THE PHYSIOLOGICAL ACTIVITY OF THE ROOT
AS INDICATED BY VITAL STAINING

By

Harriet M. Fogg

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Frank C. Rockefeller

Head of Department of Botany
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THE PHYSIOLOGICAL ACTIVITY OF THE ROOT

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INTRODUCTION

In attempting to correlate what has already been learned by investigators in seemingly isolated fields about the absorption of materials from the soil by the plant, and about vital staining, and to explain further such absorption by determining the electrical charge of the protoplasm in different cells by vital-staining methods, this paper enters a field as yet untouched except to a slight extent by Keller. Three lines of research will be followed and the relations between these, as they appear to the present investigator, shown. They are:

1. Staining technique, especially as it bears on the electrical charge of the cell, or cell part.

2. The intake of solutes as determined by the charge of the cell or cell part.

3. The absorption mechanism and the path followed by nutrient substances.
Bethe (3) in early studies established the fact that histological preparations of spinal cord dyed more intensively when the alkalinity of the dye solution was increased, and less intensively if its acidity was increased.

M. H. Fischer (10) performed model experiments on the absorption of acid and basic dyes by powdered blood fibrin. He found that the fibrin absorbed toluidin blue strongly from an aqueous solution, but that if acid were added to the dye solution scarcely any toluidin blue was taken up. On the other hand sodium indigo-sulphonate, an acid dye, was taken up only if acid was added to the dye solution. From a mixture of neutral red (basic) and indigo-sulphonate (acid), which makes a purple solution, the basic dye was taken up by the fibrin from the aqueous solution, while the negative dye was taken up from the acid solution. When neutral red and toluidin blue (both basic) were mixed, both were taken up from the aqueous solution and neither from the acid solution.

In later studies Bethe (4) experimented with the staining of gelatin sols and found that they stained with basic dyes much more quickly from alkaline solution. Yeast cells stained more quickly by acid dyes in acid solutions. Amphoteric dyes he found stained equally both in acid and in alkaline solutions, in acids behaving as basic dyes and in alkali as acid dyes.
The first important experiments with living plant cells, which are the concern of this paper, were those of Rohde (23). He studied, in order to test an hypothesis of Bethe's:--

1. The staining of gelatin of different concentrations and reactions.

2. The vital staining of plant cells of different reactions.

3. The vital staining of Infusoria of two types, one found in weakly alkaline-reacting intestines of frogs and the other in weakly acid-reacting.

That highly concentrated gels stained more deeply than less concentrated colloids; that both acid and basic dyes probably penetrated all cells but were stored only if the cell content reacted with the dye; that acid dyes were stored in large amounts by acid cells, little by neutral and not at all by basic, while basic dyes were stored by all cells but in decreasing amounts with increasing acidity of the cell; and that by the use of buffers the reaction of the living cell could be changed and the dyeing thereby altered, were Rohde's conclusions. These results are exactly what were anticipated in Bethe's hypothesis. (It might be stated at this point that Rohde's references to the inner reaction of the cell always refer to the vacuolar sap and not to the protoplasm).
Collander (7), working with the living cells of many plants and with acid dyes only, found that the stains penetrated very little, although certain tissues, to be referred to later, did take the stain. A high hydrogen-ion concentration was favorable to dye intake while a low hindered it.

Loeb (16) brought powdered gelatin to pH values ranging from 3.3 to 6.2 and treated them in a dark room with M/64 AgNO₃. He then washed the gelatin in cold water to remove the silver not in combination with the gelatin. The gelatin was liquefied, poured into test tubes, and exposed to the light. The gelatins of pH greater than 4.7 turned dark in a half hour while the gelatins of lower pH did not change in more than a year.

Experiments with acid and basic dyes showed that acid dyes stained gelatin at pH values lower than 4.7, while basic dyes stained it at pH values higher than 4.7. The isoelectric point, or the critical hydrogen-ion concentration of gelatin for acid and basic dyes, and for silver nitrate, was shown to be 4.7.

Robbins (24) performed experiments with gelatin and with potato tuber tissue. He found that on the acid side of the isoelectric point the gelatin formed salts with anions only, while on the alkaline side it reacted with cations only. With living potato cells and acid dyes a gradation of color was obtained by the use of buffers of different
hydrogen ion concentrations. Basic dye, however, was taken up so vigorously at all pH values that no difference could be seen. Homogeneous tissues killed with alcohol before buffer and dye treatment identical with that given living tissues showed only a quantitative, never a qualitative, difference. All dead tissues stained more deeply than living. In later studies Robbins (25) was able to show that potato tuber tissue reacted to dyes like a protein with an isoelectric point of about pH6.0. Pearsall and Ewing (18) discovered several maxima and minima of swelling in potato tissue tuber, the several minima probably being the isoelectric points of the constituent proteins, which seems to suggest that Robbins' point may be only one of these several points found by Pearsall and Ewing.

Haylor (17) stained sections of root tip tissues killed and fixed in chrom-acetic acid and imbedded in paraffin in the usual way. He used buffers ranging from pH 3.5 to pH 7.3 and double stains. Macroscopic examination showed a fine gradation of color with eosin and toluidin blue, from red at the acid end, through purple to blue at the alkaline end, and from blue through purple to red with methyl blue and safranin. Microscopic examination revealed an exceedingly fine differentiation of cell parts.
Pischinger (20) correlated the reaction range in which the power of holding dye showed rapid diminution (the I. E. P.) for cyanol on the alkaline side, for toluidin blue on the acid, with the isoelectric points of the substances as determined by cataphoresis. He recommended the dropping of the terms acid and basic in favor of negatively and positively charged.

As there is considerable confusion in terminology, it seems wise to define the terms acid dye and basic dye as they will be used in this paper. Bancroft (2) defines an acid dye as one which contains the color group in the acid radical and dissociates to give colored anions. These anions are adsorbed to the positively charged fiber or tissue. The term negative, then, as applied to dye, means that the color group is negative and should be applied to acid dyes. The basic dye dissociates to give colored cations, which are adsorbed to negative tissues, and is therefore positive. Keller (14) does not agree with other investigators in so far as he emphasizes the fact that the charge of many stains may be reversed by addition of acid or alkali. In his tables he lists many so-called basic dyes, as for example safranin, as negative, and many so-called acid dyes, as eosin, as positive. He emphasizes the fact, however, that positive dyes are taken up by negative tissues and negative dyes by positive tissues.
Thus far we have discussed positive and negative dyes, in relation to negative and positive tissues, as determined by staining methods. A check on staining as a means of discovering tissue charge is the electrostatic method. Peterfi and Ettisch did the first work of this sort, but Gicklhorn and Umrath (12) simplified and perfected their methods. By the use of a very sensitive recording apparatus and extremely fine nonpolarizable electrodes which were inserted into the tissues to be examined, they were able to determine the potential differences and relative electrical charges of the tissues studied. These charges agreed in general with determinations made with dyes.

