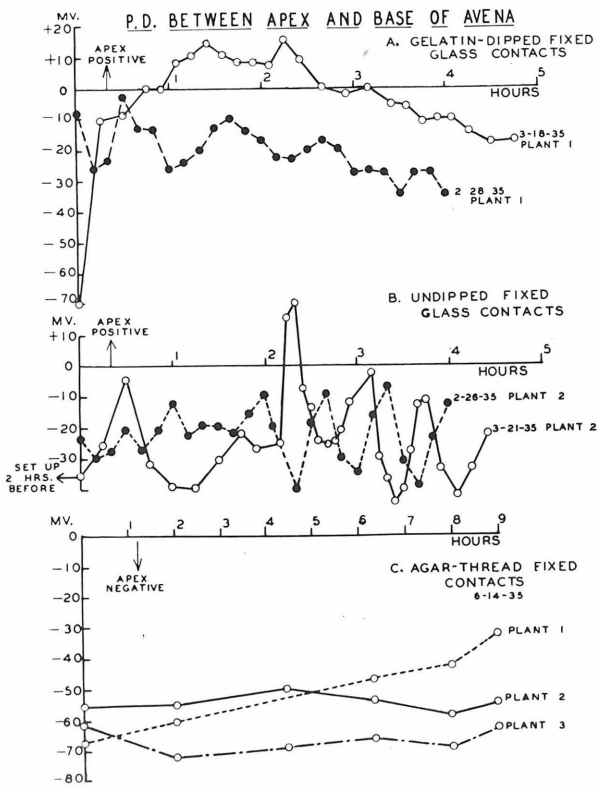
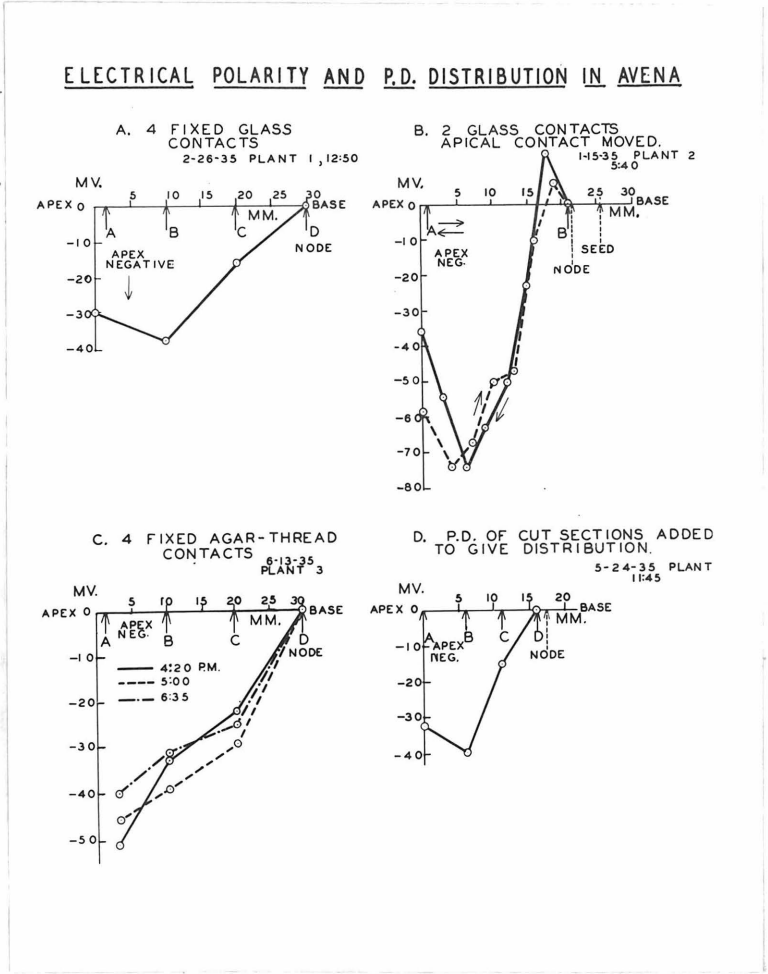


Figure 6 A illustrates the electrical polarity and P.D. distribution in the Avena coleoptile of intact plants as determined with the four fixed, gelatin-dipped glass contacts, the plant being in total darkness, and constancy having been obtained. The electrode cups each contained a $Zn-ZnSO_4$ electrode, each of which was isoelectric to all the others, both before and after each experiment. The P.D. between each contact position to the plant was obtained by manipulation of mercury-in-paraffin switches outside the experimental chamber in which the plants were housed. Figure 6 B illustrates a similar gradient taken with only two glass contacts, the basal contact being fixed, and the apical contact being moved toward the base by means of the micro-manipulators. Every few mm., a reading was taken. After the contacts touched each other, the apical contact was again moved upward, readings being taken every 5 mm. The movements of such fluid contacts up and down the coleoptile did not wet the surface and thus invite electrical shunting (cf. Rosene (1935)), because of the fatty nature of the cuticle. It will be noticed that the gradient taken by the manipulation down the coleoptile differs from that taken on moving the contact back up the coleoptile. After such manipulations it is found that the P.D.'s vary considerably, and sometimes the polarity is reversed for considerable time (cf. figure 4). Figure 6 C represents a similar gradient, the contacts in this case being four fixed agar threads as described above. The three curves in C represent three gradients taken on the same plant at different times, showing that the P.D. distribution remained constant for a considerable time. Figure 6 D represents the P.D. distribution calculated from the individual P.D.'s of

cut-sections of a coleoptile (cf. section on P.D.'s of cut-sections). In all of these curves, electronegativity of the tip is represented on the ordinates, and the length of the coleoptile is represented on the abscissae, A, B, C, and D representing points of contact to the coleoptile from tip to base. The potential at D is taken as the reference zero. The P.D. from A to D is always equal to the sum AB + BC + CD.

FIGURE 6



From the above experiments it is clear that the tip of the Avena coleoptile is normally electronegative to the base.

b. Electrical Polarity and P.D. Distribution in Pisum, Impatiens, and Zea

Pisum sativum seedlings were studied for the normal electrical polarity and P.D. distribution. Contacts were made to etiolated plants with the linen threads previously described, the threads being wrapped around the zone to be measured. There were several electrode contacts to each plant, represented as A,B,C, etc. in figure 7 A, 1,2,3. The P.D. obtained between each contact was found to be fairly constant for several hours at a time. The measurements were made in the dark room in weak red light. Figure 7 A represents typical gradients. It will be noticed that nodal zones are electro-positive to the internodal zones, and that there exists an electronegativity of the tip with respect to more basal regions. This confirms Rehm's (1936b)findings on Phaseolus, and disagrees with Ramshorn's (1934) finding that the tips of Asparagus seedlings are electro-positive to the basal regions, although the nodal zones were positive to internodal zones.

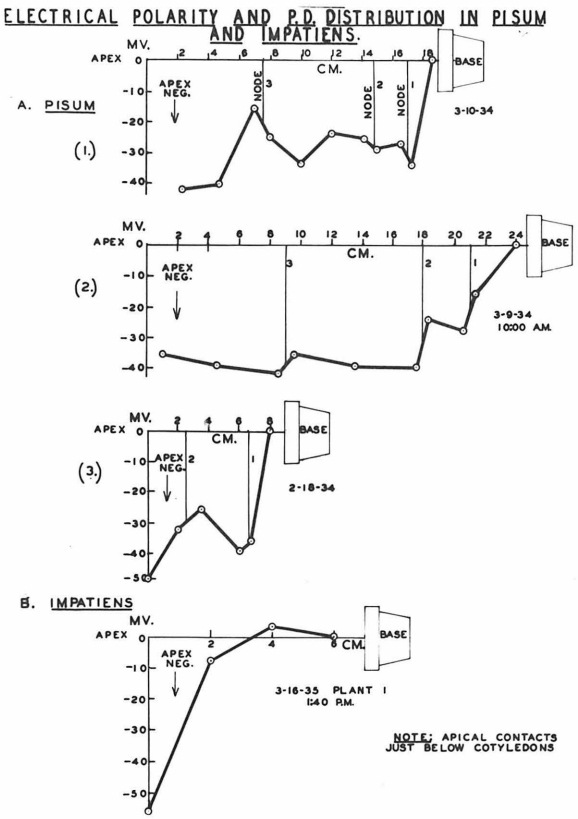
Etiolated Impatiens balsamina seedlings were set up in the same way as Pisum seedlings were, and the P.D. distribution recorded. The hypocotyls are without nodes, and the plants were only seven centimeters in height. Figure 7 B represents the distribution found.

Zea mays seedlings 5 to 7 cm. in height were set up in the same way as has been described for the Avena seedlings, four fixed contacts being made by means of agar threads. The polarity and P.D. distributions found corresponded very closely with those recorded for Avena.

It is concluded from these observations that in Pisum, Impatiens,

and Zea, the tip of the etiolated seedling is normally electronegative to the basal regions. This shows that the electrical polarity of the Avena coleoptile is not unique in its apical negativity.

FIGURE 7

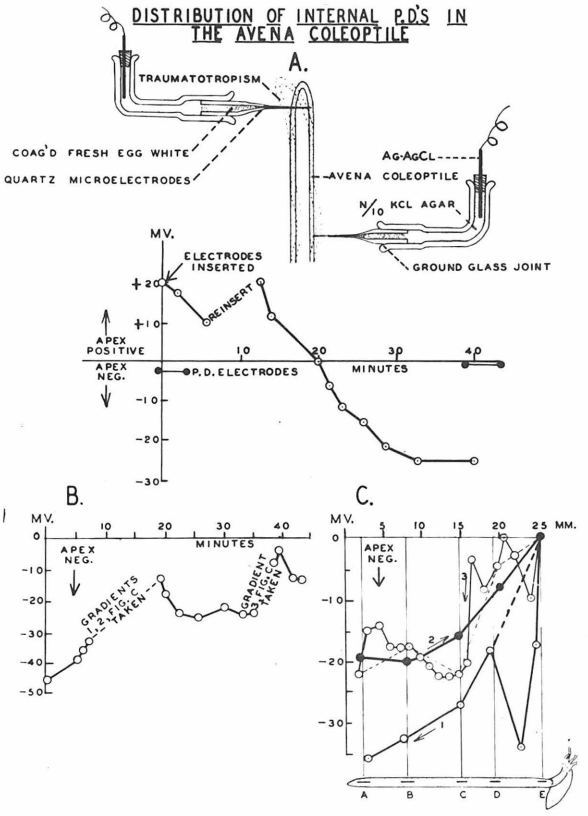


c. Internal Electrical Polarity in the Avena Coleoptile

The above discussion concerns the electrical polarity and P.D. distribution measured on the cuticle, thus the external polarity. It is conceivable that the distribution of internal P.D.'s might be different, as suggested by Lund's (1930,1931b) findings in the Douglas fir. Here the apical wood was electronegative to basal wood, while the apical cortex was electropositive to basal. For this reason a few experiments were performed on the Avena coleoptile, in which the P.D. distribution beneath the cuticle was examined. Quartz microelectrodes (cf. descriptions in this paper) were inserted in coleoptiles by means of micro-manipulators, and the electrical polarity was measured. The electrodes are illustrated in figure 8. They maintained an isoelectric condition very satisfactorily for two or three hours. Figure 8 A shows the P.D. between two such electrodes inserted in a coleoptile. One electrode was inserted in the wall of the coleoptile at the apex, and one at the base, both electrodes being in the same side-wall, but being inserted from opposite sides of the coleoptile. Traumatropisms toward the sites of insertion occurred after about 30 minutes, as diagrammed in figure 8 A. Immediately after insertion, the apex of the coleoptile became electropositive to the base, but after 20 or 30 minutes, as seen in figure 8 A, the tip became negative. It was considered impracticable to insert several such electrodes, or to re-insert the same two electrodes at several different loci on the coleoptile in order to obtain the P.D. distribution. This would have resulted in even more P.D. variation than is depicted in the figure; hence, in order to obtain the distribution of internal

P.D.'s, a different technique was employed. Several longitudinal slits were made down the coleoptile using a sharp razor. Each slit had its counterpart on the opposite side of the coleoptile in order to compensate the wounding effects. Such coleoptiles will remain straight, whereas the ones in which the microelectrodes were inserted showed traumatropisms. Glass-loop contacts, such as were described above, made contact with the plant, one at the tip and one at the base of the coleoptile. The basal contact was racked up the coleoptile toward the apex by means of the micro-manipulator. When a contact was centered over a slit, presumably the potential internal to the cuticle was measured. Figure 8 C shows the distributions obtained in this way. The vertical lines, a, b, c, d, and e represent the loci of the slits in the coleoptile. Curve I represents the distribution obtained by racking the basal contact up to the apex, whereas curve 2 represents the distribution obtained by racking it back down a few minutes later. Curve 3 represents the distribution obtained by racking it back up 20 minutes later. Figure 8 B illustrates the changes in P.D. between the apex and base of the coleoptile before and after each distribution was determined. It will be noticed in curve 2 of figure 8C, that the readings were taken with the contacts centered on the slits. The distribution is very similar to that obtained on the intact cuticle. Curve 1 shows the same thing, with the exception that one contact was centered between two slits, thus on the intact cuticle (between d and e). Curve 3 shows the type of curve obtained when no attention was paid to the position of contacts with respect to slits, i.e., the distribution was taken at more

FIGURE 8


DISTRIBUTION OF INTERNAL P.D'S IN THE AVENA COLEOPTILE
A.
TRAUMATOTROPISM
COAG'D FRESH EGG WHITE
QUARTZ MICROELECTRODES
Ag-AgCl
AVENA COLEOPTILE
N/10 KCl AGAR
GROUND GLASS JOINT
MV.
ELECTRODES INSERTED
REINSERT
APEX POSITIVE
APEX NEG.
P.D. ELECTRODES
MINUTES
B.
C.
GRADIENTS FIG. 1-2 TAKEN
GRADIENT FIG. 3 TAKEN
MM.
A
B
C
D
E

frequent loci, regardless of the slit positions, The general electrical polarity is the same as that on the intact cuticle, but the curves are not smooth. This indicates a radial P.D. between cuticle and the internal tissues. The curves are smooth if contacts and slits coincide.

The following section will show that cut sections are of the same electrical polarity as has just been described and, as seen in figure 6, the sum of the section P.D.'s of a coleoptile give a P.D. distribution for the coleoptile which is comparable to that in an intact plant.

The evidence presented indicates that the distribution of internal P.D.'s in the Avena coleoptile is the same as that on the outside (cuticle). The polarity is the same, i.e., the tip is electronegative to the base.

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d. Electrical Polarity of Cut Sections

The P.D.'s and electrical polarity of cut sections of the Avena coleoptile were then determined in several different ways. One method involved cutting the sections with two parallel razor blades separated by a brass strip. The cut surfaces were washed by placing the sections upright on wet filter paper for an hour. The sections were then carefully transferred to the experimental chamber, using eye-forceps. Contact was made to individual sections with the agar thread method, or with agar strips. 0.1 N KCl agar was used in most cases. The strips or threads led through paraffined glass tubes to electrode cups outside the chamber. $Zn\text{-}ZnSO_4$ electrodes were placed in these cups, and the P.D.'s measured. The chamber was maintained at a high vapor pressure by means of strips of moist filter paper.

Several hundred measurements on 3 mm. sections revealed that the cut apical surface was always electronegative to the cut basal surface from one to fifteen millivolts, the magnitudes depending upon the time at which the measurements were made.

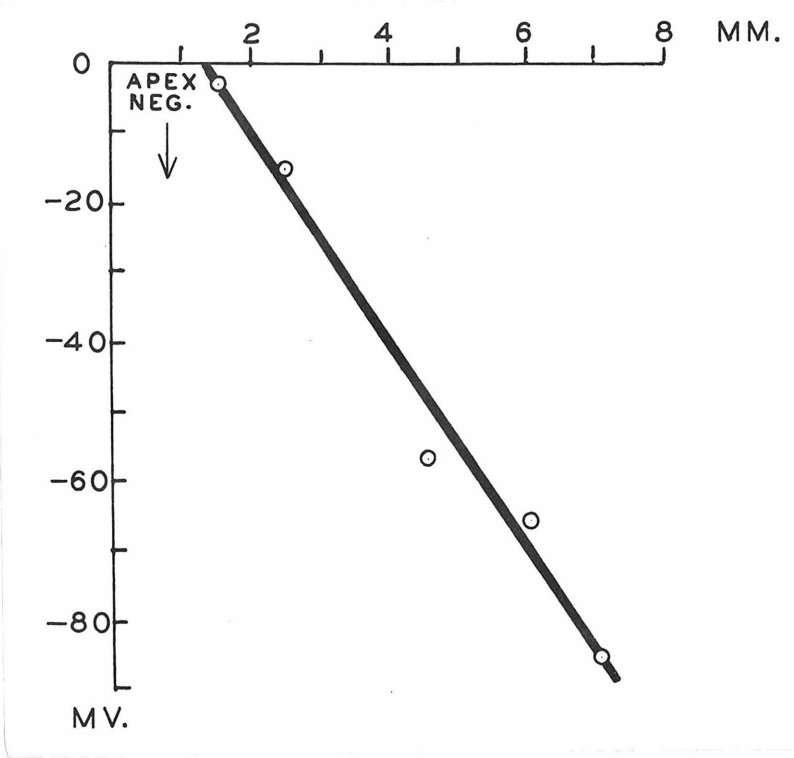
Another method involved placing from 12 to 20 sections on an agar block, and placing a similar agar block on the tips of the sections. 0.1 N KCl agar strips made contact with these blocks and to the cups outside the chamber. Here, therefore, the average P.D. of several sections in parallel was measured. The same result was obtained, i.e., apical negativity. This latter method will be discussed again in a later section of this paper.

A third method involved making contact with several places on longer sections with agar threads held in place with a drop of gelatin, as described in an earlier section of this paper. The cut surfaces were usually electronegative to the intact cuticle (another indication of a "radial" polarity), but the apical cut surface was always negative to the basal cut surface.