The Intake of Solutes as Influenced by the Cell or Cell Part

The results obtained by Keller, Fischeringer, and Gicklhorn and Umrath lead naturally to the conclusion that the kind and amount of dye taken up depends upon the electrical charge of the cell part, cell, or tissue in question. Just as in diffusion the law holds that if a substance can dissolve in a membrane it can penetrate it, so in adsorption, which is a special physico-chemical affinity to the adsorbens, the law holds that, if a substance can be adsorbed to a membrane, it can penetrate it.
Rohde (27) demonstrated the dependence of intake on electrical charge with live frogs. He fed the frogs dyes. If the dye was acid, the frog lived. This was because both the negatively and positively charged dyes could be adsorbed to the membrane of the kidney epithelium and be given off into the urine. Feeding the frog boric acid hastened the excretion of the dye. If, however, he fed the frog soda, which made its blood more alkaline and thus changed the charge of the kidney epithelium, the dye was not excreted but was accumulated in the blood until the amount became toxic and the frog died. Probably this was a case of irreciprocal permeability.

Irreciprocal permeability, when one side of a tissue has one charge and the other side the opposite, was much more clearly shown by Wertheimer's (29) work. He made sacs of the living skin of frogs' legs. In some of these sacs the skin was in the normal position, the outside of the skin serving as the outside of the sac. In others the skin was turned so that the outside of the skin was the inside of the sac. These sacs were filled with dye and suspended in Ringer's solution, or filled with Ringer's solution and suspended in dye. When the normal sac was filled with methylene blue the dye went out through the membrane and colored the Ringer's solution in which it was immersed. If eosin was put in the bag it did not pass through the membrane. If, however, the
same dyes were put in turned sacs, eosin passed through while methylene blue was retained. If the dyes were on the outside and the sacs filled with Ringer's solution, methylene blue did not enter the normal sac; eosin did. Methylene blue entered the turned sac; eosin did not. If a mixture of methylene blue and eosin was used, methylene blue always passed from the physiological inside of the membrane to the physiological outside, and eosin in the reverse direction. These phenomena can be easily explained by postulating a negative charge of the protoplasm on the inside of the membrane, to which the positively-charged basic dye was adsorbed and a positive charge to the outside cells of the membrane to which the negatively-charged acid dye was adsorbed. Wertheimer in explaining his results says that the outside of the membrane has a negative charge and the inside a positive; that particles of methylene blue which are stopped by the positively-charged inner membrane suffer no retardation but pass right through the negatively-charged outer membrane. The former explanation seems much more logical to the writer. As has been stated before, and as shown by Michaelis and his collaborators, only positively-charged particles can pass negatively-charged membranes (dry colloidion membranes), whereas the negatively-charged ions are immobilized. Though Pischinger showed that adsorption slows up the rate of diffusion, we are concerned here only with the question whether or not substances can pass a membrane. As
has been said in the foregoing, dyes of the same charge as the membrane do not pass at all, whereas dyes of the opposite charge can pass it, although the rate of diffusion is slowed down by the adsorption.

The Absorption Mechanism and the Path of Absorption

De Vries (9), in 1886, made a very complete study of the root which resulted in his calling that part of the root where secondary endodermis was not yet formed and where root hairs functioned, the absorbing root. By the use of a mercury pressure apparatus he was able to show that as long as the endodermis was left intact no water could be forced out of the central cylinder until the successive transverse slices which he cut had finally removed the endodermis. On its removal water rushed out. Eosin also was confined within the central cylinder. The impermeability of the endodermal cylinder he assigned to three kinds of resistances: (1) The high turgor pressure of living protoplasm; (2) the presence of the cork bands; (3) the intimate contact of the cork bands of the radial walls with the living protoplasm.

He also found that the endodermis of the lateral roots connected with that of the main roots, forming a closed system.

An earlier worker had reported protoplasmic streaming in the root hairs of sixty-five genera of widely
separated plant families. De Vries discovered it also in the endodermis and correlated its cessation in this tissue with the formation of secondary endodermis. Epidermis, exodermis, cortex, and undifferentiated vessels showed the same streaming. De Vries assigned great importance to this phenomenon and stated that water from the root hairs passed through the cortex, endodermis, and pericycle, conducted by protoplasmic streaming.

De Lavison (8) carried on two series of experiments with living, intact roots by which he proved that:

1. Substances which could pass through cellulose but not through protoplasm were stopped by the Casparian strips of the root endodermis, both in passing from outside into the root and from inside out.

2. If no Casparian strips were present, such substances passed the endodermis.

3. That substances which could pass through protoplasm as well as through cellulose, crossed the endodermal layer by way of the protoplasm.

Priestley (22) discovered the mestematic tissue at the root apex to be relatively impermeable to the passage of most solutes, though it stained readily with basic dyes in high, probably toxic, concentrations. Acid dyes were not adsorbed at all by living roots grown in dye. Much later (22) he stated that the absorbing region of the root lay between the apical meristem and the regions with completely
suberized membranes, essentially the same, then, as de Vries, and discounted the importance of root hairs except under abnormally low soil water conditions. The possibility of differential entry of salts into the stele opposite the relatively acid xylem and alkaline phloem was suggested by the presence of conspicuously dyed patches of cells opposite the xylem and extending both inside and outside the endodermis and including the latter (23). When basic dyes were forced through the xylem under pressure they were retained within the endodermal cylinder, while acid dyes penetrated the endodermal protoplasts relatively easily and escaped into the cortex.

This paper will attempt to re-examine absorption in the light of all this previous work and explain it in terms of the electrical charges of cells as indicated by their vital staining.
EXPERIMENTAL WORK

Materials and Methods

Many preliminary experiments were performed before a satisfactory procedure was developed. Roots of *Vicia faba* and *Helianthus* were used. To procure these roots seeds were soaked for 24 hours, planted in moist sand, and kept under bell jars or germinated on moist filter paper in cool chambers in the dark. These methods proved satisfactory, both from the standpoint of obtaining normal roots with pith intact, and from that of the ease with which they could be moved without injury. Roots studied were from three to six days old and ranged in length from 3 to 6 centimeters and in diameter from 1-1/2 to 3 millimeters.

If the roots were not perfectly turgid when removed from the sand or damp chamber, they were allowed to stand in distilled water until turgid before sectioning. They were then cut into tap or distilled water and examined. The region chosen for study was that part of the root in which the xylem was well developed but where the bundles remained distinct. Usually there were well-defined caps at the ends of the xylem arms and the Casparian strips had formed. Sclerenchyma was often present and there was rarely a well-defined cambium. Occasionally, for some special reason, older or younger sections were studied.
Three methods of dyeing and rinsing were tried:

1. The sections were put directly into the dye after the examination mentioned above, then rinsed until all surplus dye was removed in tap or distilled water, either with or without a little dilute KH$_2$PO$_4$, or in dilute buffer mixtures of varying hydrogen-ion concentrations.

2. The sections were buffered for varying periods of time, rinsed in distilled water, and dyed. They were then rinsed with the buffers.

3. The buffers were added to the dyes and the sections thus buffered and dyed simultaneously. They were then rinsed with dilute buffer mixtures of the same pHs as those used in the dyeing.

Dyes of a very wide range were sampled and their charges determined in a way to be described later. The dyes finally decided upon as most satisfactory were: Positively charged—safranin, toluidin blue, Bismarck brown, neutral red, thionin, methyl violet, and methylene blue; negatively charged—eosin, erythrosin, light green, and cyanine. Of these safranin and toluidin blue, light green, and cyanine were most used.

The dyes were employed in concentrations ranging from $10^{-2}$, as used by Naylor but on dead tissues, and $8 \times 10^{-3}$ used by Collander, to $10^{-4}$ and $10^{-5}$. These last two concentrations proved most successful and were used in all the experiments which are described in this paper.
The dyes were mixed at first with distilled water, but very peculiar and, at the time, unexplainable results made it seem wise to attempt to obtain a dye mixture which was nearly neutral. Therefore the dyes were mixed with tap water and the pH of each determined by a quinhydrone set. The determinations for concentrations of $10^{-5}$ tap water dyes were as follows:

- Bismarck brown............. 7.26 (basic)
- cyanine..................... 7.21 (acid)
- eosin.......................... 7.0 (acid)
- erythrosin................... 7.35 (acid)
- light green................... 7.27 (acid)
- methylene blue.............. 6.8 (acid)
- methyl violet............... 7.10 (basic)
- neutral red................... 7.18 (basic)
- safranin..................... 7.0 (basic)
- thionin...................... 7.35 (basic)
- toluidin blue................ 7.45 (basic)

Mollvaine's standards of secondary phosphate and citric acid buffer mixtures were used because their long range did away with the complicating factors which might have arisen through the use of two or more buffer series of shorter range and different constitution. The series as used ran from pH 3.0 to pH 7.5. The pH of each buffer was tested colorimetrically and by use of the quinhydrone set. Different proportions of buffer and water or dye were tried and that finally adopted was 1 cc. of buffer to 15 of water or dye. The pH of the solution was tested times enough before and after using to prove that there was too little change to be of any significance in the experiment, the change being from 0 to 0.2 of a Sorensen unit.
The time of buffering and dyeing varied greatly, the general result being that longer buffering meant shorter dyeing, and vice versa.

Very uneven results were obtained until a shaking machine was available, but as soon as this was procured and the buffering made more effective, the results became very uniform and were in accordance with those of other experimenters. The machine was a very simple one, being a cylindrical box of galvanized iron 5-3/4 inches in height and 10 inches in diameter, in the bottom of which was fitted an inch board with thirteen 1-7/8 inch circular holes. These holes were lined with cotton, the requisite number of 100 cc. bottles used in the experiment were put in them as evenly spaced as possible, and the cover was secured by nuts screwed on long bolts at the sides of the box which extended through holes in iron bars across the bottom and cover. An electric motor revolved the box on a horizontal axis counter-clockwise at a moderate, even speed.
Preliminary Experiments

In the summer preceding this study, Dr. Bakhuysen had observed a differential staining with acid dyes of the cells at the ends of the xylem arms in cross sections of young roots. Other investigators had made brief mention of this phenomenon. Collander (7) had found that the cells surrounding the vascular bundles in young white hyacinth petals dyed with cyanol; Naylor (17) that certain cells in the vicinity of the xylem reacted differently from other cells; and Priestley (22) had discovered patches of cells opposite the xylem arms which stained differentially though with acid and basic dyes both. This region seemed to offer interesting material for study.

Preliminary experiments with dyes of high concentration—$10^{-2}$ and $8 \times 10^{-3}$—called attention to these very regions. The root hairs, epidermis, cortex, xylem, and pith stained always in the same way from the very beginning of the work, but the endodermis, and what will be called the xylem caps and cortex caps, seemed for some time to follow no rules. Most of this paper will be devoted to those regions only.

The parts of the root which stained regularly and the sort of dye with which they stained were:
<table>
<thead>
<tr>
<th>Part</th>
<th>Stained with</th>
</tr>
</thead>
<tbody>
<tr>
<td>Root hairs</td>
<td>both negative and positive dyes</td>
</tr>
<tr>
<td>Epidermis</td>
<td>both negative and positive dyes</td>
</tr>
<tr>
<td>Cortex</td>
<td>positive dye</td>
</tr>
<tr>
<td>Xylem</td>
<td>both negative and positive dyes</td>
</tr>
<tr>
<td>Solerenchyma</td>
<td>both negative and positive dyes</td>
</tr>
<tr>
<td>Phloem</td>
<td>only positive</td>
</tr>
<tr>
<td>Pith</td>
<td>only positive</td>
</tr>
</tbody>
</table>

As these parts were so uniform in their staining they will be largely disregarded except in so far as they are used as a check on the charge of the dye. Here these tissues are used as a test-object just as Keller (14) used for a test-object, in checking the charge of his dyes, cross sections of young ivy stems.