Using the method by which 20 sections were measured in parallel at the same time, the relation of the length of the section to the P.D. of the section was determined. It was usually found that time was required before the maximum P.D. was established. (Presumably this was due to the diffusion of ions from the sections into the agar blocks). For this reason, the P.D.'s were allowed to reach their maximum values before plotting against length. This time function was more pronounced in longer sections. Figure 9 shows the length of the section in millimeters plotted against P.D. in millivolts, after this maximum P.D. had been reached in all sections (5 hours).

FIGURE 9

RELATION OF SECTION LENGTH TO P.D.



In the section on P.D. distribution in intact plants (cf. fig. 6), it was seen that the sum of the several P.D.'s along a coleoptile was equal to that measured from apical contact to basal contact. In figure 9 it is seen that this principle of summation again holds, since the magnitude of the P.D. of cut sections is directly proportional to the length of the section (cf. Lund (1928, 1931), and Rosene (1935), on the principle of summation of P.D.'s)).

Cut sections of Vicia faba and of Pisum sativum showed the same electrical polarity as Avena coleoptile sections. In the section on dye-uptake, it was seen that Impatiens cuttings also showed apical negativity. Thus the phenomenon seems quite general. In Vicia and Pisum, the P.D. magnitudes varied from a few millivolts to 30 or 40 millivolts, depending upon the length of the section.

e. Geoelectric Effect

During the measurements of section P.D.'s, it was noticed that inverted sections exhibited an inverted electrical polarity. A section seemed to show negativity of the end oriented upward regardless of whether this end was morphological tip or base.

The experimental procedure usually consisted in inverting single sections and measuring the individual P.D.'s of these sections, or by placing 12 to 20 sections on one agar block, making contact with this block and with the other cut surface with a similar agar block, thus obtaining the average P.D.'s of the lot of sections in parallel. Measurements were made immediately, when possible, upon inverting the sections. The polarity of the inverted sections showed an immediate inversion of electrical polarity. The time

relations of the inversion have not been cafefully studied, but the establishment of the inverted polarity seemed to take less time than the geoelectric effects of Brauner (1927). It was noticed, however, that this geoelectric effect was not maintained indefinitely, particularly in the shorter sections. The original polarity (apical negativity) returned within 60 to 120 minutes, depending upon the length of the sections. Figure 10 A shows the course of the P.D.'s of inverted sections during a period of time. The sections were cut at 9:30 A.M. and placed on wet filter paper in an inverted position. The experiment was set up at 11:30 A.M., the first readings being taken at 11:40 A.M. During this time, the inverted polarity had attained a considerable magnitude.

FIGURE 10

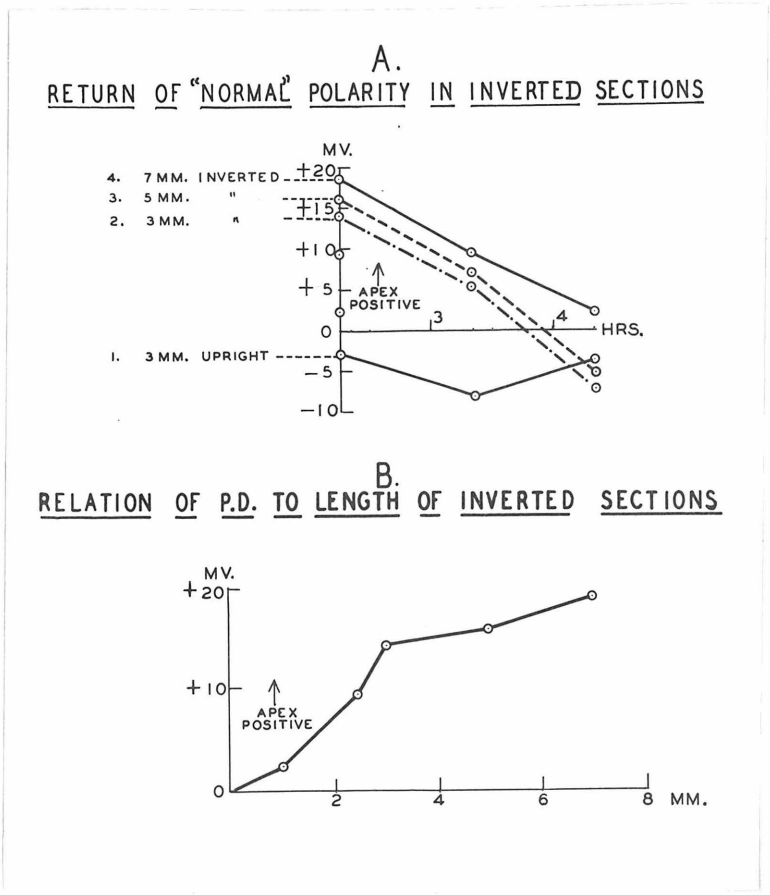


Figure 10 A, curve 1, shows the change in P.D. of control upright 3-mm. sections. They exhibit normal apical negativity. Curve 2 is for inverted sections 3mm. in length; curve 3, 5 mm.; curve 4, 7 mm. in length. It is seen that the longer the sections, the greater the magnitude of the inverted polarity; and that the greater the magnitude of this inverted polarity, the longer the time necessary to reestablish the normal polarity. The abscissae are in hours after cutting the sections. Figure 10 B represents data from the same experiment. The P.D.'s of sections with inverted polarity are plotted against the length of the sections in millimeters. The P.D. values used are those of maximum magnitude, i.e., at first measurements, as represented on the zero ordinate of figure 10 A. It is seen that a nearly direct proportionality exists between length and the P.D.'s of inverted polarity, thus indicating a similarity to upright sections which exhibit the same direct proportionality.

The possibility remains that the normal polarity of upright sections of plants is partly a result of the position of the plant or section with respect to gravity, i.e., that the electrical polarity is, partly, a geoelectric effect.[1] This scheme is complicated by the return to normal polarity with time. It is not known what the effect of inverting intact plants, e.g. roots or shoots, has on their electrical polarity. The establishment of positivity of the under side of inverted sections recalls to mind the similar establishment of positivity of the under side of horizontally-placed plants (Brauner (1927)),

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[1] See section on irreversible narcosis, p. 65.

That the geoelectric polarity or inverted polarity is not a phenomenon confined to Avena sections was shown by the fact that 5 mm. inverted Pisum sections developed the inverted polarity. In this case, however, more time was required to establish the inversion. On first inverting, the polarity was found to be inverted a few millivolts, the maximum inverted polarity becoming established only after 3 or 4 hours. Since Brauner (1928) showed that the geoelectric effect varied in the seed coat of various plants, depending upon the membrane structure, this is not surprising. It is likely that other plant sections would exhibit their own peculiarities. This individuality was also observed by Brauner (1927) for geoelectric effects in horizontally placed plants.

The linear relation between section length and P.D. of either normal or inverted sections may be similar in nature to the fact observed by Brauner that geoelectric P.D.'s in membranes were greater in thicker membranes.[1.] He did not, however, find any such linearity.

From the section (f)on electrical polarity, it was concluded that the normal, inherent electrical polarity of intact plants and cut sections of Avena, Zea, Pisum, and Impatiens, was an electronegativity of the apical parts with respect to more basal parts. This polarity exists internally as well as externally, and is not directly related to growth.

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1. Brauner (loc. cit.) also found that increasing gravity by centrifuging increased the geoelectric effect.

f. The "Light-electric Reaction"

It has been demonstrated in the previous sections that gravity may change the electrical polarity of cut sections of various plant organs. It is well known that gravity has no effect on polar transport of auxin (Went (1928); van der Weij (1932); Pfaeltzer (1934)). This leads us to the conclusion that electrical polarity has no causal relation to polar transport.

That light has no effect on polar transport has been shown by Boysen-Jensen (1932). The question arises, does light affect the electrical polarity of the Avena coleoptile? The following section is concerned with this question.

The effect of radiant energy on bioelectric potentials in plants has been little investigated chiefly because of the complexity of interpretation of the results. Most previous work of this type has been confined to bioelectric responses of green organs (Haake (1892); A.D. Waller (1900 a, b); Bose (1907); J.C. Waller (1925, 1929); Brauner (1927); Sheard (1929, 1930); Glass (1933)). Thus the interpretation becomes difficult as photosynthesis itself presumably causes changes in potentials which are superimposed upon the normal bioelectric potentials.[1]. The present preliminary note will briefly describe bioelectric responses of the coleoptile of the etiolated seedling of the oat, an organ totally lacking in chlorophyll (Clark (1937c)). In this case it was hoped to obtain the more or less direct effect of light on the potentials of a plant organ.

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1. Marsh (1936) and Blinks (1937b) have recently conclusively shown a close linkage between bioelectric changes and the photosynthetic mechanism in Valonia and Halicystis.

The experiments were carried out with etiolated seedlings of a pure line of Avena sativa (Victory oats) grown at 24°C. and a relative humidity of 90. When the seedlings had reached a length of 30-40 mm., one of them was transferred without injury and totally intact to the experimental chamber. Only weak red light was employed for the observations. The chamber contained two partitions, one for the experimental plant and one for the control plant. Each partition contained a special holder for the seed whose roots were suspended in a vial of water. The coleoptile extended upward through four glass loops placed equi-distant from one another ranging from the coleoptilar node to the tip. Each glass loop held a tap water meniscus which in turn made water contact to side-arms of cups of water in which Zn amalgam-saturated $ZnSO_4$ half-cells were placed. Thus four fixed electrical contacts were made to each coleoptile so that the potential difference between any two of them could be measured. They are illustrated as A, B, C, and D in figure 11. These contacts are essentially the same as those used by Glass (loc. cit.) in his study of the effect of light on the bioelectric potentials in Elodea leaves (cf. figure 2). No $ZnSO_4$ reached the plant through the side-arms within the time of any one experiment. All contacts were isoelectric to one another. The four contacts made connection by well insulated wires to mercury-in paraffin switches outside the light-proof chambers. The measurements were made with the string electrometer described earlier in this paper.

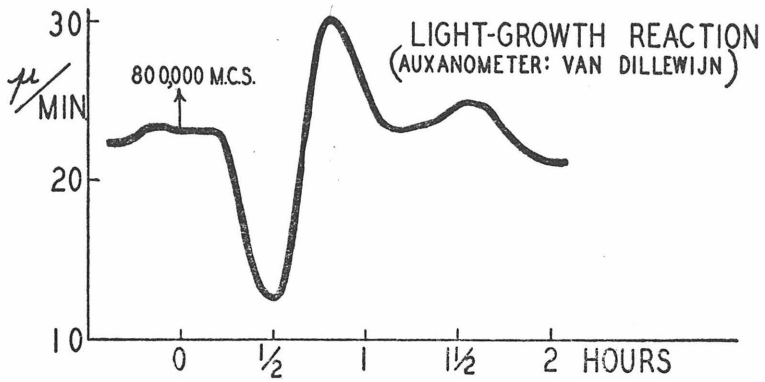
The light source for the stimuli was a 100-watt Mazda incandescent lamp 40 cm. above the tip of the seedling, being horizontally directed to the plant from three sides by means of three mirror

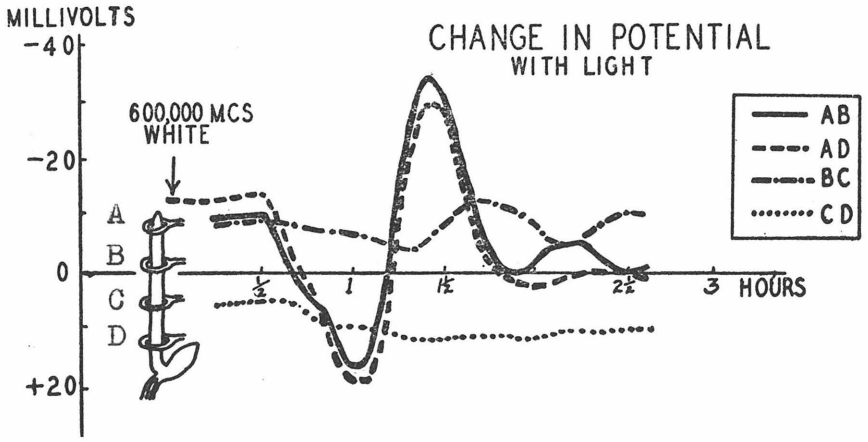
strips placed 120° from one another and 45° to the horizontal. The heat radiation was filtered out by the interposition of a layer of one centimeter of running water between light source and chamber, so that the temperature of both partitions of the experimental chamber remained constant within 0.1°C. while either partition was being illuminated.

As described in the section on electrical polarity, preliminary measurements of plant electrical potentials obtained by contacts moved up and down the plant by means of micro-manipulators and under red or orange light, and at constant temperature, were extremely variable and it was impossible to obtain constancy over any considerable length of time after the most careful manipulation. Only those plants with four fixed glass or agar thread contacts and in complete darkness gave the constancy necessary before the electrical responses to any external stimulus could by determined. With four such glass contacts, the potential differences (P.D.) between A and B, B and C, C and D and their sum A and D (cf. figure 11), were determined by manipulation of the mercury switches. These values were plotted as millivolts. Figure 11 shows the results. Approximately 90 to 120 minutes after setting up the plant elapsed before constancy of the P.D.'s obtained. The changes previous to this usually consisted of a slow fall followed by a slow rise in negativity of the coleoptile tip with respect to more basal contacts. With respect to time relations and shape of the curve, this reaction corresponds closely with the growth reactions obtained after setting up a similar plant in a Koningsberger auxanometer(Koningsberger (1922)) as was seen by examination of many auxanometer records (see also figure 5 A).

Figure 11

THE "LIGHT-ELECTRIC REACTION"





After constancy was obtained, the light was turned on one plant in one partition while the other plant set up in exactly the same way was kept in complete darkness in the other partition. Measurements were made in a few seconds every five or ten minutes. After the reaction of the illuminated plant was recorded and relative constancy again reached, the other plant was illuminated for its reaction, and so on. Usually two such reactions were recorded in one day's experimentation. The time of illumination was varied, but it was found that with the times most studied, namely 5, 10, and 30 minutes, the curves were similar. Since it was very difficult to obtain a set-up wherein the potentials were constant before illumination, many experiments were necessarily performed before a few good ones were obtained[1].

Of the experiments performed, seven wherein the period of illumination was thirty minutes gave similar curves. These are typified by the curve given in the figure.

It is noticed that the electrical response comes after the light is turned off. (One hundred watts at 40 cm. for 10 min. = approximately 600,000 meter-candle-seconds, assuming 167 for the mean horizontal candle-power of the 100-watt gas-filled incandescent lamp). The tip of the coleoptile is normally electronegative to the base, in contradiction to Ramshorn (1934), who used manipulators to move the contacts up and down the plants, making observations by red light. After illumination, this negativity at first decreases,

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1. As discussed in the part on electrical polarity, this condition was somewhat offset by dipping the glass loops in gelatin or agar before mounting the contacts on the plants. Presumably "handling reactions" are caused by the plant rubbing against the solid glass loops.

then increases to a maximum after which it falls again to the original level, usually fluctuating somewhat thereafter. The largest reaction obtains in the sub-apical region, AB, with smaller delayed, reactions in the more basal regions. The algebraic sum of the P.D.'s between A and B, B and C and C and D is always equal to that between A and D. The time relations, the magnitude of the effect (up to 90 millivolts) and the shape of the curve all strongly suggest a relationship to the light-growth reactions of the Avena coleoptile (van Dillewijn (1927); Went (1925)). A curve from van Dillewijn for the light-growth reaction is given in the figure for comparison of shape of the curves and time relations. Whether the electrical reaction is a cause, an effect or a parallel phenomenon possibly associated with growth reactions to light remains to be shown.

It is concluded, among other things, that light may cause a change of electrical polarity in the Avena coleoptile, this change even amounting to a reversal of the normal polarity. Since light has no effect on polar auxin transport (Boysen-Jensen (loc. cit.)), the theory of the correlation between electrical polarity and polar transport is again thrown into question.

g. Effect of Applied E.M.F. on Inherent E.M.F. and Electrical Polarity

If one wished to link a physiological function, such as polar transport of auxin (or ion accumulation, secretion, etc. See discussion) with a phenomenon like bioelectric polarity, it would be desirable, among other things, to alter this polarity by means of applied E.M.F.'s and to measure the effect on the function in point. The latter question will be discussed in a later section (p 72). The present discourse is concerned solely with the effects

of applied currents on inherent bioelectric polarity, and the possible significance of these effects.

(1)Historical Review

Blinks (1930 a,b,c,; 1936 a,b,c) studied the changes in resistance and potentials in several plants, including the large single-celled algae Valonia, Halicystis, and Nitella. With regard to the measurements made across the protoplasmic layers of these cells, he found : (1) Valonia ventricasa and macrophysa; The vacuole is electropositive to sea water. When small E.M.F.'s are applied so that current flows either outward or inward across the protoplasmic layer, no back E.M.F.'s or large resistance rises occur. Similarly no changes are observed when fairly large currents pass outward from sap to sea water. But when large currents pass inward, thus opposing or in series with the inherent polarity, large back E.M.F.'s (or resistance rises) ensue; (2) Halicystis; The vacuole is 70 to 80 mv. electronegative to the sea-water. An inward current slightly increased the sap negativity, while an outward current decreased it. This latter decrease is 7 to 8 times less than the increase due to inward current; (3) Nitella: The sap is electronegative to the fresh-water surface, thus like Halicystis the vacuole negativity is increased (200 to 300 mv.) if the current is inwardly applied, and decreased proportionately less if directed outward.

Hence Blinks has shown that regardless of the polarity exhibited between sap and external medium, that inward currents (below certain thresholds) change the polarity much more than equal and oppositely directed currents. This suggests a "unipolar conductance",

but in truth is due to a differential polarization capacity of the two protoplasmic surfaces.

Marsh (1930) showed that the E.M.F.'s of the onion root are changed by small applied direct currents in such a way that: (1) if the applied voltage opposes that of the root (in parallel), the latter is increased; and (2) if the applied voltage is in series with that of the root the latter is decreased. These findings confirm those of Blinks' with the exception of Valonia.