The xylem caps are those cells, usually three or four rows, between the protoxylem and the endodermis. They make their appearance in the sections at about one centimeter from the tip and occur in every section up to the place where the xylem has formed a complete ring. Their morphology will be explained later, in Plate I.

The endodermis divided itself by its manner of staining into two regions— that opposite the xylem and that opposite the phloem. Sometimes one stained, sometimes the other; sometimes both stained evenly, and sometimes one more deeply than the other.
Although consistent results were not obtained in these first experiments, the general tendency seemed to be for the xylem caps to remain unstained with basic, or positive, dyes and to stain with acid, or negative, dyes; and for the endodermis to stain by the phloem when cortex and phloem stained, that is with positive dyes, and by the xylem when the caps stained.

Since those tissues which stain with negative dyes are believed to be positively charged, and those stained with basic dyes negatively charged, the following diagram shows the tissue charges of *Vicia faba* root as they seemed to be from these preliminary studies.

It was clearly demonstrated that a careful study of the questionable regions was needed and that its event might throw much light on the question of absorption by the root.
Further Experiments

With Sections

For more accurate results buffers were used. A typical experiment with basic dye was the following: Sections were cut into tap water and examined; the good ones—that is, those flat, even, and thin enough for the making of accurate observations—were retained. These were then put into the 100 cc. bottles, each of which contained 75 cc. of $10^{-5}$ toluidin blue in tap water and 5 cc. of one buffer from the range 3.2, 4.0, 5.3, 6.2, and 7.5. A sixth bottle contained 80 cc. of the same dye mixture, and a seventh 80 cc. of the distilled water dye mixture. These bottles were placed in the shaking machine and shaken for one hour. The sections were then removed from the bottles and rinsed in the same concentration of buffer which was used in the dyeing and examined. Macroscopically they showed a good gradation of color from dark blue at 7.5 to almost colorless at 3.2. Microscopic results were even more striking and may be clearly seen in the table.
**TABLE 1**

STAINING RESULTS WITH TOLUIDIN BLUE (POSITIVE DYE)

In 75 cc. of $10^{-5}$ tap water dye mixture with 5 cc. of buffer.

In shaking machine for 1 hour.

<table>
<thead>
<tr>
<th>pH</th>
<th>Root hairs</th>
<th>Epidermis</th>
<th>Cortex</th>
<th>Endodermis and Pericycle</th>
<th>Phloem</th>
<th>Xylem</th>
<th>Xylem cap</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.0</td>
<td>S</td>
<td>S</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.3</td>
<td>SS</td>
<td>SS</td>
<td>LS</td>
<td>LS</td>
<td>IS</td>
<td>S</td>
<td></td>
</tr>
<tr>
<td>6.2</td>
<td>SS</td>
<td>SS</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td></td>
</tr>
<tr>
<td>7.5</td>
<td>VSS</td>
<td>VSS</td>
<td>VSS</td>
<td>SS*</td>
<td>SS</td>
<td>S</td>
<td></td>
</tr>
</tbody>
</table>

* -- opposite phloem. ** -- opposite xylem.

Explanation of symbols:

- LS -- lightly stained.
- S -- stained.
- SS -- strongly stained.
- VSS -- very strongly stained.
- - -- unstained.

The results anticipated in the preliminary experiments are here very evident. With the basic dye the xylem caps were unstained throughout, and the endodermis was more strongly stained opposite the phloem than opposite the xylem.

A check on the charge of the dye was found in the fact that cortex and phloem stained. The isoelectric point of cortex, phloem, endodermis, and pericycle seems, according to this
table, to be between 4.0 and 5.3. A similar experiment with safranin gave the results in Table 2.

**TABLE 2**

**STAINING RESULTS WITH SAFRANIN (POSITIVE DYE)**

In 75 cc. of \(10^{-5}\) tap water dye mixture with 5 cc. of buffer.

In shaking machine for 1 hour.

<table>
<thead>
<tr>
<th>pH</th>
<th>Root hairs</th>
<th>Epidermis</th>
<th>Cortex</th>
<th>Endodermis</th>
<th>Pericycle</th>
<th>Phloem</th>
<th>Xylem</th>
<th>Xylem cap</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.2</td>
<td>SS</td>
<td>SS</td>
<td></td>
<td>S</td>
<td>LS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>SS</td>
<td>SS</td>
<td>VIS</td>
<td>VLS*</td>
<td>LS</td>
<td>S</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.3</td>
<td>SS</td>
<td>SS</td>
<td>LS</td>
<td>LS*</td>
<td>LS</td>
<td>S</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6.2</td>
<td>SS</td>
<td>SS</td>
<td>LS</td>
<td>LS*</td>
<td>S</td>
<td>S</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7.1</td>
<td>SS</td>
<td>SS</td>
<td>LS</td>
<td>S*</td>
<td>S</td>
<td>S</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* - stained by phloem.

Explanation of symbols:

- **S** — stained.
- **LS** — lightly stained.
- **SS** — strongly stained.
- **VSS** — very strongly stained.
- **VIS** — very lightly stained.
- **-** — unstained.

At 3.2 the protoplasm was collapsed and the cells dead.
Methylene blue, methyl violet, and thionin gave results which were very puzzling until the first time that the two additional bottles, one of distilled water dye without buffer, the other of tap water dye without buffer, were added to the series. Very enlightening results were then obtained.

Thionin is listed as a basic dye and as such should dye cortex and phloem and leave the xylem caps unstained. These three observations always serve as an accurate check on the charge of the dye. How thionin stained is shown in Table 3.

**TABLE 3**

**STAINING RESULTS WITH THIONIN (POSITIVE DYE)**

*Tap and distilled water mixtures.*

<table>
<thead>
<tr>
<th>Mixture</th>
<th>Root</th>
<th>Epi-hairs</th>
<th>Cortex</th>
<th>Endo-dermis</th>
<th>Phloem</th>
<th>Xylem</th>
<th>Xylem caps</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled water</td>
<td></td>
<td>S</td>
<td>S</td>
<td>SS</td>
<td>S*</td>
<td>S</td>
<td>-</td>
</tr>
<tr>
<td>Tap water</td>
<td></td>
<td>S</td>
<td>S</td>
<td>LS</td>
<td>S**</td>
<td>-</td>
<td>S</td>
</tr>
</tbody>
</table>

* -- opposite phloem. ** -- opposite xylem.

Explanation of symbols:

S -- stained.
SS -- strongly stained.
LS -- lightly stained.
- -- unstained.
Thionin in tap water mixture acted like an acid or negatively-charged dye, in distilled water it acted like a basic or positively-charged dye. In the series of sections stained by tap water-thionin with buffers, the xylem caps were stained throughout, darkest at 3.2 and lightest at 7.5. That is to say, the charge of the dye was reversed. With methylene blue and methyl violet these same staining results had always been obtained, and must have been due to a reversal of charge brought about by substances present in the tap water.

Experiments were carried on in exactly the same way with acid or negatively-charged dyes, except that examinations at the end of one and two hours showed such light staining that the sections were returned to the dye and buffer mixtures and shaken longer. Often several hours were required for good differentiation with a concentration of $10^{-5}$. With $10^{-4}$ a shorter time sufficed and in all later experiments with acid dyes this concentration was used. The best results were with light green, and are shown in Table 4.
TABLE 4
STAINING RESULTS WITH LIGHT GREEN (NEGATIVE DYE)

In 75 cc. of 10^{-4} tap water dye mixture with 5 cc. of buffer.
In shaking machine for 3 hours.

<table>
<thead>
<tr>
<th>pH</th>
<th>Root hairs</th>
<th>Epidermis</th>
<th>Cortex and Parenchyma</th>
<th>Phloem</th>
<th>Xylem cap</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.2</td>
<td>SS</td>
<td>SS</td>
<td>-</td>
<td>SS*</td>
<td>SS</td>
</tr>
<tr>
<td>4.0</td>
<td>S</td>
<td>S</td>
<td>-</td>
<td>S*</td>
<td>SS</td>
</tr>
<tr>
<td>5.3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>S*</td>
<td>SS</td>
</tr>
<tr>
<td>6.2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>S*</td>
<td>SS</td>
</tr>
<tr>
<td>7.5</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>S*</td>
<td>SS</td>
</tr>
</tbody>
</table>

Explanation of symbols:

- * — stained opposite xylem.
- S — stained.
- SS — strongly stained.
- LS — lightly stained.
- VLS — very lightly stained.
- - — unstained.
One more table may bring out more clearly the comparison between the staining by positive dyes with that by negative dyes.

**TABLE 5**

**COMPARISON OF STAINING BY POSITIVE AND BY NEGATIVE DYES**

<table>
<thead>
<tr>
<th>Dye</th>
<th>pH</th>
<th>Epi-dermis: Cortex: Endo-dermis: Phloem: Xylem: Xylem caps</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>5.3 to 7.5</td>
<td>S: S*: S: S: S*: S: S: -</td>
</tr>
<tr>
<td>Negative</td>
<td>3.2 to 5.3</td>
<td>S: -: S**: -: S: S</td>
</tr>
</tbody>
</table>

* -- by phloem. ** -- by xylem.
S -- stained. -- -- unstained.

In the staining of sections there was sometimes a differential staining of semi-circular areas of cells outside the endodermis in the cortex. These, together with the xylem caps, formed a symmetrical region which, when it appeared, usually stained more heavily with acid dyes and more lightly with basic dyes.
Whole Roots

Staining from Outside-In -- Intact Roots.

Method:

The staining of sections gave no direct evidence as to the path followed by the dye in entering the plant. In order to trace its course it was necessary to grow roots in the dye. A simple apparatus was devised for this. Six 8-inch test tubes were filled with $10^{-4}$ tap water dye. In the top of each tube was placed a bean seedling wrapped loosely in absorbent cotton. The root was immersed in dye about half-way up to the seed. A strip of gauze was wound around the cotton about each seed and its free end immersed in a bottle of water. This capillary device furnished just enough moisture to keep the seed from drying—a real difficulty in the earlier experiments. The roots chosen were always the same age, were straight, had no lateral roots, and were about 4 centimeters long. Such a plant had a rapidly developing epicotyl, the leaves usually expanding during the experiment. The more flourishing the top, the more marked were the dyeing results, due undoubtedly to the aid furnished by the transpiration pull. Every 24 hours one root was removed, sectioned, and studied, and the dye left in that tube was used to replace in the other tubes the amount of dye taken up by the plant and evaporated. Thus the concentration of the added dye was probably about that of the dye in the tubes which still contained living plants to which it was added.
Results with Positive Dyes:

With positive dyes, as safranin, the course of the dye was easily followed. The meristemata cap was stained deeply and evenly. The region above, about 8 millimeters in length where neither xylem caps nor Casparian strips were developed, showed deeply-stained root hairs, epidermis, and cortex, to within two to ten cells from the endodermis. The inner cortical cells, usually a region two cells in width opposite the phloem to ten opposite the xylem (the cortex cap), were more lightly stained. The endodermis opposite the phloem was stained more deeply than the cortex and pericycle and equally with the cortex and pericycle opposite the xylem. Both xylem and phloem were stained.

Beginning at about the end of the first centimeter from the tip, each section showed well-defined xylem caps and well-developed Casparian strips. In this region the dye stopped in the endodermal cells and, no matter how long the experiment ran, was never found in the stele except in xylem and sclerenchyma. To make sure that the dye entered the endodermis from the outside, frequent examinations were made at the top of the stained portion where the path of absorption from epidermis to cortex was extremely short as compared with that from root tip up the vessels to the same point, and where, because of the extremely slow upward passage of positive dyes in the vessels, the vessels were still unstained. As the endodermis in this region always stained before the vessels, this point was
satisfactorily settled. If dye mounted in the cortex, as well as passing through it transversely, the ascent was so slight as to be unobservable.

With methyl violet (without buffer) the same results were obtained. Root hairs, epidermis, and most of the cortex were deeply stained, cortex caps (the semi-circular regions outside the endodermis opposite the xylem arms which stain differentially) were more lightly stained, endodermis deeply stained (no Casparian strips developed here), pericycle lightly stained, and xylem and phloem stained. Where caps and Casparian strips were developed the dye stopped in the endodermis.

In young lateral roots the same staining was observed, though it was usually impossible to absolutely identify the endodermis except by its position. There was always a region near the tip stained clear through, then a stained cortex, with stele unstained except for the xylem vessels. In the vessels the stain was very deep and could be traced to their union with the vessels of the primary root.