Guha (1927) described a "preferential conductance" in the styles of various plants. One platinum electrode[1.] was inserted into the stigma of the plant (Narcissus, Aesculus, Primula), and another into the ovary. A direct current was passed from a two volt source, the conductivity of the style being measured by deflections on a galvanometer. Current passed more easily from stigma to ovary then in the reverse direction. Pollination abolished this "polar conductance".

Brauner (1930) found a "polar permeability" of the soaked seed-coat of the horse chestnut (Aesculus). The filtration rate of water passing inward across the seed-coat was found to be 60% greater than the outward rate. Soaked seed-coats exhibited a P.D., the epidermis being the electropositive pole (a few millivolts). Brauner stated that the coat is made up of a double membrane, and that the P.D. facilitates the inward passage of water, inhibiting the outward passage.

Metzner (1930) attempted to demonstrate a "polar conductivity" in the cutinized epidermis of fruits (apple, tomato, Physalis, Clivia);

1. Guha's work is probably unreliable, as platinum contacts polarize very easily and would obscure the results. Also, Guha may have been measuring differential polarization capacities (cf. Blinks (1936e)).

seed coats (Pisum, Phaseolus, Aesculus); parenchymatous tissue such as discs of the mid-rib of Rheum leaves, or Daucus roots; and in the algae Nitella and Chara. He used these objects as "rectifiers" to an alternating-current (50 cycles), measuring the "rectified" direct current with a D.C. galvanometer in series with the calomel half-cells making contact with the tissue being studied. No data are given.

Amlong and Bünning (1934) passed currents through Helianthus annuus roots, measuring the resulting changes in the potentials of the areas through which the alternating current passed.

The source of the stimulating current was an opening and closing shock from the secondary of an inductorium. The primary was connected to a four volt battery and the secondary connected to the electrodes. The intensity of this current was not measured, in contrast to Marsh's experiments described above. It was, however, probably higher, since Marsh used stimulating currents of the order Amlong and Bünning used to measure resistance changes after electrical stimulation. Amlong and Bünning claimed such small "testing" currents effected no P.D. changes. This may have been due to physiological differences between Helianthus and onion roots.

Amlong and Bünning found that such currents increased the negativity of any region stimulated. This negativity was correlated with loss of turgor, and decreased resistance. The former was measured by change in plasticity, the later by change in resistance to flow of sub-liminal direct currents. Thus the applied electric current increased permeability, which in turn gave rise to the non-propagated "action-current", decrease in resistance, and loss of turgor. Mechanical, chemical, or any other type of stimulus

of sufficient intensity does the same thing, hence Amlong and Bünning have not studied the electrical properties of membranes in the light of the experiments of Marsh or Blinks. Amlong and Bünning radically stimulated the root-cells, giving rise to large permeability changes lasting ten minutes or so, while Blinks' effects lasted only for a few seconds at the most, and being physiologically manifested only as polarization changes similar to those occurring at the surface of a non-polarizable electrode when it becomes polarizable because of large current passage. Metzner's results, as will be shown below, were probably due to stimulation rather than "unipolar conductance".

The following experiments were originally performed in order to determine whether or not there is such a thing as "unipolar" or "unidirectional" permeability in the Avena coleoptile, which might relate to "unidirectional" transport.

A later section of this paper (p. 72) will deal with reversal of electrical polarity as related to polar transport.

(2) Experimental

It was shown in an earlier section (p. 42) that coleoptile section P.D.'s are proportional to section lengths. In order to effectively magnify electrical phenomena in sections, it was found very convenient to pile sections one on the other in series. (This was also done with the view in mind to perform transport tests at the same time as the electrical measurements were made). Twenty five 5 mm. Avena coleoptile sections were placed upright on a tap-water agar block, a similar agar block being placed on

top of the sections. Another twenty five sections was placed on this block, and so on; so that finally four tiers each consisting of twenty five sections were prepared. N/10 KCl agar made contact to the tip and bottom blocks of this preparation, the N/10 KCl agar leading to N/10 KCl solutions in cups outside of a moist chamber in which the preparation was situated. Calomel-saturated KCl electrodes were placed in these cups, and were used both for applying and measuring P.D.'s and current passage. Currents of the order used in the experiments below were found to never polarize these electrodes.

Several experiments were performed, using Pisum sections as well as Avena sections, and in which the inherent P.D. was measured with a string electrometer. The resistance of the set-up with no sections present was measured by applying a known voltage and measuring the direct current passage by means of a galvanometer. (sensitivity about 1 mm. deflection for 10^{-8} amperes at 1 meter scale distance, period 5 sec.). Knowing this resistance, the resistance of the sections could be calculated by Ohm's law, from the electrometer measurements and galvanometer deflections caused by the current flow resulting from the plant P.D.'s. The changes in P.D. and resistance were then studied when (1) alternating current (A.C.) was applied, (2) direct current was applied, and (3) ether vapors were applied.

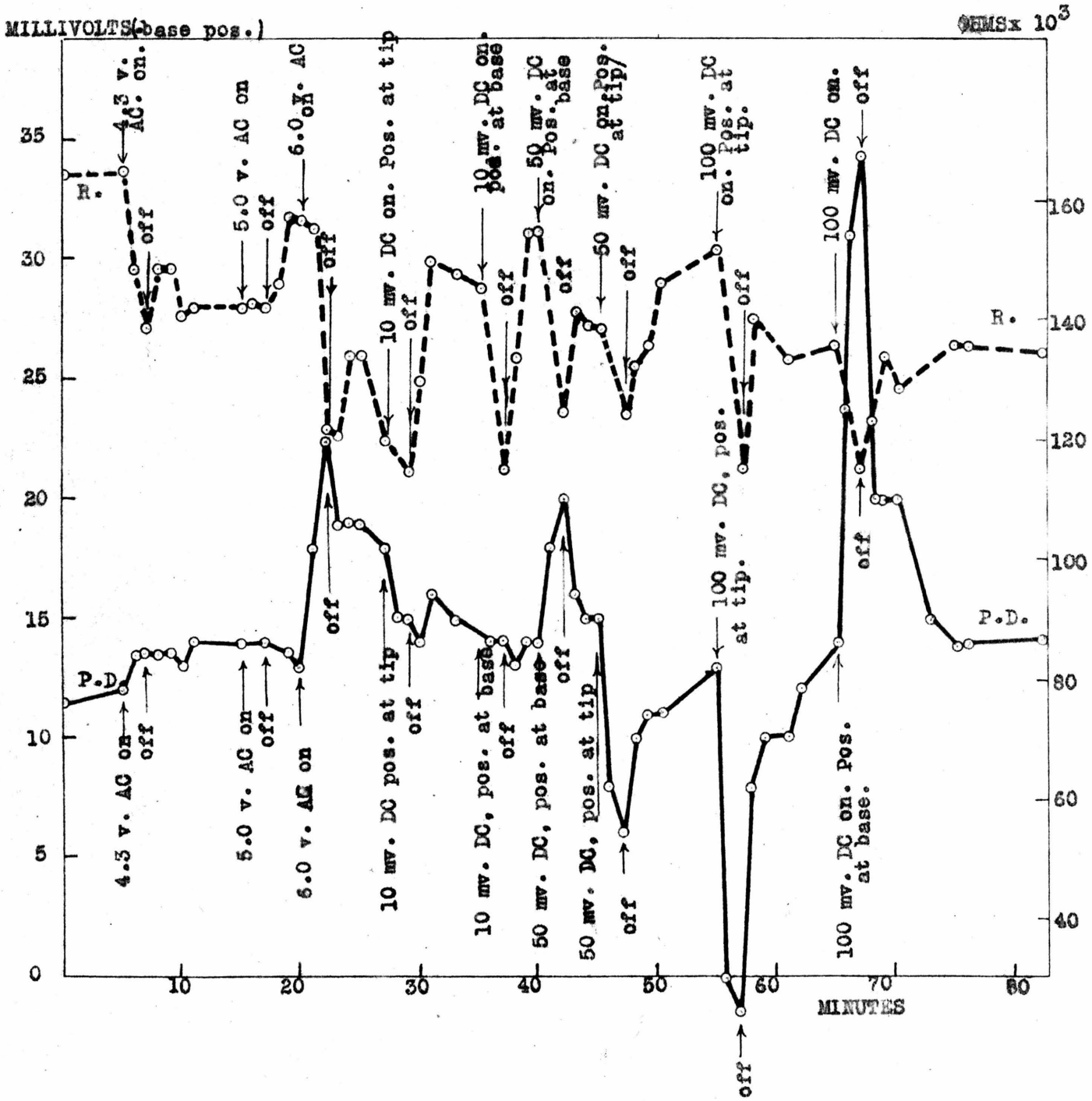
It was found that small direct currents (0.6 to 2 X 10^{-7}A.) caused decreases in resistance essentially equal to those caused by larger direct currents (6 X 10^{-7} A.). This decrease was, in one experiment (4-9-37), 35,000 $\pm$ 5000 Ω for 0.6 X 10^{-7} A. as

well as for 6 X 10^{-7} A. The P.D., however, changed only one or two millivolts when 10 mv. (0.6 X 10^{-7} A.) was applied, [illegible]; while it changed 15 to 20 mv. when 100 mv. (6 X 10^{-7} A.) was applied. In this experiment, which represents a typical case, the morphological bases were, as usual, electropositive to the apices. When 10 mv. were applied, in series with the section P.D., the section P.D. dropped 3 mv.; when in parallel with the section P.D., no perceptible change resulted. 50 mv. in series decreased the inherent P.D. approximately 10 mv., and when in parallel, increased it about 7 mv. But 100 mv. in series decreased it 15 mv., reversing the normal electrical polarity; and when in parallel increased it 20 mv. The time relations of these changes are shown in figure 12. The return or depolarization curves show that the processes affected by applied E.M.F.'s are reversible. The normal polarity is quickly restored when the applied E.M.F. is removed. The section of this paper (p. 72) on applied E.M.F.'s and transport deals with longer periods of current application. In such cases, polarization is more permanent, depolarization occurring very slowly. This reminds one of similar results described by Blinks and Marsh (loc. cit.).

The important conclusion to these observations is that the resistance drops when E.M.F.'s are applied, regardless of polarity. This means the protoplasmic surfaces are changed so that current is carried more easily by ions, i.e., that permeability has increased. The sensitivity of the resistance-measuring apparatus used in this particular experiment was really not high enough to

FIGURE 12.

THE EFFECT OF APPLIED DIRECT AND ALTERNATING CURRENTS ON THE INHERENT P.D. AND RESISTANCE OF COLEOPTILES.



quantitatively link resistance changes with P.D. changes, but it is certainly clearly indicated that the resistance drops just as much with 10 mv. applied E.M.F., as with 100 mv. (or with 6 volts A.C.).

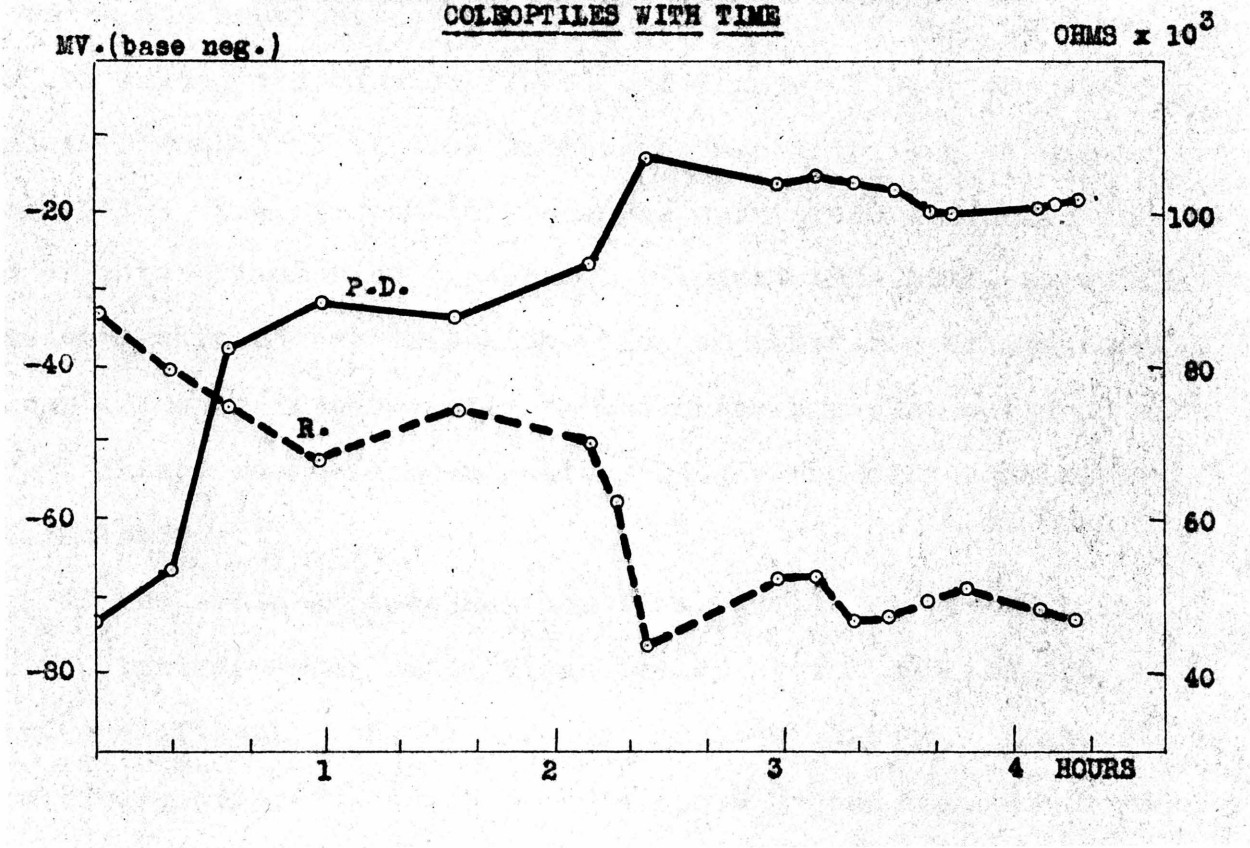
It would be highly desirable to study more closely the resistance and potential changes with applied E.M.F.'s, especially since Blinks (1936 e) and Osterhout (1936) have shown that compound membrane P.D.'s may be separated into simpler component P.D.'s by this method; or in other words, differential compound membrane permeabilities may be analyzed into simpler components. This may prove to be the only way to attack the electrical problem of polar transport, ion accumulation, etc., since mere measurements of normal inherent P.D.'s mean little, as will be proven in this thesis.

Figure 13 represents electrical behavior of coleoptile sections which happened to have an inverted polarity which was slowly righting itself ("Handling reaction"). In this experiment, the sections were not piled in series. Coleoptiles were decapitated, the primary leaves removed, and the whole coleoptile removed from the rest of the seedling by cutting it above the node. Forty such whole coleoptiles, each 14 mm. long were placed in parallel, the P.D. between their cut surfaces being measured in this experiment.

It is seen that the resistance faithfully follows the P.D. but in an opposite direction. The resistance was much lower than in the experiment described in figure 12, and hence the measurements were more accurate because the galvanometer deflections were greater.

FIGURE 13

THE VARIATION OF INHERENT P.D. AND RESISTANCE OF COLEOPTILES WITH TIME



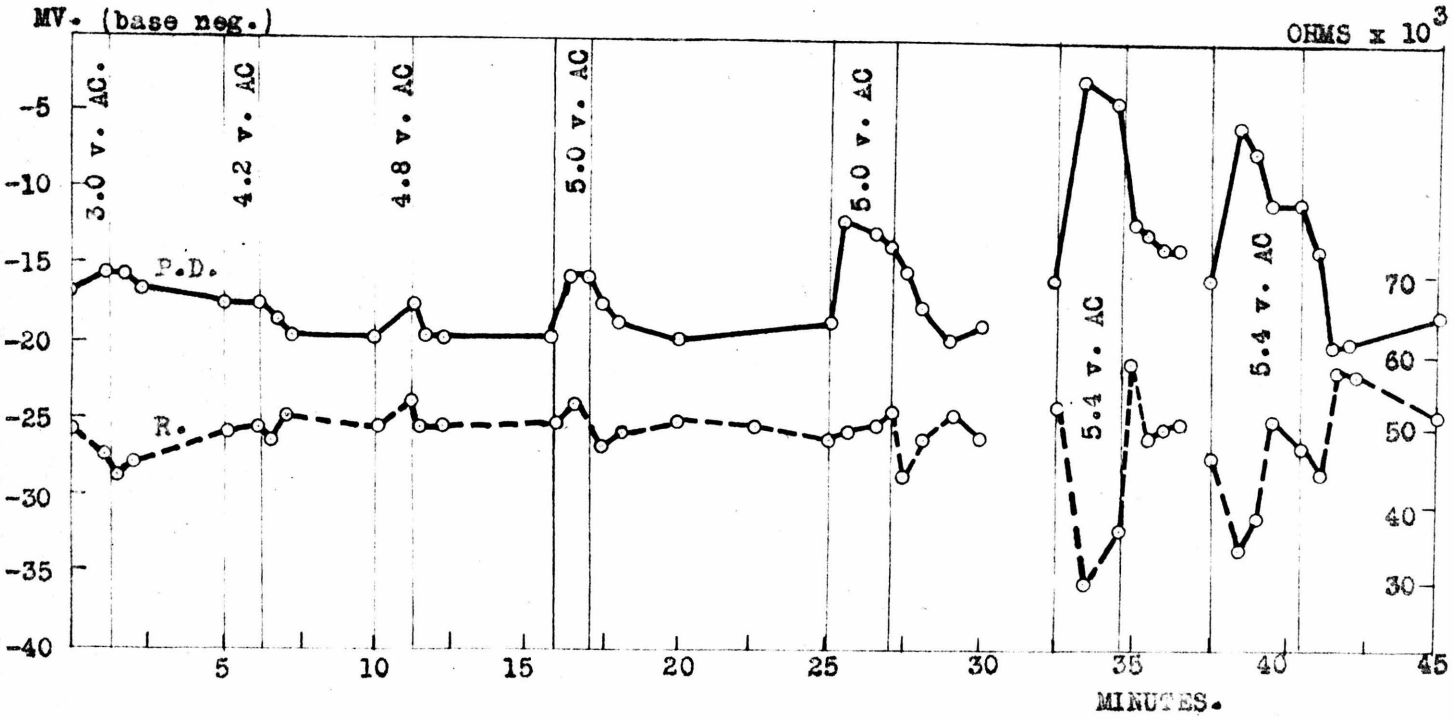
Desiring to see if an A.C. rectifying effect due to a unipolar conductance or permeability exists in the sections as described by Metzner (1930), alternating currents (60 cycles) were applied to the sections for 1 to 2 minutes. Figure 14 shows that 3 volts A.C. or less have little effect on the P.D. or resistance, whereas 5.0 volts A.C. reversibly decreases the resistance and increases the negativity of the apical surface. This confirms the experiment in which the sections had a normal polarity; and also confirms the work of Amlong and Bünning (1934) and Bünning (1934) who clearly demonstrate the correlation between permeability and bioelectric potentials. The permeability changes only after a certain threshold of A.C. stimulation is applied (liminal threshold) at which some change in the surface properties of membranes brings about a sudden increase in permeability, and hence a resistance drop and a P.D. change due to concentration changes and differential ion mobilities.