**Results with Negative Dyes:**

With the exception of the root hairs and epidermis, which showed very light staining, an intact root absorbed no negative dye, at least in amounts which were microscopically visible.

In these experiments the roots always remained perfectly turgid, increased in length, sent out the
normal number of lateral roots in the normal place, and the stems and leaves grew vigorously.

Staining from Inside-Out

Method:

The same apparatus was employed as in the experiments with intact roots. The only difference was that about a centimeter at the tip of the root was cut off under water and then only the cut end was immersed in the dye. It was necessary to replenish the dye frequently. With only the extreme end of the root in the dye, it is apparent that any dye found in the cortex, above the very limited region where it could enter from the sides due to the very slight immersion in the dye and possible slight upward ascent through the cortex, must enter from the vessels.

Results with Positive Dyes:

Roots with one centimeter cut off, and with only the cut end immersed in dye, showed no staining except in that portion, two or three millimeters at most, which was immersed in the dye.
Results with Negative Dyes:

Negative dyes rose very rapidly through the vessels to the seed.

With light green, 10−4, at the end of four days the xylem, xylem caps, and endodermis opposite the xylem were stained, the endodermis much more deeply than the xylem caps.

On one occasion the inner side of the endodermis opposite the xylem was much more deeply stained than the outer side. This is extremely important.

Cyanine stained the vessels green, the endodermis opposite the xylem deep light blue, the xylem caps and cortex caps faint light blue (metachromasy).

The roots in these experiments remained turgid, did not increase in length, but sent out large numbers of lateral roots.
DISCUSSION

Charges

A study of the plates showing the staining of root sections by basic and acid dye points at once to differential entry of materials into the vascular cylinder. Priestley (23) suggested this possibility, basing his supposition on two facts brought out in his work and mentioned earlier in this paper: (1) That the Casparian strips develop earlier opposite the phloem than opposite the xylem, and (2) that there were patches of cells outside the xylem arms that stained more deeply than the neighboring cells. Priestley got this differential staining, however, with both acid and basic dyes, magdala red and neutral red, though at a seemingly high concentration.

This investigation has gone further. We have discovered five facts that seem to be of major importance: (1) That the xylem caps remain unstained with basic dyes; (2) that the xylem caps stain with acid dyes, especially at a low pH; (3) that on one occasion the inner side of the endodermis opposite the xylem stained with light green, a negative dye, much more deeply than did the outer side; (4) that only the part of the endodermis opposite the xylem stained with acid dyes; (5) that with basic dyes the endodermis stained sometimes only opposite the phloem, sometimes all the way around, either
evenly or more deeply opposite the phloem than opposite the xylem, but never opposite the xylem and not opposite the phloem.

These discoveries seem to the writer to point to a difference in charge on the outer and inner side of the endodermis opposite the xylem resulting in irreciprocal permeability of the endodermis at that place. Whenever such a condition is present, one side of a tissue or membrane shows a higher combining power for a given substance, either water or solute, than does the other side. The general rule under such conditions is that the substance in question is transferred from the place of higher combining power to the place of less. The greater the difference in combining powers of the two sides, the greater is the amount of transfer from the outer solution to the inner solution.

Let us see what this means in the one instance where staining showed very clearly a difference in the charge on the inner and outer sides of the endodermis. With light green, i.e., an acid or negative dye, the cells of the endodermis opposite the xylem were much more deeply stained on their inner than on their outer sides, which means that there was a much stronger positive charge on the inner side than on the outer and therefore a greater combining power with a negative dye on the inner than on the outer side. Because of this difference between the charge of the inner and outer sides, particles of the opposite charge—that is negatively-charged particles—would be strongly adsorbed to the inner side of the
endodermis and transferred to the outer side where they would be given off, transfer being determined not by protoplasmic streaming alone, as de Vries (9) thought, nor because of the increase of permeability due to contact with acid stelar sap, as Priestley (28) thought, but because of the irreciprocal permeability of the endodermis in this region.

That acid dyes pass from the vessels through the xylem caps and endodermis was proved in the experiments with living roots the tips of which had been cut off and the ends immersed in dye, under which conditions both light green and cyanine were found in the vessels, xylem caps, endodermis opposite the xylem, and on two occasions in the cortex caps, while the intact living root showed adsorption of dye only in root hairs and epidermis. Apparently acid dye particles, at least in visible amounts, can not pass through the cortex from the outside.

It will be remembered that the endodermis divides itself into two definite, differently staining regions--that opposite the xylem and that opposite the phloem. The staining with acid dyes has already been discussed. With basic dyes the staining is usually heavier, or only, opposite the phloem. This indicates a negative charge in this region of the endodermis. The fact that sometimes the endodermis stains evenly all the way around is not explainable at the present time, for the conditions under which such staining takes place
are not yet known or under control. It has been found that when sections are stained for a long time or at high concentrations with basic dye, everything is stained and there is little differentiation. It may be that with a short enough period of staining and low enough dye concentration a perfect differentiation of the endodermis could be obtained. The results of Rohde and Keller suggest this. Such differences in staining as were found in the endodermis were found in no other tissue and must be significant.

Absorption

If the diagram showing the charges of the tissues as determined by the way in which they stain is studied, the following explanation of absorption in terms of charges of tissue and dye may be postulated.

Both acid and basic dyes enter the root hairs. As has already been mentioned, Haylor (17) found that with a double stain of toluidin blue and eosin, or methyl blue and safranin, sections at pH values of from 4.3 or 4.6 to 5.6 stained purple; that is, they took up both the acid and the basic dyes. In increasing the pH within this range basic dye is adsorbed in regularly increasing amounts, while the acid dye is adsorbed in decreasing amounts. This region is the isoelectric range. Root hairs and epidermis are evidently in this range. Whether in the root hairs and epidermis an irreversible permeability also exists, i.e., whether the electrical
charge is different on outside and inside, has not been deter-
mined.

The dyes next come in contact with the
strongly negatively-charged cortex parenchyma cells. The nega-
tively-charged acid dye particles can not be adsorbed by this
tissue and their entrance into the root is stopped here. The
positively-charged basic dye particles, on the other hand, are
strongly adsorbed and can pass through the cortex. A gradually
increasing negative charge from outside to inside of the cortex
as a whole could be assumed here. This would correlate well with
the osmotic suction increase shown by Ursprung and Blum.

The basic, or positive, dye has now arrived
at the endodermis, which we are sure has a stronger positive
charge on the inner side than it has on the outer side opposite
the xylem, and which has a negative charge opposite the phloem.
Since the endodermis even by the xylem is sometimes stained by
positive dyes as well as by negative, we may assign to that
part of it, but on the outer side only, a relatively negative
charge. Because of these charges the positive, or basic dye,
is readily adsorbed, strongly opposite the phloem, and equally
or less strongly opposite the xylem; but though the inner side,
opposite the xylem, is more positively charged, the passage of
the basic dye is apparently stopped here. No matter how long
the experiments with intact roots ran, no positive dye was ever
seen in the xylem caps.
This is all that can be directly observed of the intake of basic dyes.

To follow the course of acid dyes we must begin with the vessels. The lateral inward passage is stopped at the outer cortex cells, but it will be remembered that such dyes rise rapidly in the vessels and also that they pass laterally from the vessels into the xylem caps and into the endodermis opposite the xylem. Apparently on this side of the endodermis the charges are exactly reversed, the xylem caps being positively charged and adsorbing negative dye. Therefore, the explanation of the passing of dye through the xylem caps into the endodermis is exactly like that of the passing of dye through the cortex, except that the direction and charges are the opposite. That is, the negative dye is adsorbed to the positive tissue and passed in because there is a gradually increasing positive charge which culminates in the high positive charge of the inner side of the endodermis. The dye passes from the cap into the endodermis because of the strong adsorbing power inherent in its strong positive charge, but stops there notwithstanding a negative charge on its outer side or is released in very minute quantities.

It becomes necessary at this point to explain the passing of the dye across the xylem cap, although in the experiments with intact roots positive dye was never seen in the caps. However, we know that the dye entered the endodermis opposite the xylem and that the combining power for it
was greater on the outer than on the inner side of the endodermis. It must therefore be given off on the inner side. Probably the difference in potential is too slight to desorb such heavily charged particles as those of the dye, at least in visible amounts. The fact that we can not see dye in the xylem caps need not necessarily prove that no dye is there, however. Dye is never seen in protoplasm but we can not conceive of its entering the vacuole without passing through the protoplasm. The decreasing positivity, which would mean increasing negativity of the xylem cap, would account for its crossing to the xylem vessels.

The basic dye is always present in the vessels and sclerenchyma. The vessels of an intact root are stained with a basic dye, only as high or very little higher than the cortex is stained. This would seem to point to the fact that the dye either enters the vessels from the cortex, or enters the cortex from the vessels. The cortex is stained only as far up as the root is immersed in the dye. Roots with their tips cut off and the ends immersed in basic dyes stained only as far up as they were immersed in the dye. Dye did not rise in the vessels. As absence of cross walls and pull of transpiration would both help the dye once in the vessels in its upward climb, it would seem that if the dye actually did enter the vessels from the tip it would rise more rapidly through the vessels than through the cortex where it must encounter many cross walls and pass through many protoplasts
without the direct help of the transpiration pull. Therefore, it seems that it must enter the root through the root hairs, epidermis, cortex, endodermis, and xylem caps to the xylem.

The cortex caps are not understood. With methylene blue and methyl violet, in intact roots immersed in dye, they stained more lightly than the rest of the cortex. This differential staining was found only where the Casparian strips were still undeveloped. If these results were consistently obtained with basic dyes, the charges of which were not easily reversed as those of methylene blue and methyl violet are, they would indicate a less negative charge in the inner region of the cortex. Cortex caps were, however, seen with only these basic dyes, and once with safranin. Twice cyanine and light green passed through the endodermis from the vessels and xylem caps and stained the cortex caps. Priestley shows photographs of differential staining of these regions, but the concentration of dye used was high and the cells are very apparently dead. Possibly when the Casparian strips are still undeveloped the line dividing the charges is less definite and that part of the cortex more positive.

The phloem stained with basic dyes in sections, but remained unstained when living roots were grown in dye. Since the phloem is used mainly for transport of elaborated foods down from the stem, there is no reason to expect it to be dyed by materials passing up through the root. It does, however, stain in sections, and when it stains, the
pericycle and endodermis opposite it stain, as does the cortex. This would seem to indicate that if food materials get into the phloem in the leaf or stem, they may pass out of it into the cortex.

One tissue remains unmentioned, the solerenohyma. This tissue stained more quickly in sections than did any other tissue, and dye rose in it in roots immersed in dye more rapidly and higher than in any other tissue. Keller mentioned this fact and thought the solerenohyma of importance in conduction. Except to confirm his staining results, this paper can add nothing. It does not seem to the writer to be a conducting tissue.

The staining of the meristematic cap by basic dyes can be nicely correlated with the fact that these cells must have elaborated food materials for their synthetic metabolism. Although in the experiments described here the dye was absorbed from the outside solution, since this tissue is undifferentiated, it could probably absorb basic substances equally well from any side to which they were presented. Basic substances, then, on their way down from the leaves, which were retained within the endodermal cylinder to its end at the meristematic cap, could be adsorbed by this negatively-charged region and there used.
Absorption of Salts

It is not possible yet to directly connect these results with dyes with the absorption of salts by the root, but it does seem that the work with dyes at least indicates the route and the mechanism of intake.

Blüh (5), summarizing the work which has been done by several experimenters in determining the electrical charges of salts, states that KCl, NaCl, BaCl₂ and other salts show migration toward the cathode; that chlorides wander toward the cathode more than toward the anode; and sulphates toward the cathode as much as toward the anode. Since these salts are all positively charged (the sulphates very slightly so) they must act like positively-charged dyes with the exception that their charges are so slight as compared with those of the dyes that the potential differences between tissues and salts must be much less than those between tissues and dyes, and the intake of salts, therefore, must be both much less and much slower.

There are many complicating factors, as for instance the presence in the soil solution not of one positively-charged substance, but of many, some of which are much more strongly adsorbed than others. We have to consider, then, not only the charge, but also the valency, and not the charge and valency of one ion or undissociated salt, but of several, as
well as the effect of the ions of the lyotropic series. If we simplify the whole process as it never is simplified in nature, we can see that one positively-charged nutrient salt would be taken up by root hairs and endodermis and passed through the cortex by the same mechanism as was one basic dye. This salt would pass through a cortex of gradually increasing negative charge and be taken up by the outer still more negatively-charged endodermis, to be transferred to the inner side, where the holding capacity for the salt is less, and given off there, though given off in very small amounts, so small in the case of the dye as to be invisible. The salt is then transferred to the xylem vessels, just as we assumed the dye was.