If one would believe Metzner (loc. cit.), one would think the D.C. galvanometer deflections caused by the applied A.C. meant rectification of the A.C. by the plant tissue. That this is an entirely erroneous conclusion is shown by the P.D. measurements. No galvanometer deflections occur unless the plant P.D. is altered, and hence the galvanometer deflection is caused by <u>stimulation</u> by the A.C., and not rectification of the A.C.

It is seen in figure 12 and in figure 14 that alternating current stimulation increases the negativity of the apical end of the sections. This may mean that the apex is more easily stimulated than the base, with subsequent greater increases in permeability

FIGURE 14

THE EFFECT OF APPLIED ALTERNATING CURRENT ON INHERENT P.D. AND RESISTANCE OF COLEOPTILES.

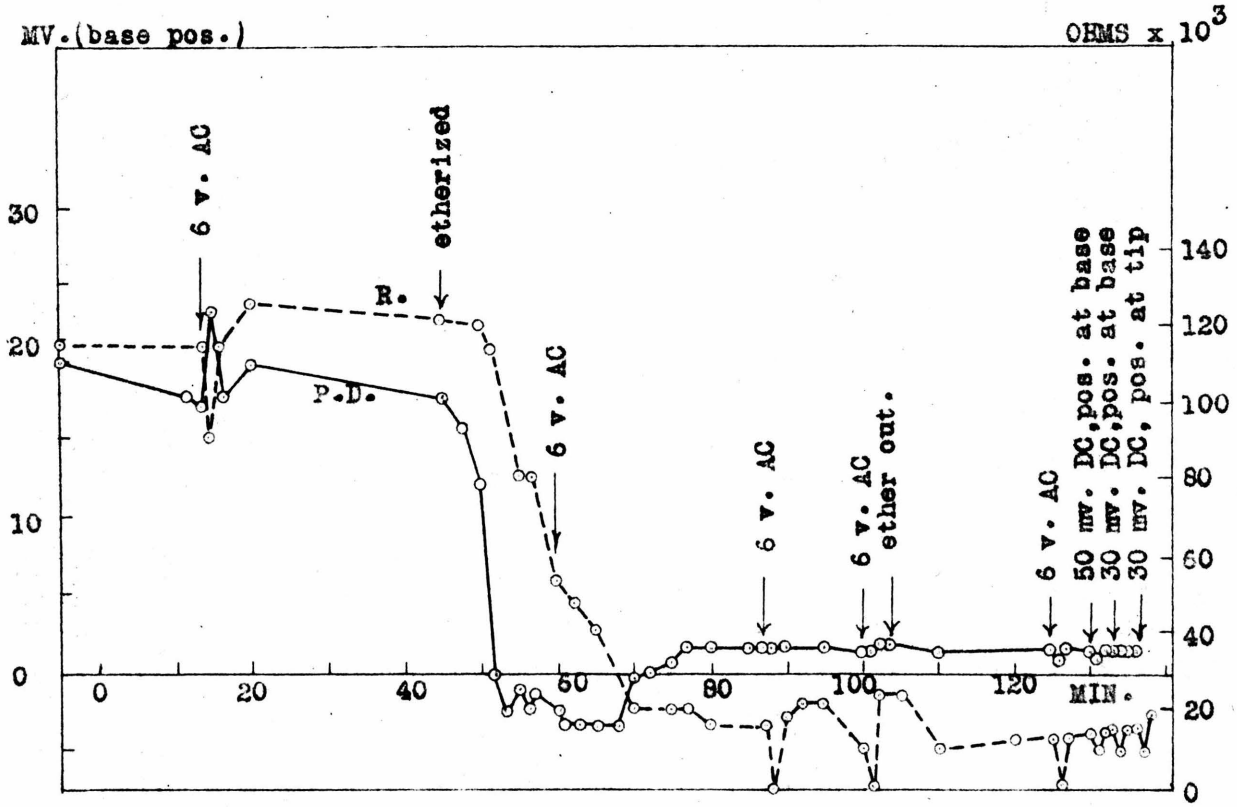


and resulting greater apical negativity. That increased permeability (caused by induction shocks) gives rise to negativity was shown by Amlong and Bünning (1934).

Ether narcosis irreversibly abolishes electrical polarity if the narcosis results in complete loss of turgidity and death. Figure 15 shows the drop in resistance and P.D. when air saturated with water vapor was bubbled through pure ether and passed through the experimental chamber containing the sections. After forty minutes, a residual apical negativity remains which probably represents the true geoelectric effect found by Brauner (1928, 1933) in dead as well as living membranes. Figure 15 also shows that 6 volts A.C. does not change the P.D. in the narcotized tissue. The D.C. galvanometer, however, showed some deflection. Here, therefore, one might think Metzner's unipolar conductance is being manifested in a rectification of the A.C. Brauner (1930), as mentioned in the historical review of this section, demonstrated a unipolar permeability in certain dead plant tissues (dead seed-coats). Brauner did not determine whether such an object could rectify an A.C. In the narcotized sections, it is more probable that the applied A.C. merely increases permeability still more, and hence the resistance falls. The reason why no further P.D. changes result probably rests on the possibility that the narcosis has irreversibly destroyed selective ion permeability. No ohmic unipolar conductance exists in the narcotized sections as shown by equal galvanometer deflections regardless of the polarity of applied, small direct currents (approximately 10^{-7} A.).

FIGURE 15

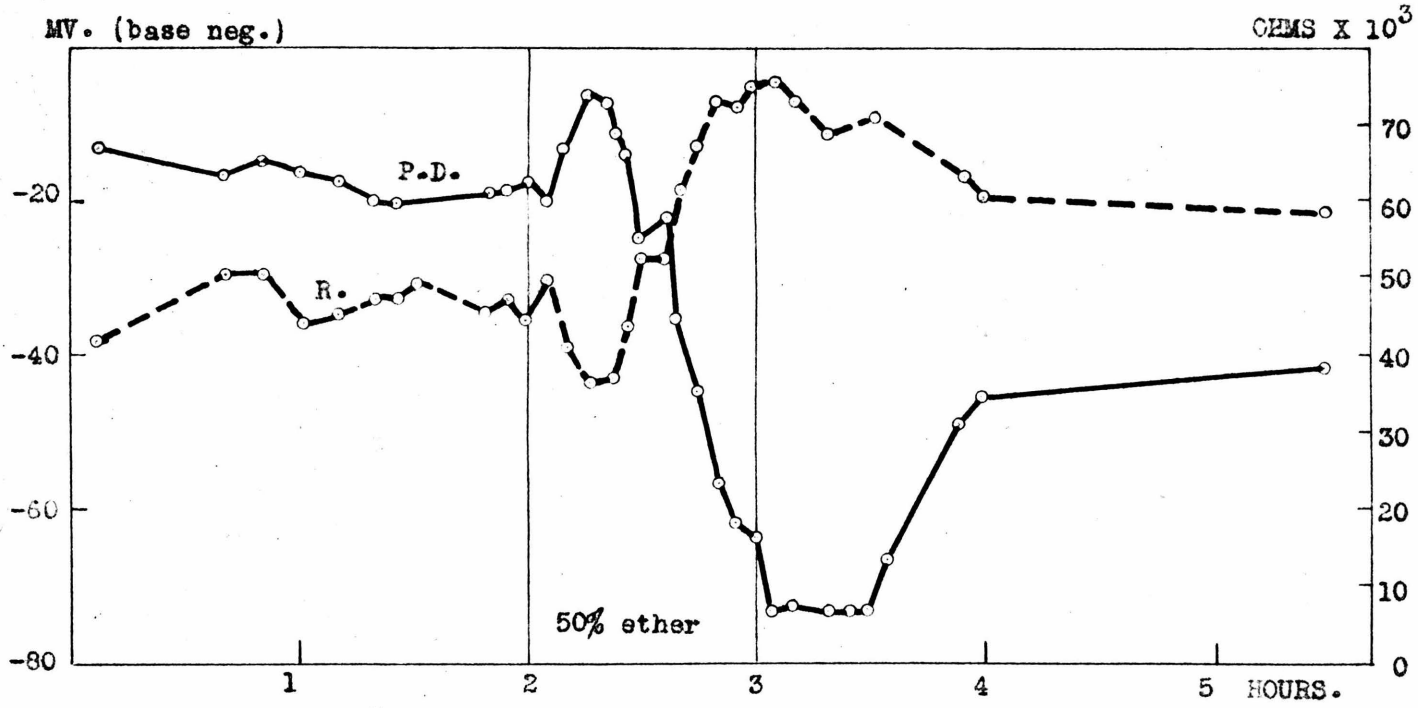
THE EFFECT OF IRREVERSIBLE NARCOSIS ON THE INHERENT P.D. AND RESISTANCE OF COLEOPTILES.



Reversible ether narcosis was obtained in the sections with inverted electrical polarity by passing air through water half-saturated with ether and then through the experimental chamber.

As seen in figure 16, the apical negativity was first increased (stimulation) for a brief period, then decreased, just as in complete narcosis. The resistance changes in the opposite way, and follows the P.D. changes faithfully. The cause of the opposite changes in resistance in reversible and in irreversible narcosis is not known, as no more experiments of this nature were performed. Electrical properties possibly associated with polar transport may be separable from those shown in the later part of this paper (p.72) not to be correlated with polar transport. Reversible narcosis increases the resistance of sections, and decreases their apical negativity. Complete, irreversible narcosis decreases both. Hence the possibility remains that the increased resistance during reversible narcosis signifies a decreased permeability. This decreased permeability may be causally related to decreased polar transport. v an der Weij (1934), as pointed out in the introduction, reversibly abolished polar transport by ether narcosis. His Avena coleoptile sections were narcotized by ether vapours from water half-saturated with ether vapours. This same concentration of ether-vapour was used in the reversible narcosis described above (figure 16). Due to lack of further experimental data, no conclusions can be drawn concerning the possible relation of reversible narcosis to P.D.'s and transport . The problem will be more carefully investigated in the near future.

FIGURE 16
THE EFFECT OF REVERSIBLE NARCOSIS ON INHERENT P.D. AND RESISTANCE OF COLEOPTILES.



SUMMARY OF PART I

1. Theories of the cause of polar transport of auxin in plants are discussed. The electrical theory has been accepted by many workers as one of the most plausible. (cf. part II).

2. Went's bipolar dye-uptake experiments on Impatiens cuttings, used to substantiate his electrical transport theory, are repeated and confirmed. Positively charged dyes are taken up most by bases, negatively charged dyes by apices, of Impatiens cuttings. This is in agreement with the fact that electrical measurements show that Impatiens cuttings have apical electronegativity.

3. Intact Avena and Zea coleoptiles, Pisum stems, and Impatiens hypocotyls exhibit apical negativity when constancy of P.D. measurements is obtained. Various methods of measuring this polarity are discussed.

4. Cut sections of Avena and Zea coleoptiles, and of Pisum and Vicia stems exhibit the same polarity found in Impatiens cuttings, i.e., apical negativity. Time is required to establish this polarity. The P.D.'s of sections are directly proportional to the length of the sections.

5. The internal electrical polarity of the Avena coleoptile is the same as the external.

6. Inverting sections inverts their electrical polarity, i.e., the morphological apices become electropositive to the bases. This inverted polarity disappears with time. It is proportional to the length of the sections as in the case of upright sections; and the time of disappearance of the inverted polarity is proportional to the length of the sections.

7. White light from all sides changes the electrical polarity of etiolated Avena coleoptiles. The polarity reverses 30 minutes after the light is turned off, after which it increases again to reach a higher level than before stimulation. Following this increase, it falls again.

8. The photoelectric effect is localized in the apical end of the coleoptile. The time relations and shape of the "light-electric reaction" are similar to "light growth reactions" such as described by van Dillewijn (1927).

9. Sections of Pisum stems and of Avena coleoptiles are reversibly polarized by applied direct currents beyond certain thresholds of applied current. The normal electrical polarity may be reversibly increased or decreased, or even reversed, by these currents, depending on the polarity of the applied voltage.

10. Apical negativity is increased on stimulation by alternating currents, but only after a certain threshold of applied current is used.

11. Avena coleoptiles cannot rectify an alternating current, as stated by Metzner (1930), nor do they display a unipolar resistance to direct current passage. This means that a possible asymmetry in membrane permeability theoretically linked with polar transport (accumulation, secretion) cannot be analyzed by simple unipolar or rectification measurements.

12. The apical negativity is reversibly decreased by reversible ether narcosis, and the P.D. drops to that caused by gravity when narcosis is irreversible.

13. The resistance of Avena coleoptiles reversibly decreases on passage of small direct currents and alternating currents.

14. The resistance changes follow the inherent P.D. changes, but in the opposite sense, i.e., when apical negativity increases, the resistance decreases. An exception occurs in complete, irreversible ether narcosis, where both resistance and P.D. decrease.

PART II

Polar Transport of Auxin and Electrical Polarity

It was concluded in the first two sections that electrical polarity existed in seedlings and that the apices were electronegative to the basal regions both externally and internally. This polarity could be altered and reversed by light , by gravity, and by applied P.D.'s. The electrical theory of polar transport demands the polarity which was found. On the other hand, the theory is directly contradicted by the fact that neither light nor gravity affect longitudinal transport. It is a recognized fact that light and gravity affect lateral transport; however, this paper is concerned only with longitudinal transport.[1] Indeed, lateral and longitudinal transport seem to be entirely different in nature. The following pages are concerned with experiments in which attempts were made to link transport and electrical polarity. Auxin transport and electrical polarity were measured and the electrical polarity was altered to see if there were parallel changes in transport.

1. The Effect of Applied P.D.'s on Auxin Diffusion in Agar

In accordance with an electrical theory of transport of the plant growth hormones, it was of primary importance to first demonstrate whether or not the hormone is electrically transported in vitro.

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1. The theory of the causal relation of lateral displacement of auxin by applied P.D.'s to electrotropisms has not been directly verified by experimental measurement of this displacement.

Dolk(1930, 1936) attempted to demonstrate such a transport by the following method. Zea mays growth substance (auxin-a) was collected in agar blocks by diffusion from coleoptile tips. Two such blocks were placed between platinum electrodes and a P.D. of from 2 to 10 volts applied across them for one hour. At 10 volts, a strong electro-osmosis of water occurred such that one block swelled while the other diminished in size. Dolk stated that such electro-osmosis would distort the results,(since the water current would carry the auxin to the negative pole). He also said that electrolytic destruction of auxin would probably occur at the electrodes. At 2 volts no appreciable electro-osmosis occurred but the auxin concentration in the two blocks remained the same. (Tested by the Went avena technique, cf. below). Dolk concluded that auxin was not electrolytically transported under the conditions of his experiments.

Koch (see p. 9) also investigated this problem. He stated that auxin-a is transported to the positive pole in agar. It will be recalled that his conclusions are to be criticized in that he used only two test plants for each concentration of auxin determined. (see below, p. 76).

(a) Experimental

The present author has reinvestigated this problem. He used synthetic heteroauxin prepared in these laboratories (indole-3-acetic acid). Since this substance is a weak acid, it dissociates into a large anion and a hydrogen ion. The anion should be transported in an electrical field to the anode. That heteroauxin conducts a current can be determined by actual conductance measurements.

To demonstrate that the heteroauxin used in the experiments about to be described actually conducts a current, conductance measurements were made so that the transport number and absolute mobility under unit potential gradient could be ascertained. The sodium salt of indole-3-acetic acid was prepared by accurately neutralizing the acid and recrystallizing from absolute alcohol. (The salt preparation was kindly made for this purpose by Dr. Koepfli of these laboratories). The salt was then made up in different concentrations and their conductances determined in a calibrated conductance chamber at 25°C., using a conductance bridge with a Vreeland oscillator at 1000 cycles. The cube roots of the concentrations were plotted against the equivalent conductances. This curve was extrapolated to the limiting conductance at infinite dilution. Since the limiting conductances of the hydrogen and the sodium ions at 25°C. are known, the limiting conductance of the acid could be determined from that of the experimentally determined conductance of the salt. Knowing the limiting conductance of the acid, the dissociation constant could be calculated in order to check the accuracy of the conductance measurements. The dissociation constant is known to be about 2.0×10^{-5} (Kögl (1935)). From the limiting conductance of the auxin anion, its transport number and mobility could be calculated.

Two experiments gave limiting conductances of the salt of 79.5 and 78.5, average 79.0 since,

Λ Na @ 25°C. = 50.9 (Noyes and Sherrill (1926))

and Λ H @ 25°C. = 350

hence Λ Auxin anion = 79.0 - 50.9 = 28.1

and Λ Auxin (acid) = 28.1 + 350 = 378 @ 25°C.

Since the dissociation constant, K, is proportional to the degree of dissociation, $\therefore K = \frac{C a^2}{1 - a}$, where $a = \frac{\Lambda}{\Lambda_o}$, Λ = the conductance at different concentrations, Λ_o = the limiting conductance, and C is the molal concentration. For three concentrations used, the dissociation constants were:

Normality	K X 10^{-5}
.004	3.0
.008	2.8
.01	2.8
	Av: 2.9

For a comparison, Kögl's values for the dissociation constant at 25°C. are given. His determinations were based on pH measurements.

N	K X 10^{-5}
.001	2
.005	3
.01	1
	Av: 2

Hence it is seen that the conductance measurements were quite accurate, and in fact gave better constants than Kögl's determinations.

The transport number of the heteroauxin anion is equal to the ratio of the limiting conductance of the anion to that of the sum of the anion and cation, 28.1/378.1 = 0.07. Hence only 0.07 of an electric current is carried by the heteroauxin anion in solution. The absolute mobility of the anion under unit potential gradient of 1 volt per centimeter in a volume of one cubic centimeter is 28.1/96,500 = 2.9 X 10^{-4} cm./ sec. = 10.5 mm./hr.

The above calculations prove that the heteroauxin used is capable of conducting an electric current.

Before experimenting with applied P.D.'s in plants, it was of interest to see how much of a potential gradient is necessary to establish a measurable difference in transport in a liquid or agar medium. To this end the following experiments were performed. Five such experiments were run, the following protocol being typical:

Protocol 1

Effect of Applied P.D. on Auxin Transport in Agar

Tap-water agar blocks 11 X 8 X 1 mm. were soaked one hour in heteroauxin of a known concentration. One of them was then placed atop 3 tap-water agar blocks of the same dimensions, and Ag-AgCl electrodes applied at each end of the stack of blocks. A P.D. of 300 millivolts was applied for one hour such that 9 microamperes flowed. Another stack of blocks was set up in the same way but with the polarity of the applied P.D. reversed. A control was run in which no current was passed. At the end of the hour, the blocks were removed, separated, and each one cut into 12 smaller blocks and tested for their auxin content by the standard Went technique. The technique involves placing a smaller block on one side of the stump of decapitated Avena coleoptiles, so that the auxin from the block will diffuse down one side of the coleoptile more than it does the other side, thereby causing that side to grow more than the other side, resulting in a curvature away from the side on which the block was placed. This curvature is proportional to the concentration of auxin in the block, the size of the block, the time between decapitation and placing the block on, and the time the block is left on. The method is so controlled, that within certain limits of auxin concentration, the curvatures in degrees give an accurate analysis of the concentration such that 1^{o} corresponds to about 10^{-7} mg. of heteroauxin. Usually 12 to 24 plants are used for each determination, the averages being taken. (For further details, cf. Went, (1935); Schneider, (1937)).

The results of the protocol described above are as follows:

The effect of Applied P.D. on Auxin Transport Through Agar Blocks (Exp. 4)

300 millivolts
Transport time one hour
9 micro-amperes current flow

	Control	Anode	Cathode
Top block---	12°	9	10
	6	7	5
	5	2	4
Botton block---	2	2	2
		Cathode	Anode
Sum	25°	20	21

The arrows indicate the theoretical effect of the current on the auxin transport. In another experiment, 450 millivolts applied for one hour, and at 28 microamperes current flow, gave considerable electrolytic destruction of auxin. The concentration used in the tip blocks was 20°. At the end of one hour only about 5° remained in all four blocks. The blocks nearest the anode showed white discolorations which on microscopic examination proved to be due to minute gas bubbles, presumably of oxygen. When 10° auxin was used and 600 millivolts applied, complete destruction occurred.

Protocol 1 shows clearly that under the conditions of the experiment, in which no electro-osmosis or auxin destruction occurred, an applied E.M.F. has no effect on the transport of auxin in agar. Since higher potential gradients seemed necessary in order to detect a measurable effect, it was thought that electrolysis could by overcome by the use of flowing electrodes or completely non-polarizable electrodes.

Experiments were then conducted using automatic siphons to wash away the products of electrolysis at the electrodes. Zn electrodes were used, and the solutions at the electrodes were separated from the agar blocks with agar-gelatin seals. An auxin solution was used to wash the top electrode, its concentration being the same as that in the top block. Tap-water was used to wash the bottom electrode. In this way the concentration in the top block would remain constant while transport occurred. Four agar blocks were used; 22.5 volts applied, and 0.1 milliampere current flowed for one hour. Analyses of the blocks showed 26°, 24°, 19°, and 12° from anode to cathode,

while in the case of the blocks used for the opposite polarity no auxin entered the blocks at all. The control showed 6°, 3°, 2°, 1° from top block to bottom block, showing that transport by diffusion was much less than electrolytic transport. This P.D. represented approximately a 50 volt per centimeter gradient, and definitely causes auxin to be electrolytically measurably transported to the positive pole. The results would not be particularly distorted by electro-osmotic flow since water travels to the negative pole in agar and would only tend to decrease the amount of auxin carried by electrolysis. That water is carried to the negative pole was easily demonstrated by applying a P.D. of one or two volts across an agar thread 20 X 1 mm. The thread immediately swelled at the negative pole, and shrunk at the positive. Therefore, in the above experiment, auxin traveled to the positive pole <u>despite</u> any flow of water in the opposite direction.

2. Electrical Polarity and Polar Transport in the Avena Coleoptile

It has been demonstrated that sections of coleoptiles exhibit an electrical polarity which is in the right direction for the electrical transport theory. It would be necessary to directly demonstrate that one of these factors varies in the same way that the other is when it is altered. The following section is concerned with measurements of transport when the electrical polarity is varied. A later section (p. 90) is concerned with measurements of electrical polarity when polar transport is varied.

a. Transport Technique

The transport experiments were performed as described by van

der Weij (1932), except that no holders were used to support the coleoptile sections as this solicits auxin leakage through water films in the holder. Sections were cut, usually 3mm. long in all experiments, from 72 hour Avena coleoptiles. The coleoptiles were grown in sand from hulled seeds of Avena sativa of a pure line stock (Victory oats) in the experimental dark room at a relative humidity of 90, at 24°C., and in orange light not causing photo-tropisms. The section-cutters were made of two razor blades separated by a brass strip. Usually two sections were cut from the actively growing sub-apical zone of a coleoptile. The sections were then placed in an upright position on wet filter paper by means of eye forceps. This washes out enzymes which destroy the auxin. (cf. van Overbeek (1936)). After an hour, excess moisture was removed from the sections with filter paper. Twenty sections were then placed on the 11 X 8 X 1 mm. agar blocks (tap water agar) and another agar block containing the auxin placed on top of the sections. Agar blocks can be made to contain auxin in any desired concentration by soaking them in the solutions for about an hour. The transport time was usually one to two hours depending upon the concentration of auxin in the top block. After this time, the two blocks were removed, cut into 12 smaller blocks, and tested by the Avena technique. The entire procedure took place in the dark room. The sum of the values obtained for each block should approximate the amount originally in the top block. The results were usually tabulated as the percentage of the sum of the auxin concentration found in top and bottom blocks which was present in the bottom block.

b. Shunting Experiments

Rosene (1935) demonstrated that the P.D. between apex and base of an onion root could be decreased by liquid shunts interposed between the contacts and at a distance from them. On the basis that the P.D. between the two ends of a coleoptile section could be lowered by a metallic shunt, it was thought that the auxin transport might be simultaneously lowered. Hence metal-foil (tin) was placed on the top and bottom agar blocks of a transport experiment, the foil making a shunt between the 2 blocks. Controls were run with foil not shunted. A typical experiment showed the following result. The top block of the control contained 8°, and 4.5° in the bottom block. The shunted experiment contained 8°, in the top block, and 3° in the bottom block. The percent of top plus bottom entering the bottom block of the control was 36% in the control, 27% in the shunted experiment. The difference, 9%, is well within the experimental error. In five such experiments the differences (expressed as was the above result) were as follows: +12%, +9%,-7%,-5%,-2%, average +1.4%, which is well within the experimental error.

The conclusion is that shunting the P.D. between the two ends of a section has no effect on the auxin transport. It is realized that such a shunt is probably very ineffective in reducing the individual P.D.'s probably maintained across each cell boundary. The electrical resistance of the coleoptile section is very high (nearly a hundred thousand ohms for the above set-ups), and the P.D. between the two ends is relatively very minute. (a few millivolts). Hence any current dissipated by metallic shunts

between the two cut ends would by quite small, and the individual cell P.D.'s would probably be unaffected. If the resistance of the tissue were quite small, the situation might have been different.[1].

c. Applied P.D.'s and Transport in the Coleoptile Sections

Transport tests such as described in the previous section were made while a P.D. was applied between the two blocks such that the electric current would theoretically augment transport in one case and inhibit it in the other. Ag-AgCl electrodes were applied directly to the agar blocks and P.D.'s of from 25 to 750 millivolts applied for one to two hours. The electrodes were the same size as the agar blocks and were replated every experiment. A typical result is shown in protocol 2.

Protocol 2

Effect of Applied P.D. on Transport in Coleoptile Sections

Heteroauxin concentration 40° in top block at start.
Transport and current application one hour.
P.D. 300 millivolts.
20 three mm. sections for each transport experiment.
After one hour, the top blocks were diluted 3 X for analysis.

	Control	Anode	Cathode
Top block	35°	31° ↑	31° ↓
Bottom block	8°	7° ↑	7° ↓
		Cathode	Anode
Sum	43°	38°	38°
% of sum to bottom	18.6%	18.6%	18.4%

The arrows indicate the theoretical effect of the current flow on the auxin transport.

1. Clark (unpublished data) and Francis (1933) showed that the P.D. across frog skins (20 ohms resistance) is maintained

Of twelve such experiments in which the applied P.D. varied from 25 to 300 millivolts, the total average of the percent of the sum of the concentrations found in top and bottom blocks which was present in the bottom blocks was : with the current (anode at bottom block), 21%; against, 20%; control,25%. It is obvious that the differences are within the experimental error and that no effects of applied P.D.'s on transport were found.

Since no results were obtained in the above experiments, it was thought that perhaps the potential gradients were not steep enough. The absolute mobility of the heteroauxin anion is 10.5 mm. per hour at one volt per centimeter. We should therefore expect 300 millivolts per 3 millimeters which is nearly one volt per centimeter, to affect the auxin transport in one hour, regardless of the fact that the conducting path in the experiments just described is not exactly like the standard cube usually referred to when the term "absolute mobility" is used. It was found, however, in the experiments using Ag-AgCl electrodes that a potential difference of more than 300 millivolts caused destruction of the auxin. It must also be remembered that hydrogen ions, and ions of other salts

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when externally shunted until very low values of external resistance are attained, after which the P.D. falls. Here the cells responsible for the P.D. are fewer in number, and have lower resistances than the plant cells discussed above, and an external shunt can lower the individual cell E.M.F.'s more effectively. Rosene(1935) and Marsh (1930) have also shown how dissipation of electrical energy by external shunts may lower bioelectric potentials.

(in agar and tap water) will carry the current, the auxin carrying only 7% of the current. Hence it would be necessary to apply larger currents for longer periods of time, expecially when high resistances are encountered (e.g., 50 volts/cm. in the case of agar).

Since electrolytic destruction of auxin occurred when P.D.'s above 300 millivolts were used, recourse to flowing electrodes was taken. The same type of siphon flow electrodes were used as were described in the section on transport in agar (p. 72), with the exception that the apparatus was made so that the electrode vessel and top agar block could be racked down to the sections on the bottom agar block by a set-screw mechanism. This despended with clumsy manipulation and the danger of upsetting or injuring the sections by loading them down with cups, siphons, agar-gelatin seal, and fluid. The fluid used for washing the top electrode was N/10 KCl and auxin, and N/10 KCl for the bottom electrode. Current was passed so as to theoretically augment transport in one case and to decrease it in the other (opposite polarity). No difference was found, however, in the amounts of auxin transported, even when P.D.'s of 3 volts and a current of 0.04 milliamperes was used. The sections, after current treatment, were washed in an upright position on wet filter paper for an hour, and tested in further transport experiments to see if there were latent effects of the current treatments, but none were observed. It was not easy to wash out the auxin remaining in the sections left there by the first treatment, so that any latent effects occurring were frequently masked by contamination. Hence sections were current-treated in the apparatus <u>without</u> auxin application,

and then immediately tested in transport experiments for any changes in their ability to transport normally. A typical experiment follows:

Protocol 3

Latent Effect of Applied P.D. on Transport in Avena Sections Flowing electrodes

40 three millimeter sections from subapical zones of coleoptiles per experiment, i.e., for each polarity and for the control. P.D. 1.5 volts. Current 0.8 milliamperes. Transport time and treatment one hour.

Each lot of 40 sections in each experiment were then halved for duplicate transport experiments, using an auxin concentration of 45° in the top blocks. The average results of each duplicate set are shown as follows:

	Anode	Cathode	Control
Top block	7° ↑	7° ↓	7.2°
Bottom block	5° Cathode	4.6° Anode	6°
Sum---------	12°	11.6°	13.2°
% of sum to bottom	42%	40%	45%

The average of four such experiments gave as the percentage of the sum transported to the bottom block; anode at top (opposing transport), 37%; cathode at top (with transport), 39%; control, 38%. These results signify no effect of the current on transport in the sections. Higher currents, i.e., above one milliampere, caused the sections to become flaccid and hence could not be used in the case in agar.

Effect of Applied P.D.'s on Inherent P.D.'s and Transport in Coleoptile Sections

The question now arose, does the inherent P.D. of the sections

change appreciably when such P.D. applications were made?

A priori, this question can be answered in the affirmative, since on p. 59 , it was shown that 100mv. can reverse the polarity of many sections in series. To assume that the polarity in the following experimental procedures is reversed, however, would be unjustified, since in the experiments described on p. 59 , only short times of current passage were studied. To definitely answer this question, therefore, transport tests were made in which auxin transport, inherent and applied P.D.'s, were all measured simultaneously. Several such experiments were performed, the following protocol being typical:

Protocol 4

Effect of Applied P.D.'s on Inherent P.D.'s and Transport

Three millimeter sections were cut and washed in an upright position for onehour on wet filter paper. 20 such sections were placed on tap-water agar blocks in a moist chamber, electrical contact being made to the block with a strip of N/10 KCl agar which led outside the moist chamber to a cup filled with N/10 KCl in which the $Zn-ZnSO_4$ electrode could be placed. The top agar block containing auxin had a similar KCl agar strip leading to another cup and electrode outside the chamber. The control transport experiment had KCl agar strips of the same size contacting the top and bottom blocks. The inherent P.D.'s of such set ups, as well as the applied P.D.'s used were measured with the string electrometer, the current passage with a micro-ammeter. It was found that the electrodes did not polarize with the currents employed, so that the same electrodes used for applying the P.D.'s could be used for measuring the change in inherent P.D.'s of the sections. In such transport experiments it is seen that we are measuring the average P.D.'s of 20 coleoptile sections in parallel. The external P.D.'s were applied, measured, cut off after awhile, and the inherent P.D.'s immediately recorded. About 2 seconds elapsed between two such readings, but it was observed that the inherent P.D.'s maintained their levels

several seconds after current treatment of a minute or so (see p.60). In other words, depolarization was not so rapid that the effect of the applied P.D.'s on the inherent P.D.'s could not be accurately determined with the short period string electrometer.

In the experiment being described, the transport time was 90 minutes, the applied P.D. 3 volts, and the current flow 10 microamperes. Before the P.D. was applied, the inherent P.D.'s were: Cathode at the top block (current flow augmenting transport), apex 2 millivolts electronegative to the base; anode at the top (current flow theoretically inhibiting transport), apex 4 millivolts electronegative to the base. Hence the same polarity existed as found and described in the first part of this paper. Five minutes after the current was turned on, the inherent P.D. was measured after briefly cutting off the applied current. every five minutes thereafter during the course of the experiment, the inherent P.D. was measured and found to be the same throughout, namely: cathode at the top (augmenting transport?), apex 43 mv. negative to base; anode at the top (inhibiting transport?), 55 mv. positive to base. The blocks were analyzed for their auxin content at the end of the 90 minutes and the following results were obtained.

In the case where the inherent apical negativity was increased from 2 to 43 mv.,

	Cathode	Control
Top block ----------	3°	4°
Bottom block --------	4°	5°
	Anode	
Sum ----------	7°	9°
% of sum to bottom --	57%	55%

In the second case, where the inherent polarity was inverted from an apical negativity of 4 mv. to a positivity of 55 mv.,

	Anode	Control
Top block ----------	4°	4°
Bottom block --------	3°	3°
	Cathode	
Sum ----------	7°	7°
% of sum to bottom --	43%	43%

Five such experiments gave the same result, namely that increasing or inverting the ingerent electrical polarity had no effect on the polar transport.

d. Geoelectrical Polarity and Polar Transport

As mentioned in the introduction, Went (1928), van der Weij (1932), and Pfaeltzer (1934), clearly demonstrated that gravity has no effect on the polar transport of auxin in Avena coleoptile sections. The present paper shows that inverting sections inverts their electrical polarity for awhile (p. 44). Should it be demonstrated that this inverted electrical polarity has no effect on polar transport, the evidence would be yet stronger against the thesis of inherent electrical transport. To this end, transport experiments were performed in which sections were inverted and the inherent electrical polarity and transport measured. Protocol 5 illustrates one such experiment.