The fact that basic dyes are retained within the endodermal cylinder correlates well with the fact that the salts absorbed by the plant are used largely in the chlorophyll-containing cells and therefore must not escape in the root. Priestley found that in the stem, conditions were the reverse of those in the root, and that here basic dyes easily escaped from the cylinder. Blüh found sucrose, dextrose, and levulose positively charged, which would permit of their being taken up by phloem. The fact that phloem, pericycle, endodermis, and cortex stain together has already been suggested as pointing to the fact that positively-charged substances might escape into the cortex to be used there, when
coming down from the stem. Too little work has been done on the charge of salts and sugars to allow too positive statements about them as yet.

Relation to Other Theories

The relation of this theory of electrical adsorption and irreciprocal permeability to other theories of absorption is that it supplements and explains those theories.

De Vries (9) accounted for the transfer of material from the outside of the root to the inside by protoplasmic streaming. He did not, however, give any reason why streaming protoplasm should take up water on one side of the cell and give it off on the other. The theory of irreciprocal permeability can explain that. It is only necessary to prove a difference in water and solute-holding capacity on the outer and inner sides of the cell, that is, a difference in charge, and we have a mechanism which will take up water and solutes on one side and give them off on the other.

De Lavison showed that if Casparian strips were developed, substances could pass the endodermis only if they could be absorbed by the endodermal protoplasts, and that they entered the root freely where the strips were undeveloped. He did not attempt to explain absorption in any other terms than diffusion along cellulose walls and permeability of endodermal protoplasts.
Priestley (23) concluded that both diffusion and osmosis were inadequate theories to explain the passage of salts across the endodermis into the stele. He suggested that there was an accumulation of ions by the endodermis from the soil solution on the outside, and a liberation of these ions on the inner side. His theory was built on results obtained by Hoagland and Davis who had found that cells of *Nitella* lost ions if the pH of the external medium became too low, and on the hypothesis that the stelar sap, because of the presence of relatively large amounts of carbon dioxide, was very acid and made possible the liberation on that side of the endodermis of the ions which were collected from the soil solution on the outside. He does not explain why acidity should increase the permeability of a membrane. It seems that Priestley was approaching by reasoning what has been discovered in this study by experimentation. Direct evidence has been presented here of the irreciprocal permeability of the endodermis, which accounts for the accumulation and liberation of substances by that tissue.

Priestley explained the high suction tension on the outer side of the endodermis, and low suction tension on the inner side, which Ursprung and Blum's work brought out, in terms of accumulation and liberation of ions. The theory of irreciprocal permeability would base this accumulation and liberation of ions (and water) not on an unexplained
increase of permeability by increased acidity, but on a proved potential difference between the two sides of the endodermis.

**Technical Dyeing**

It was interesting to find that the dyeing results obtained with the living cells of plants and animals checked perfectly with the dyeing of textiles. Although it had long been known by practical dyers that the addition of acid or alkali to the dye bath affected the taking up and fastness of the dye, the first scientific investigation of dyeing was carried on by Pelet-Jolivet in 1910. He proved that a dye could not be absorbed by a fabric bearing the same charge as the dye, but that the opposite charge could be effected in the fabric by the addition of acid or alkali and the dye then absorbed.

Bancroft (2) proved that the dyeing of textiles was an adsorption process, though in rare instances chemical combination might take place. He defined acid and basic dyes as they have been defined in this paper and showed that an acid dye was taken up most strongly in acid, less in neutral, and least in alkaline solutions. The reverse was true of basic dyes.

Briggs and Bull (6) worked in detail quantitatively on the same subject. They used wool, hydrochloric
acid, the acid dye Scarlet R, and the basic methylene blue. The results were again the same. Hydrochloric acid increased the amount of acid dye adsorbed and decreased the amount of basic. The opposite was true of methylene blue. With an alkali the reverse results were obtained. This study was a very careful quantitative one with all varying factors carefully controlled.

This brief review of technical dyeing shows that the same physico-chemical laws are operating in the dyeing of living cells as in the dyeing of fabrics.
1. The charges of root tissues can be determined by vital staining methods.

2. The entrance of dyes into the root is dependent upon the charges of the tissues and of the dyes.

3. The endodermis possesses powers of selective adsorption because of its irreciprocal permeability.

4. Most of the salts used by the plant have been proved by cataphoresis to have a positive charge and therefore may be expected to penetrate the root somewhat as the basic dyes do.

5. Because of the irreciprocal permeability of the endodermis, positively-charged substances are largely retained within the endodermal cylinder in the root.

6. The adsorption of dyes by tissues follows the same physico-chemical law as the adsorption of dyes by textiles.
BIBLIOGRAPHY


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EXPLANATION OF PLATES

Plate I.

Figure 1.—Photograph of drawing and diagram from Haberlandt showing xylem caps, the inner portion of which is cambium, the outer portion dividing pericycle. (In younger sections no cambium is present).

Figure 2.—Living root of Vicia faba stained in light green 10^{-4} at pH values of 3.2, 4.0, 5.3, 6.2, and 7.5. At 3.2 the stele is strongly stained, the stain decreasing with increasing pH. At 7.5 the section is almost invisible in the photograph, only its shadow showing clearly.

Plate II.

Figure 3.—Living Vicia faba root stained with light green as in Figure 2, at a pH of 4.0. The xylem caps are strongly stained and the phloem is unstained.

Figure 4.—The same section at higher magnification. The endodermis can be recognized and seen to be stained opposite the xylem and unstained opposite the phloem.

Figure 5.—Still further enlargement of the same section. This shows lack of stain in endodermis and pericycle opposite the phloem, and in the phloem itself.

Figure 6.—Living root of Vicia faba stained with 10^{-5} toluidin blue at pH 7.5, showing xylem cap unstained; endodermis and pericycle opposite phloem, and the phloem, unstained.
Figure 1.

Figure 2.
PLATE II.

Figure 3.

Figure 4.

Figure 5.

Figure 6.