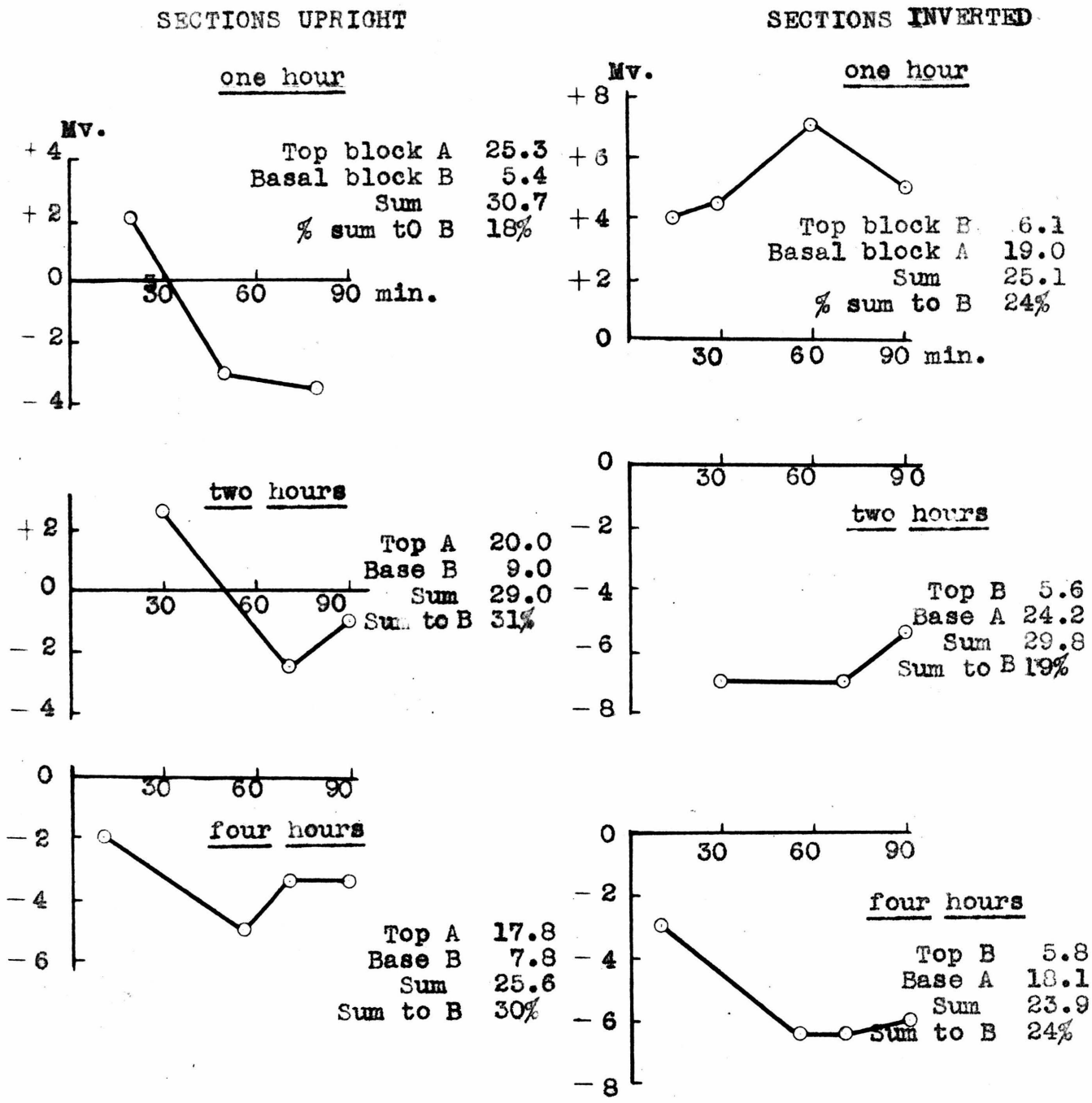
Protocol 5

Effect of Gravity on Electrical Polarity and Transport

> Sections were cut and divided into two lots. one lot was placed on wet filter paper in an upright position, while the other lot was placed inverted. After one hour, twenty sections from each lot were removed and a ninety minute transport test made on each lot, the sections remaining in the position they had on the filter paper. The auxin agar blocks were placed on the upright apical surfaces of the upright lot and another on the inverted apices of the inverted lot. During the transport, the P.D. between each block of the two transport experiments was measured several times. The curves in figure 17 represent the change in these P.D.'s with time. At the end of two hours, two more lots were removed from the wet filter paper and the process repeated, as was also the case for two more lots left standing 4 hours after sectioning. In this way, the effect of gravity could be

FIGURE 17.

The effect of gravity on electrical polarity and transport



followed over a period of several hours. Figure 17 contains the results for the upright and inverted sections left in those respective positions for 1, 2, and 4 hours, thus containing six experiments in all.

The P.D.-time curves of each of the six experiments are shown in figure 17. Diagrams representing the auxin analyses of the top and bottom blocks, the tabulated sums of the top and bottom blocks, and the percentage of the sums which were found in the bottom blocks, are also shown in figure 17.

In the above protocol, the time-P.D. curve of the upright sections shows the usual characteristic, that time elapses before the apical negativity is established, namely less than one hour for freshly cut sections; and in the case of the sections left standing 4 hours, the apical negativity was apparent on the first measurement. It is seen that the sections left inverted one hour show the usual inverted electrical polarity, i.e., apical positivity, and that this positivity is maintained throughout the transport test; hence if the electrical transport theory is correct, the transport polarity should also be inverted. After two hours of morphological inversion, the sections have regained their normal polarity, a characteristic already discussed on p. 45 . After four hours, the normal polarity was even more pronounced.

It is seen at once that, regardless of the fact that the electrical polarity was inverted in the case of sections left inverted one hour, the amount of auxin transported is the same as in the upright sections with normal electrical polarity.

In this section, therefore, it was demonstrated that although auxin can be electrolytically carried to the positive pole in agar, it is not transported basally in coleoptiles because of the measured inherent positivity of the morphological base.

3. The Specific Inhibition of Auxin Transport

It has been shown in the previous section that the longitudinal transport of auxin is independent of measured electrical polarity. This was demonstrated by altering the polarity and showing that transport was unaffected. This section will show that transport may be specifically abolished without apparently changing any other properties yet investigated. Among these later were semi-permeability, cell-elongation, protoplasmic streaming, respiration, and electrical polarity.[1].

Ponder and Macleod (1937) showed that the P.D. across a frog skin could be completely abolished by certain lysins, without affecting respiration at all. It was thought, therefore, that such substances might abolish the electrical polarity of *Avena* coleoptiles without affecting polar transport, since the latter is dependent upon respiration (van der Weij (1932, 1934); Bonner (1936) (1934)).

It was entirely unexpected, therefore, when the results showed just the opposite. The lytic substance used abolished transport, but not electrical polarity, or any other observed properties

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1. van der Weij (1932, 1934) showed that change of temperature altered transport, and that ether narcosis could reversibly inhibit it (see introduction). These two environmental changes, however, would have many other effects beside altering the mechanisms directly responsible for polar transport. There are probably many factors which are limiting in polar transport, among which might be mentioned, food-supply, oxygen, pH, water-supply, and temperature. Light, through its effects on protoplasmic streaming (Bottelier (1934)), may influence the rate or intensity of transport, but probably not polarity. Temperature and narcosis affect many other processes in the plant remotely related or entirely unrelated to the polarity mechanism of transport.

until the concentrations of lysin were high enough to be very toxic. The following pages will describe the experimental procedure.

a. Experimental

On the theory that certain lysins (saponin, sodium glycocholate, or taurocholate) abolish bioelectric potentials without influencing respiration in the frog's skin, it was thought that these substances might inhibit polar transport of auxin in Avena coleoptiles, if membrane P.D.'s are related to transport polarity.

One way of testing the transportability of various auxins is to see if they will cause curvatures in Avena when applied in agar blocks on one side of the decapitated coleoptile. If no curvature ensues, and yet the substance is active in the pea test, the substance is not longitudinally transportable (Haagen Smit and Went (1935); Went and Thimann (1937)). For this reason, saponin and sodium glycocholate were applied in agar blocks to the decapitated apices of Avena coleoptiles for 3.5 hours, after which a second decapitation was made in which about one millimeter of coleoptile tip was removed. Indole-3-acetic acid in agar blocks was then applied on one side of the decapitated stump, the resulting curvatures being recorded 90 minutes after the auxin application. The control curvatures averaged 15°. The following table represents the results (12 plants per test).

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1. See page 95.

Table 3

Effect of Saponin (S) and Sodium glycocholate (Na-G, Merck) on Avena Tests with Heteroauxin

Agar block on stump 3 hrs., and containing:	Curvatures
Tap-H_2O (control)	15.9°
Na-G 1:10^5	12.7°
" 1:10^4	12.2°
" 1:10^3	13.7°
" 1:50	13.2°
Tap-H_2O (control)	13.5°
S (sat'd) 1:1000	15.0°
" 1:100	10.7°
" 1:10	15.0°

Although some variation occurred in this test, it is clear that no inhibition of Avena curvatures was obtained. The conclusion was tentatively made that the large glucoside molecule, saponin; and the sterol-amino molecule, Na-G, did not enter the coleoptiles.

For this reason, direct transport tests were made in which the sections were infiltrated with S and Na-G, with a water-infiltrated lot for controls. Four mm. sections from Avena coleoptiles were cut with the van der Weij section-cutter (1932) and placed with their hollow centers on the teeth of combs, to enable the experimenter to determine their morphological polarity after treatment in various solutions. The combs holding the sections were placed in Na-G and S solutions, with a water control, and infiltrated for two minutes by removing air from the intracellular spaces by negative pressure produced in a dessicator by an aspirator, after which the solutions replaced the air when the pressure was released. The combs were then removed, the

solutions carefully blown from the hollow centers of the sections, the sections blotted, and 6 of them placed with their morphological bases downward on tap-water agar blocks. Agar blocks containing 0.98 mg. indole-3-acetic acid per liter were placed on their apices. After two hours, the bottom blocks were removed, cut into twelve smaller blocks, and tested by the Avena test, using the double decapitation method with three hours between decapitations, and ninety minutes between time of putting the agar blocks on and photographing (Schneider (1937)). Table 4 shows the results. One column in this table shows the result of a microscopic examination for protoplasmic streaming in the sections after the transport tests had been made. The sections showed protoplasmic streaming when placed intact in a drop of water on a slide; or when cut into two halves with a razor blade and examined under water (900 magnifications, white light). Another column describes the macroscopic condition of the sections.

Table 4

Concentration test (0.073 mg. per l.) 8.4°
" " 7.3°
Av: 7.8°

7.8 = 1/40 stock soln. (2.98 mg./l.) ∴ stock = 7.8 X 40 = 312°.
Top blocks = 1/3 stock = 312/3. ∴ Top blocks contained 104°.

Analysis of bottom blocks of transport tests in which sections were infiltrated with:	Curvature	Protoplasmic streaming	Appearance
Na-G 1:100	No curvature	-	flaccid
Na-G 1:500	" "	+	turgid
S (sat'd) 1:20	8.9°	-	turgid
S 1:100	11.0°	+	turgid
H_2O	8.8°	+	turgid

It is concluded from this table that : (1) glycocholate is toxic in a concentration of 1:100. It is not toxic in 1:500. The criteria for toxicity were taken to be cessation of protoplasmic streaming, and flaccidity caused by loss of semi-permeability and turgor.

(2) Glycocholate in non-toxic concentrations completely abolishes auxin transport. This is not due to cessation of protoplasmic streaming.

(3) Saponin in a concentration of 0.25-saturated stopped protoplasmic streaming, but had no effect on auxin transport. This is interesting, as Went (1928), Bottelier (1934), (see introduction) point out that protoplasmic streaming may limit transport.

Saponin in higher dilution has no effect on auxin transport, turgor, or protoplasmic streaming.

It is immediately conceivable that the effect of glycocholate may not be limited to the membrane mechanism responsible for polar transport. In higher dilution (1:500) it does not obviously affect permeability, as judged by maintenance of turgor; nor does it affect protoplasmic streaming. It might, however, inhibit some other mechanism which limits transport, for example, cell oxidations, and presumably, therefore, the sources of energy for the performance of concentration work in polar transport.[1]

Bonner (1936) has shown that growth by cell elongation in

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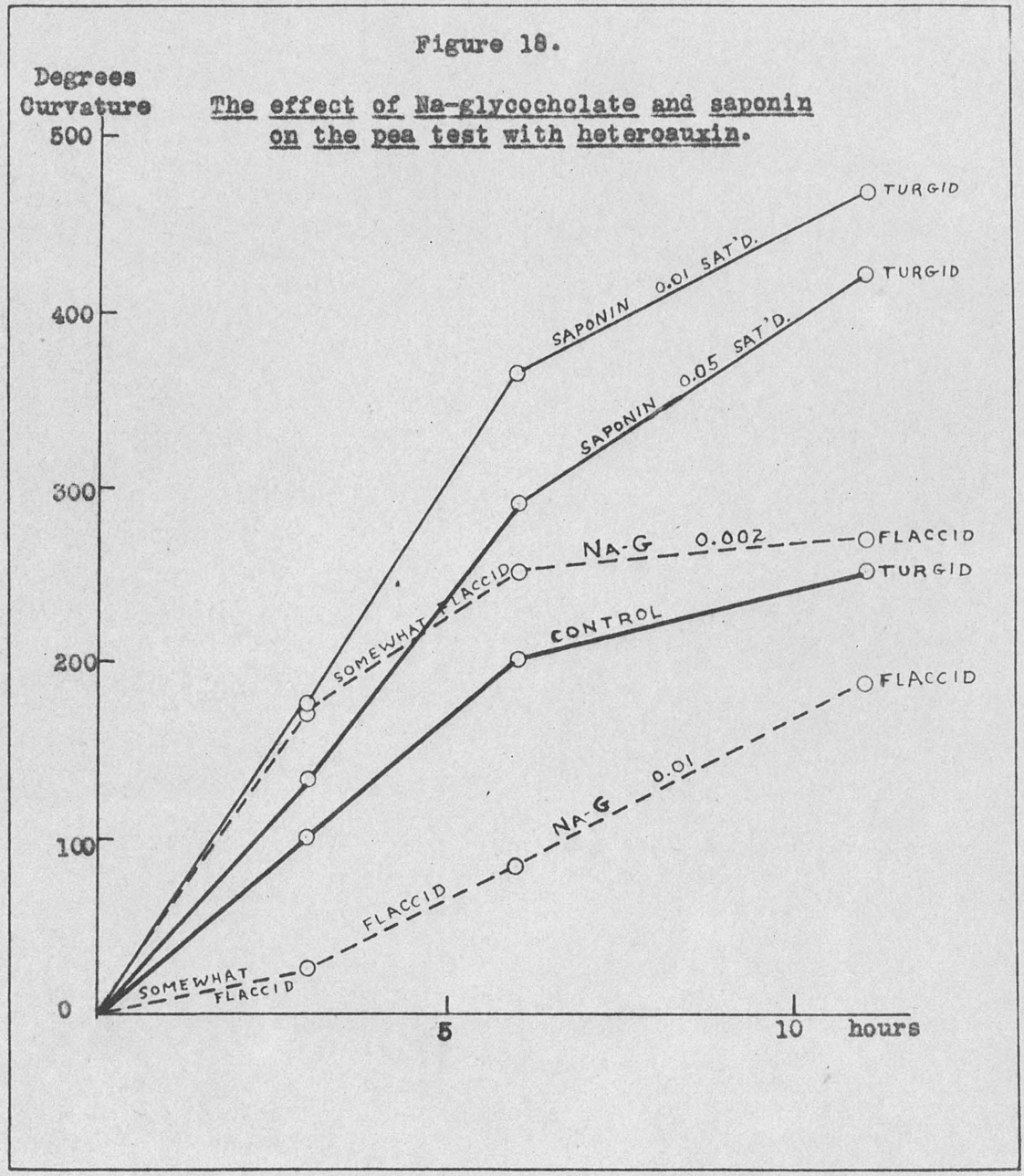
1. cf. van der Weij (1934), Hoagland, Steward, and Davis (see p. 4 introduction), who show that polar transport and ion accumulation are limited by oxidative processes.

the Avena coleoptile is limited by respiration. If, therefore, glycocholate affects respiration, it should affect growth. Since, however, growth in Avena coleoptile sections is limited by transport (Haagen-Smit and Went (1935)), the effect on growth must presumably be studied by means of the pea test (Went and Thimann (1937); Went (1934); van Overbeek and Went (1937)). In this test, polar transport does not limit growth. Hence the effects of glycocholate and saponin on heteroauxin curvatures in the pea test were investigated. Etiolated Pisum seedlings were used about seven days after soaking and planting. The first leaves were removed, and the apical end of the stem split longitudinally about 5 cm. The stem was then cut off just below the split part, and the split stems put in the solutions to be tested. Auxin enters the intact sides of the split ends more than on the cut side (van Overbeek, loc. cit.), increasing growth there, so that the two halves curl inward. The extent of this curvature is roughly proportional to the activity of auxin on cell elongation.

The split pea stems were put in solutions containing auxin alone, saponin alone, glycocholate alone; and in auxin solutions containing saponin and glycocholate. The curvatures were measured after 3,6, and 11 hours, the resulting curvatures being plotted in figure 18. The concentration of heteroauxin used was 1.96 mg./liter. Seven tests per solution were made.

Figure 18.


Degrees Curvature
The effect of Na-glycocholate and saponin on the pea test with heteroauxin.
500
400
300
200
100
0
5
10
hours
TURGID
SAPONIN 0.01 SAT'D.
TURGID
SAPONIN 0.05 SAT'D.
NA-G 0.002
FLACCID
SOMEWHAT FLACCID
TURGID
CONTROL
FLACCID
NA-G 0.01
FLACCID
SOMEWHAT FLACCID

It is seen that both glycocholate and saponin in concentrations which do not cause a decrease in turgor, not only do not inhibit growth , but actually accelerate the growth rate, producing final auxin-curvatures between 50 and 60% higher than the auxin controls. Glycocholate alone had a very small activity on the pea test, but saponin had none.

The method of the facilitation of auxin activity by these lytic substances is not yet known. It might be conjectured that they (1) increase the membrane permeability to auxin, which would increase the rate of curvature; and (2) facilitate the action of auxin in the processes governing increased cell wall plasticity and therefore cell elongation.

The important conclusion for the purposes of this section is, however, that apparently non-toxic concentrations of lysins do not inhibit the action of auxin on plant growth, while one of them (glycocholate) completely abolishes auxin transport.

This conclusion has been justified by repetition of the above experiments, using glycocholate in concentrations down to $1:10^5$. The following experiment illustrates this justification:

Four millimeter coleoptile sections were infiltrated, dried, and two hour transport tests performed with heteroauxin of a concentration of 356^o in the apical agar blocks of six upright sections. The basal blocks were tested by the Avena test, using the double decapitation method described above. The sections were infiltrated with aqueous glycocholate solutions of the concentrations indicated in table 3.

Table 3

Effect of Sodium glycocholate on Auxin Transport

Sections infiltrated with:	Analysis of basal block	Protoplasmic streaming	Appearance
H_2O	11.4°	+	turgid
Na-G 1:10^5	no curvature	+	"
" 1:10^4	"	+	"
" 1:1.5X10^3	"	+	"
" 1:10^3	"	-	"
" 1:5X10^2	"	-	flaccid

The conclusion is that glycocholate abolishes transport in very high dilution,[1] although protoplasmic streaming and semipermeability are unaffected.

As stated above, Ponder (1937) found respiration unaffected in frogs' skins although the P.D.'s were abolished by saponin and Na-G (1:100). It was shown above that auxin transport is presumably specifically abolished.

The following experiment shows the effect of Na-G on the electrical polarity of coleoptile sections from the same lot that were infiltrated for the transport tests. The sections were piled in tiers to give series summation of P.D.'s (see p. 57). Two tiers of ten sections each were piled up, and the P.D. between morphological tips and bases measured with the electrometer. Normal polarity existed, since the bases were electropositive. Table 4 shows the results of measurements two hours after setting up (about the same time that the transport tests were ended).

1. Dilutions higher than 1:10^5 have not as yet been tested.

Table 4

Electrical Polarity of Coleoptile Sections Treated with glycocholate

Sections infiltrated with:	P.D. in mv. (base +)
H_2O	10
Na-G $1:10^5$	10
" $1:10^4$	10
" $1:5X10^3$	5
" $1:10^3$	0
" $1:5X10^2$	0

Microscopic examination of sections treated with $1:10^3$ and $1:5X10^2$ Na-G showed a few motile bacilli in the cell vacuoles (gelatin was used to make electrical contact with the sections. The contacts were, however, isoelectric). This shows that semi-permeability was completely abolished by the more concentrated, toxic solutions of Na-G, so that large particles such as bacteria could easily pass through the cell membranes into the vacuole. This observation lends support to the observation that these sections were flaccid, had no protoplasmic streaming, and no P.D.

The unexpected conclusion was immediately made that although $1:10^5$ down to $1:5X10^3$ Na-G completely abolishes auxin transport during the two hour transport test, it has no effect on electrical polarity (or protoplasmic streaming and semi-permeability).

Pea tests confirmed the earlier observation that Na-G increases the activity of auxin. In this test, unlike the former, the split pea stems were first infiltrated[1]. with the Na-G solutions before being placed in the Na-G-auxin solutions.

1. Infiltration of the pea stems thus makes their physiological state more comparable with that of the infiltrated coleoptile sections.

The pea curvatures were measured after 12 and after 24 hours. The concentration of auxin used was 0.73 mg./liter. Ten tests per solution were made. Table 5 shows the results:

Table 5

Effect of glycocholate on Pea Test Curvatures in heteroauxin (GS.)

Solution		Curvatures			
		12 hrs.	condition	24 hrs.	condition
GS + H_2O		150°	turgid	173°	turgid
GS + Na-G	1:10^5	165°	"	224°	"
"	1:10^4	170°	"	211°	"
"	1:5X10^3	270°	"	290°	"
"	1:10^3	190°	flaccid	too flaccid to measure	flaccid
"	1:5X10^2	130°	"	"	"
Na-G control	1:5X10^3	-62°	turgid	negative curvature	turgid
"	1:10^3	70°	flaccid	too flaccid to measure	flaccid

(note: negative curvature signifies no GS-curvature).

The same facilitation by Na-G of the rate of growth and final curvature reached in auxin solutions is thus confirmed. Na-G, 1:5X10^3, again gave an increase over the GS control, in this case an increase of 60%. The control Na-G, 1:10^3, gave a GS curvature, but the sections were completely flaccid. Na-G in 1:5X10^3 gave no curvature, thus impurities in the sodium glycocholate do not cause the effect.[1].

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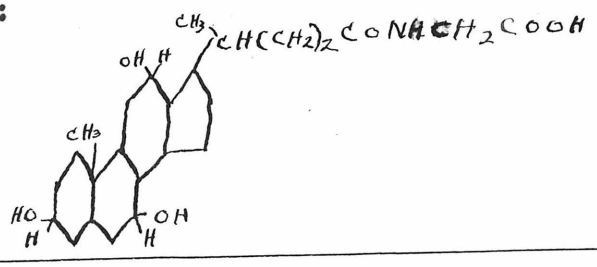
1. Sodium glycocholate, (a Merck product), has the following structure:



It was shown that glycocholate does not inhibit growth in the pea test. To make the growth test more complete, growth of Avena coleoptile sections infiltrated with Na-G and put in auxin solutions with Na-G was measured.

Four millimeter sections were cut with the van der Weij (1932) section cutter, placed on teeth of combs, infiltrated with $1:10^5$ Na-G, and placed in 0.74 mg./liter heteroauxin solutions. Twenty two sections (2 per coleoptile) were used for each test. Measurements were made 12 and 30 hours after the start of the experiment. Table 6 represents the results, expressed in $\overset{10x}{\text{A}}$ ocular micrometer scale divisions (each division approximately 0.1 mm.).

Table 6

Effect of glycocholate on Growth of Avena Coleoptile Sections in heteroauxin (0.74 mg. per liter)

Sections infiltrated with:	0 hrs.	12 hrs.	% increase in 12 hrs.	24 hrs.	% increase in 24 hrs.
H_2O	4.35	4.77	9.6%	5.12	17.7%
Na-G	4.35	4.69	7.8%	5.11	17.5%

After 30 hours, all sections used in the above experiment showed protoplasmic streaming, and were turgid.

It can be concluded that Avena coleoptile growth is not affected by sodium glycocholate which completely abolished auxin transport. At first, one might ask how coleoptile growth responses to auxin may occur when transport is abolished? As stated in the introduction, polar transport is like ion accumulation in that the polarity is the result of an excess basal transport over apical transport. Over long periods of time, therefore, polarity will

not be limiting, especially in the high concentrations of auxin used. The effect of polarity of transport(and entrance of auxin into the sections) on section growth would probably be observed only in the first few hours, where the high velocity of transport of auxin into the control sections would result in a much higher growth rate than in the sections in which polarity is abolished by Na-G. Ordinary diffusion and apical transport ultimately let enough auxin in the Na-G-treated sections to permit growth to overtake the growth of the control sections. Whether or not the above suggestion is correct, the important conclusion reached is that growth by cell elongation is unaffected in Avena coleoptiles with a concentration of Na-G which completely abolishes polar auxin transport.

It can be predicted, a priori, that glycocholate will not inhibit respiration, since growth is unaffected, and since Bonner (1936) has shown that growth by cell elongation depends on respiration. This prediction was realized by respiration measurements of Avena coleoptile sections.

The Warburg manometric technique (1926) was used. Twenty 4 millimeter coleoptile sections were placed in 3 cc. of phosphate buffer at pH 6.0 in vessels of approximately 17 cc. volume. (vessel constants known). The central wells in the vessels were filled with 0.3 cc. 25% KOH. The constants were corrected for the section volumes. The vessels were shaken in a constant-temperature bath at 25°C. in the dark, and readings on the manometers taken every thirty minutes for two hours. One vessel contained sections infiltrated with tap-water, the other with

sections infiltrated with $1:10^5$ Na-G. The sections consumed on the average of 9.9 cu. mm. of oxygen per 30 minutes in the buffer control; and 9.7 in the Na-G-treated. The Q_{O_2} (cu. mm. per section per hour, S.T.P.) was 1.21 for the controls; 1.22 for the sections treated with glycocholate.[1].

The results show that respiration of coleoptile sections is unaffected by glycocholate of a concentration which abolishes polar transport of auxin. This finding at first sight disagrees with Bonner's statement (1935) that transport of auxin into coleoptiles depends upon respiration. Bonner's conclusions were based on experiments in which sections were placed in an oxygen-free environment. van der Weij (1934) has also shown that polar transport of auxin is reversibly abolished by ether narcosis. The probable explanation is, however, that oxygen-lack and narcosis affect respiratory mechanisms limiting the polarity phenomenon, while glycocholate merely removes the connecting link between respiration and transport.[2]. It is conceivable that the respiration in certain plant cells which concentrate ions (Hoagland, Davis, Steward. See introduction) may also be separated from the accumulation mechanism, simply by treatment with such lysins.

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1. These figures are of the order found by Bonner (1936) for coleoptile respiration.

2. To prove that respiration does not limit transport, one would have to abolish respiration without affecting transport.

In other words, respiration may continue without polar transport or ion accumulation occurring, but if it is inhibited, both (1) transport (van der Weij (1934); and (2) ion accumulation (Hoagland and Broyer (1936)) are inhibited.

Thus for the first time, a method has been found which will specifically abolish polar auxin transport. Further experimentation on other physiological processes which are changed during this specific inhibition should reveal the mechanism of polar transport in more detail.

b. The Effect of glycocholate on Lateral Transport of Auxin

If lateral transport is caused by membrane potentials, it seemed possible that glycocholate would abolish lateral displacement of auxin, and therefore photo- and geotropisms. This conception was based on Ponder's (loc. cit.) observation that Na-G abolished frog-skin P.D.'s

(1) Phototropisms

Avena coleoptiles were therefore removed from etiolated seedlings, and infiltrated with (1) tap water, and (2) $1:10^5$ glycocholate (a concentration which abolishes longitudinal transport). One half of the undecapitated coleoptiles were removed from the primary leaves, the other half left with leaves in place. The coleoptiles were placed upright, and exposed to a 60 W. gas-filled incandescent lamp (tungsten filament) at four meters for two hours[1]. at 25°C.

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1. Assuming a mean horizontal candle power of 100 for the 60 W. lamp, the meter-candle-second value becomes approximately 45,000.

The coleoptiles without leaves curve at a faster rate, but the final curvatures of both sets was the same. Photographs were made of the curvatures, and the resulting curvatures measured. The results are shown in table 7, with the mean error given.[1.]

Geotropisms

Coleoptiles prepared as described above were placed on their sides for three hours (25°C., in the dark), after which photographs were taken, and the curvatures measured. Twenty four coleoptiles were infiltrated with water, and an equal number with $1:10^5$ Na-G. The resulting curvatures with mean errors,[1.] are given in table 7:

Table 7

Effect of glycocholate on Lateral Transport of Auxin

Phototropisms (~45,000 M.C.S.)	(24 coleoptiles)
Infiltrated with:	Curvatures
Water	17.8± 3.1°
Na-G $1:10^5$	20.1± 3.3°
Geotropisms (3 hrs. presentation time)	**(24 coleoptiles)**
Water	43.8± 5.3°
Na-G $1:10^5$	42.7± 6.5°

It is quite obvious that glycocholate in a concentration which quite abolishes longitudinal transport, has no effect on photo- or geotropisms. Van Overbeek (1936) has shown that auxin-a

1. $\sqrt{\frac{\Sigma(d)^2}{n(n-1)}}$ expresses the mean error, where Σd is the sum of the deviations from the mean, and n is the number of plants measured.

destruction by light may partially account for phototropisms in Avena, but lateral displacement of auxin is the most important factor in photo- as well as geotropisms, as has been quantitatively proved by Went (1928), Dolk (1930 a), Boysen-Jensen (1932), and others (see Went and Thimann (1937)).

It has been stated on several occasions in this paper that lateral and longitudinal transport of auxin in plants are due to entirely different mechanisms. This would perhaps be a priori predicted, since lateral transport is induced, and may occur toward either side of the stem or root; while longitudinal transport is inherent (polarity), and may not be reversed easily, if at all. The difference between the two types of transport was also shown by the facts that gravity, light, and an electric field affect lateral[1], but not longitudinal transport.

The difference is further indicated by the unequal effects of glycocholate on the two types of transport. Furthermore, Michener (1937) showed that geotropisms may no longer be induced in Pisum seedlings treated with ethylene.[2]

van der Laan (1934) claimed that lateral displacement of auxin in horizontally placed Vicia faba epicotyls is reversed in ethylene, i.e., that the auxin is displaced to the upper side of

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1. Lateral displacement of auxin in electrotropisms has not as yet been directly measured.

2. Ethylene increases auxin destruction in Pisum, but not enough to inhibit growth completely. If growth can occur, geotropisms should occur if lateral displacement may take place. (Michener, loc. cit.).

the stem. van der Laan also showed that ethylene has no effect on longitudinal auxin transport in Avena coleoptiles. In general, experiments with ethylene show, up to the present time, that lateral transport is inhibited or reversed but that longitudinal transport is unaffected, thus the exact reverse of the glycocholate effect.

The glycocholate effect is not one on electrical polarity, as shown by the experimental evidence presented in this section. Evidence from other sources (see p. 8) has shown a close, if not causal, relationship between lateral displacement of auxin and geoelectric P.D.'s. The irreversible narcosis experiments described in a previous section of this paper (page 65) showed that geoelectric P.D.'s probably constitute some part of normal electrical polarity. On such evidence, it could be predicted beforehand that glycocholate would not affect lateral displacement of auxin.

To what then is the effect of glycocholate on longitudinal, polar auxin transport due?

Glycocholate is a lysin, and is highly lipophilic. In high concentration, it probably dissolves lecithin and various lipoids from the cell membranes, and finally completely destroys the membranes. In higher dilutions, however, it may merely compete with the surface-active auxin for surfaces. If auxin is transported either by (1) surface-spreading (see introduction), or (2) adsorption to a larger, cataphoretically transported, negative

particle; this surface competition would abolish transport. Moreover, as Ponder pointed out, if P.D.'s are abolished by lysins, an electrical transport of the auxin anion would be abolished. As yet, no evidence exists which indicates such an electrical transport, but the methods of measurement so far used may be entirely insufficient to demonstrate the electrical properties of the individual membrane potentials possibly responsible for the transport polarity. As pointed out in the section on applied P.D.'s it is hoped that polarization capacity experiments will reveal such properties, especially when studied during reversible narcosis. Further, a homologous series of surface-active substances (e.g., the phenyl or indole series of auxins) might show an effect on longitudinal transport of auxin-a or heteroauxin, the effect being greater as higher members of the series are used. This would be interpreted as an increasing competition for surfaces. Polarization capacity measurements during such experiments might reveal further functional or structural changes in the cell membranes due to the action of the surface-active homologues.

Summary of Part II

1. Heteroauxin (indole-3-acetic acid) has a transport number of 0.07 and an absolute mobility of 10.5 mm. per hour under unit potential gradient of one volt per centimeter as measured by conductance experiments.

2. Heteroauxin is electrolytically transported to the anode in an electric field. A potential gradient of approximately 50 volts per centimeter measurably influences the transport of auxin in agar. Strictly non-polarizable conditions are necessary in order to establish such an influence.

3. Applied E.M.F.'s have no influence on polar transport of auxin in Avena coleoptiles although these applied E.M.F.'s reverse or increase the inherent electrical polarity of the same sections.

4. Shunting the P.D.'s of coleoptile sections through an external metallic conductor has no effect on polar transport.

5. Inverted electrical polarity induced by gravity has no effect on polar auxin transport in coleoptile sections.

6. $1:10^5$ sodium glycocholate abolishes polar auxin in Avena coleoptiles, but has no effect on growth by cell elongation, semi-permeability, protoplasmic streaming, respiration, and electrical polarity. Saponin stops streaming but not transport.

7. Glycocholate-infiltrated coleoptiles respond normally to gravity and light.

8. It is pointed out that the mechanisms of lateral displacement of auxin in tropisms, and polar transport of auxin are entirely different.

9. It has been demonstrated that electrical polarity either has no causal relation to the polarity of auxin transport in the Avena coleoptile, or that this relation is not obviously amenable to treatment by the bioelectric measurements mostly used in this paper.

Discussion

It has been experimentally demonstrated that the normal electrical polarity of several seedlings is an electronegativity of tip to base. Several views are held as to the mechanism of the origin of this electrical polarity.

Lund (1928) has claimed that electrical polarity is the result of oxidation-reduction potentials such that usually, but not necessarily, regions of highest rates of oxidation are electropositive to other regions (or, in the thermodynamic sense of red-ox potentials, the ratios of oxidant to reductant are different in the different ends of the structure concerned). A detailed discussion of the theory is out of place at this time (cf. Lund (1931a)) but certainly the theory is thrown into doubt by the fact that red-ox potentials can be measured only by indifferent electrodes, and not by non-polarizable electrodes such as used by Lund. Francis (1934), Beutner and Lozner (1933), Ramshorn (1934), Stern (1933), and Dorfman (1934, 1936), have all criticized Lund's theory on this basis. Stern (1933) and Marsh (1930) stated that if the living membranes, to which Lund made contact with non-polarizable electrodes, acted as indifferent, metallic conducting electrodes, the P.D. measured could be the same as Lund's hypothetical oxidation-reduction chain interposed between the contacts. While it is improbable that these membranes act as metallic conductors, yet the possibility remains that they may do so.[1].

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1. Schott and Borsook (1933) and Borsook (1935) have shown the possibility of metallic-electron conduction between enzyme centers in *E. coli*, Fetcher (1934) demonstrated the possibility of electron conduction in membranes which are composed of conductors of the second class (cf. Lilly (1936). (see p. 20).

Bioelectric potentials, as linked with oxidative processes, can be explained by other mechanisms as well as by oxidation-reduction potentials. (Stern (1933), Dorfman (1934, 1936), Beutner and Lozner (1931), Francis (1934)). Most of these explanations are based on the effects of oxidations on diffusion potentials or membrane potentials.

The oxidation-reduction polarity theory, moreover, is thrown into doubt by the experiments of Dorfman (1934, 1936), who showed that the oxidation-reduction polarity of the frog's egg was opposite in sign to the bioelectrical polarity measured with non-polarizable electrodes.

The bioelectrical polarity of the Avena coleoptile is certainly not directly linked with respiration in the different regions of the coleoptile since Bonner (1934) showed that there was no distribution of respiration in this organ. Moreover, from data published elsewhere (Clark (1937)), reduced ascorbic acid (vitamin C) is found in highest concentration in the apex of the Avena coleoptile, the concentration decreasing basally (cystine, cystein, and glutathione are not present). It was also indicated that the reverse relation held for the distribution of oxidized ascorbic acid. This does not conform with Lund's oxidation-reduction polarity (Lund (1931a)), since by this theory, the tip would usually be electropositive. This is assuming, however, that the oxidation-reduction potentials of ascorbic acid could play a part in the electrical polarity. (For exceptions to this polarity rule, see Lund (1931a)).

With regard to the disagreement between the findings presented

in this paper and those described by Ramshorn (1934), the following discussion becomes pertinent: Ramshorn made measurements of the electrical polarity of several different seedlings and roots, and showed a parallelism between growth and electropositivity. Regions of highest growth-rate were electro-positive to other regions. Temperature changed both in the same way, and applied potentials accelerated growth if the applied polarity coincided with the measured inherent polarity; and, conversely, inhibited growth if the polarities were opposed. On page 741 of his paper, Ramshorn presents a series of curves of the gradient of electric potentials from tip to base in Helianthus hypocotyls after stimulation by shaking. After stimulation, the tip became electronegative to the base presumably within a few seconds. In 15 minutes, the tip became electropositive, and in 75 minutes the magnitude of this positivity had diminished only a few millivolts. This roughly confirms Lund's (1931) finding that the electropositivity of the apex of the Douglas fir decreases or that the tip even becomes negative on mechanical stimulation.

In the present study of the Avena coleoptile, reliable constancy of P.D.'s was not obtained until 90 to 110 minutes after setting up the experiment, as much care as possible being taken not to stimulate the plants during this operation. Moreover, constancy was not good unless the plants were in complete darkness; and manipulation of contacts from point to point involved considerable stimulation.

Ramshorn's correlation between growth and electrical polarity might, in my opinion, suffer a reversal in some cases, particularly in Avena, if the time relations, contact manipulation, and light

conditions of his experiments were reinvestigated,[1] especially with regard to the constancy of observed P.D.'s over longer periods of time.

Barth (1934a, 1934b) observed that apical positivity and organic polarity in the hydroids are not correlated, but that either apical or basal positivity may be correlated with organic polarity, depending on the hydroid used. This lends no support to Ramshorn's positivity theory; nor do Rehm's (1936b) measurements on Phaseolus, in which he found the tip of the plant electronegative to the basal regions.

Czaja (1935) says that the auxin-transport itself electrically polarizes the plant, thus roughly supporting Ramshorn's statement that auxin changes the P.D.'s and growth rate. Czaja, however, is largely theoretical in his consideration, and bases his assumptions on results obtained with unphysiological concentrations of auxin. In the light of unpublished experiments of my own, the effect of auxin on plant potentials is a real one, but one probably not closely linked with the normal inherent polarity. De Haan (1936) is also if this opinion.

Experiments on the effect of gravity on the Avena coleoptile P.D.'s have revealed that the electrical polarity can be changed or inverted by inverting their morphological axes. This polarity inversion is not permanent, the original polarity returning. Hence "normal" electrical polarity is not exclusively and directly caused by geoelectric potentials, but it is possible that they are contributing factors. This possibility is being examined.

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1. Wilkes (1936) has recently confirmed the writer's observation that the Avena coleoptile has apical negativity.

The mechanism of the origin of electrical polarity was thought to be linked with the phenomenon of polar permeability observed, e.g., by Brauner (1928), Metzner (1930), and Guha (1927). Plant tissues have been said to exhibit a selective ionic permeability so that an electric current is conducted more easily in one direction than in the other. Experimental evidence, however, has shown that no unipolar conductance of direct currents, nor rectification of alternating currents exists in sections of Pisum stems, nor of Avena coleoptiles.

The purpose of the present study was originally to prove or disprove the electrical polarity theory of the polar transport of auxin in plants. This purpose has been realized inasmuch as the measured P.D.'s were concerned. If the term "electrical polarity" is extended to include the unipolar or differential polarization capacities of membranes under the influence of various environmental changes, this purpose has not been realized. P.D. measurements of intact tissues such as used in the present problem have as yet told nothing of plant membrane function. It was tentatively predicted (p. 67) that electrical polarity (P.D. distribution), polarization capacity, or selective ion permeability possibly associated with polar transport across membranes, could be separated from each other by employing various lysins and narcotics. It is also possible that geoelectric P.D.'s occurring in living, upright sections are separable from "normal" polarity by treatment on a klinostat. Reversible ether narcosis increases the resistance of sections, and decreases apical positivity; whereas glycocholate has

no effect on electrical polarity, yet abolishes transport. It is quite possible, therefore, that by employing such methods, electrical properties may be found which are causally linked with polar transport.[1.]

It has been stated throughout this paper that living membranes perform work in concentrating substances against osmotic or concentration gradients. This is not a tacit assumption, but is based on the wide-spread knowledge that plant and animal membranes do expend energy in doing so. Polar transport is one such example, differing, however, from most of the others in that the "secretion" occurs across a whole organ, and not merely into or out of single cells or organs.[2.]

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1. It is, however, also quite possible that electrical measurements will not tell anything at all about the mechanisms in question. This will be discussed presently.

2. A "secretion" phenomenon possibly functionally similar to that manifested in polar auxin transport is the absorption of water by roots, the water being transported across several layers of cortex cells. The transport from cell to cell occurs against an osmotic gradient, as shown by Ursprung and Blum (1921), who showed that suction force increases from epidermis to endodermis, where it suddenly drops in the pericambium and vascular tissue. Henderson (1934) has shown that the root absorption requires the expenditure of energy, the source being in oxidative metabolism.

A few classical examples of membrane concentration are: (1) stomach cells can secrete 0.14 N HCl from blood at pH 7.3 ($5X10^{-8}$ in H^+ ions), and hence do osmotic work, per gram mol of HCl, equal to R T $\log_e$ $14X10^{-2}/5X10^{-8}$ = about 9,000 calories (Hill (1930)).

(2) Kidney cells (epithelium of uriniferous tubules) secrete urea from blood at 0.03% to 2% in urine, performing osmotic work per gm. mol equiv. = R T $\log_e$ 2/0.03 = 2,500 calories (Hill, loc. cit.).

(3) Fish swim-bladders at 500 fathoms depth (100 atm. pressure) excrete oxygen from sea water of a partial pressure of oxygen about 0.1 atm., and perform osmotic work/gm. mol of O_2 = R T $\log_e$ 100/0.1 = about 4,000 calories (Hill, loc. cit.).

The Prague school, under Keller (1932) has tried to causally link such secretory processes with membrane potentials. Huf (1935, 1936) has also done this, showing that oxidative processes in the frog skin determine (1) absorption of water and, among other things, chlorides; and (2) the P.D. across the skin. Reversible inhibition of respiration reversibly abolishes both water absorption and the P.D. Monoiodoacetic acid poisoned skins (inhibition of glyoxalase activity in lactic acid formation from glycogen) show neither concentration work nor P.D.'s, while lactate restores both if supplied to such poisoned skins. Huf, believes therefore, that the energy supplied by the oxidation of lactate performs the concentration work. This oxidative energy is supposedly converted into electrical energy.[1].

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1. As much as 70% of the energy supplied by oxidative metabolism can be dissipated in electrical work. Clark (unpublished) has shunted frog-skins through a variable external resistance,

measuring the current flow as a function of the resistance; calculated the Joule heat of this current flow; and found the percentage of the total oxidative heat that this Joule heat could comprise, when the external resistance was nearly that of the skin itself (20 ohms). The percentage was, in some cases, as much as seventy. Francis (1933) made similar calculations, using an external resistance of much higher values (1,500 ohms). He found that the electrical energy output was 5-10% of the metabolic energy. In both Francis' and Clark's work, the latter energy was calculated by indirect calorimetry, knowing the respiration and assuming that sugar is burned.

Fürth (1933) has calculated the electrical energy necessary for performing concentration work in secretion of urea and electrolytes across kidney epithelium cells, stating that the measured bioelectric currents would suffice.

Many other examples of such experiments could be given at this time, but aside from the knowledge that concentration work is done, little is known. The mechanisms involved remain mysteries.

A singular objection to any electrical theory of secretion, ion-accumulation, or polar transport is that electrical energy must be expended. Knowledge of how such energy arises is almost completely lacking. Whether the expenditure is expressed as an electrolysis, a cataphoresis, or as an electroendosmosis; electric current must be carried as ionic charge, colloidal charge (electrokinetic potential), or as electronic charge. These charges must be carried to poles bearing opposite charges. At such poles, the charges must be removed, or equilibrium conditions would quickly ensue. The charges must therefore, flow in a return circuit either as free electrons, colloidal charges, or ionic charges. Such mechanisms have been

postulated by many workers, but experimentally verified by only one, namely Söllner (1930, a,b,; 1932, a,b,c,d). Söllner has constructed models in which electrolytes could be concentrated against an osmotic gradient (negative, anamalous osmosis) because of membrane diffusion potentials set up by selective cation or anion permeability in porous,charged membranes; the selectivity being due to membrane charge, and the pore sizes. Moreover, Höber and Hoffman ((1928), see also Höber (1937)) showed that membrane mosaics could be made which have closely juxtaposing cation and anion selectively permeable areas, which may set up positive and negative charges, respectively, which may in turn cause a negative, anomalous osmosis of electrolytes against concentration gradients. Such membranes need exhibit no P.D., or at least such a complex one that electrical measurements will clarify no functional mechanism present. Such mechanisms need attain no static equilibria, but may, in combination with a dynamic Donnan equilibrium, set up a state of steady flux which may explain secretory processes such as polar transport or ion accumulation (Brooks (1929)). It might therefore seem that the only solution to such problems must be sought in the molecular or ionic properties of substances as related to their transportability, in addition to changes in the membranes which alter this transportability.

Differential partition coefficients of substances in solvent-like membranes may also be responsible for concentration work in living cells. Such differential partitioning may be linked with dynamic states such as Donnan equilibria, the dynamic state being dependent upon oxidations (Teorell (1933); (Osterhout (1933, 1935)).

Another possible flux mechanism is that of surface orientation of dipoles, suggested by E.K. Rideal (private communication).

Rideal (Schulman and Rideal (1931); Rideal (1933, 1934)) has studied phase boundary potentials set up at water-air and water-oil interfaces by oriented dipoles. The following scheme might explain a constant flow of energy which might be necessary for membrane concentration work. A sheet of oriented molecules possessing dipole moments may be inserted at a phase boundary. This substance may be chemically changed[1]. in the interface, the change involving an ionic exchange; the product being removed from the other side of the interface as fast as it is formed. Here then would be a possible scheme for the constant "secretion" of organic dipole molecules, as well as ionic exchange involving flow of electric energy.

Many biologists (Nathansohn (1919)) have postulated a fourth mechanism to explain membrane processes associated with secretion, concentration work, etc. This mechanism is known as electrostenolysis (Söllner (1919)). Here current flow across an inert non-metallic membrane may produce actual electrolytic oxidations and reductions, involving the transport to and liberation of ions (even in the molecular gaseous state) at the membrane surfaces. Such current flow is usually derived from an externally applied E.M.F. Inherent membrane E.M.F.'s can also cause such electrolytic oxidation-reduction reactions to occur (the "Becquerel phenomenon". See Freundlich and Söllner (1928)), but Söllner (loc. cit.) has stated that semi-metallic conductors must be present in the membranes be-

1. Chemical changes at interfaces may occur as a result of adsorption phenomena, e.g., denaturization of proteins.

fore the latter phenomena can take place. Söllner's statement is questionable, but such a discussion would be too extensive to include here.

Marsh (1932) said that electrostenolytic liberation of acid and base occurred at the opposite ends of Tradescantia cells subjected to electric current flow; further assuming that such a process necessitates the assumption of "metallic" properties of the cell membranes, since oxidation-reduction potentials (and current flow) require free electron conduction[1]. (see footnote on first page of discussion).

Although Blinks (1932) has disproved Marsh's observations, and shown that the colour changes in the Tradescantia cells are due to cataphoresis of anthocyanin, he points out the possibility of such oxidative processes (membrane electrolyses)[2]. occurring in living membranes (1936c).

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1. Borsook (unpublished) has postulated the essentiality of free electron conduction between enzyme centers as one of the mechanisms involved in the utilization of metabolic energy in synthetic processes in living organisms. The conduction process has been pictured as involving electron resonation between double bonds and single bonds in the long side-chains of molecules like vitamin-A.

2. "Correlation" in living organisms is usually thought of as being brought about by humoral or nervous transmission. Polar transport of auxin is one manifestation of a correlation mechanism in plants. Bud inhibition, root initiation, induction of the transport of other substances, growth, and tropisms are a few examples of responses elicited by stimuli which correlate the "receptors" with the organs of response by means of the displacement or transport of auxin (humoral transmission). If electrical properties (P.D.'s, polarization capacities, etc.

See section on applied P.D.'s) of membranes are linked with transport, accumulation, secretion, etc., it will probably also be shown that various phases of correlation in growth and differentiations may be linked with bioelectric phenomena. It is quite possible, of course, that the electrical correlation may be caused by alteration of other properties beside the transport of hormones. This idea is not a new one and will be briefly considered at this point.

Child's theory of apical dominance and metabolic gradients (1929), Lunds theory of electrical correlation (1928, 1931a), and Borsook's theory of the utilization of metabolic energy in synthetic processes (1937, unpublished) are all compatible with such electrical correlation theories. Nathansohn (1919), however, was the first to definitely postulate such a mechanism. He discussed in detail the possibility that electrolytic oxidations and reductions of organic substances could occur at the surfaces of living membranes. By means of such reactions, organic syntheses and break-downs occur, the whole being an essential part of oxidative metabolism. The energy supply for such electrolyses is derived from electrostenolytic current flow in the living membranes; thus a vital Becquerel phenomenon (cf. p.120).

Such local electric currents in inert membranes have been experimentally demonstrated by Söllner (cf p.120).

Lund has shown that living cells (onion roots, Douglas fir, frog-skin, Obelia internodes) are electrically correlated (cf. Lund (1928)). If local membrane electrolyses control oxidative metabolism (acceleration, inhibition, synthesis, break-down) in one part of an organism, therefore, Lund (and Marsh (1930)) believes that inherent bioelectric currents will control similar processes in other parts of the organism by reversible polarization, thus supplying a method of correlation in growth and developmental processes.

Although this theory is supported by observations that growth and differentiation can be controlled by applied electric potentials (Lund (1922, 1923); Barth (1934a, 1934b); Bose (1907); Ramshorn (1934)), it remains highly speculative. The theory has only been mentioned here as a moot question which cannot be easily dismissed in favour of humoral transmission as the exclusive method of correlation in plants and animals lacking circulatory and nervous systems.

Aside from such theoretical speculations, no direct experimental proof of actual current flow has been demonstrated in living organisms without the incorporation of inserted electrodes.[1]

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1. This statement has also been made by W.L. Francis in a private communication. (An exception is the electric organ.)

A possible exception to this statement is the phenomenon of the rheoscopic nerve preparation with frog sciatic nerves, in which the action-current of one nerve actually stimulates another nerve.[1].

Thus the problems of the mechanism of (1) the polar transport of auxin, as also those of ion accumulation, absorption, and secretion in plant cells, remain as obscure as (2) the classical problems of membrane concentration in animal organisms. The two sets of problems are closely related in many respects, and in all probability will both be more easily understood when more observations have been made of either one of them.

This dissertation has definitely shown that measured electrical polarities (P.D.'s) have no apparent relation to one of these phenomena.

The significance of this statement becomes far-reaching when it is remembered that many biologists tacitly assume that electrical measurements of the nature described in the above pages bear causal relationships to the secretory functions of living organisms.

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1. The possibility remains, however, that the measured action-currents are the result of ionic changes caused by permeability increases, in turn caused by the liberation of surface-active organic substances (Höber, private communication). These substances may be the stimuli by means of which the action-current is propagated. Lillie (1936), however, believes that the action-current is an organic reaction similar to that accompanying the propagated electrolytic reduction-oxidation processes in the passive iron wire model of the nerve impulse.

Combined Summary of the Most Important Results of Part I and Part II

1. The external and internal measured electrical polarity of the aerial parts of several plant seedlings is an electropositivity of morphological base with respect to apex, as shown by bipolar dye-uptake, and by electrical measurements.
2. This polarity is demanded by the electrical theory of basal, polar transport of auxin in plants; which states that auxin migrates as a negatively charged particle to an inherent positive pole in the plant.
3. Light, gravity, and applied electromotive force may alter or invert this electrical polarity.
4. Differential ionic conductance measured by direct current passage or rectification of alternating currents does not exist in the longitudinal axis of Avena coleoptiles. Thus selective ion permeability (e.g., of auxins) cannot be measured by such methods.
5. Dissociated heteroauxin may be electrolytically carried in agar to an applied positive pole under a potential gradient of 50 volts per centimeter.
6. Applied electromotive forces up to lethal magnitudes have no effect on polar auxin transport in Avena coleoptiles even though electrical polarity is inverted.
7. Polar auxin transport in Avena coleoptiles remains when the inherent electrical polarity is inverted by gravity. It is recalled that evidence elsewhere shows similar lack of effect of light and gravity on polar transport.

8. Polar auxin transport may be specifically abolished with one part of the lysin, sodium glycocholate, in one hundred thousand parts of water, without there being any change in electrical polarity, respiration, semi-permeability, growth by cell elongation, or protoplasmic streaming.

9. Lateral and longitudinal transport of auxin in plants are due to entirely different mechanisms, as shown by the different effects of light, gravity, applied potential differences, ethylene, and glycocholate.

10. Electrical polarity expressed in terms of inherent potential differences has no apparent causal relation to polar auxin transport in plants.

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