*THE DEVELOPMENT OF DIFFERENTIAL PERMEABILITY IN ISOLATED STELES OF CORN ROOTS**

By George G. Laties and K. Budd

DEPARTMENT OF BOTANY AND PLANT BIOCHEMISTRY, UNIVERSITY OF CALIFORNIA (LOS ANGELES)

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The primary path of salt movement from root to shoot of vascular plants is through the xylem, which constitutes a group of conduits contained within the central cylinder, the stele. In roots the stele is normally delineated by a specialized layer of cells, the endodermis, and surrounded by a multilayered sheath of cortex parenchyma which lies beneath the epidermis.[1, 2] In passing radially from the root surface to the xylem, salts may be absorbed by cells at the root surface and pass centripetally through a cytoplasmic continuum, the symplasm,[3, 4] or may diffuse into the cortex through the water-free space[5] of the cell walls, there to be absorbed into the symplasm of the cortical cells. Radial movement by free diffusion through the free space is for practical purposes terminated at the boundary of the stele by the nonporous Casparian strips[2] which impregnate the radial walls of the endodermis. Thus ion entry into the stele must be through the symplasm. Plasmodesmata—cytoplasmic connections between contiguous cells—traverse the encompassing cellulosic walls, including the tangential walls of the endodermis, and provide an internal path of ion movement from epidermal cells and cortex to within the central cylinder.[1] With respect to the deliverance of ions to the xylem, the large vacuoles of the cortical and stelar parenchyma represent a diversionary sump—a salt repository which is in parallel with the xylem.[6, 7]

The concentration of salt, hence of cations and anions alike, in the xylary fluid is frequently well in excess of that in the surrounding medium.[1, 8, 9] The foregoing phenomenon is the basis of root pressure. Since mature xylem is nonliving, and in effect represents a standpipe, it is evident that osmotic work must be done between the root surface and the xylem when the total concentration of salt in the xylem exceeds that of the external solution. The choice regarding the site of osmotic work has involved primarily two alternatives. On the one hand, it has been suggested that the endodermis or neighboring stelar parenchyma represents secretory tissue which pumps ions into the xylem.[7, 10, 11] On the other hand, it has been proposed that ions are accumulated in the thermodynamic sense in the cytoplasm of the epidermal and cortical cells, following which, movement into the stele takes place passively through the symplasm in response to a gradient of chemical potential.[1, 12] In the latter view ions must ultimately *leak* from stelar parenchyma into the vessels of the xylem. In both cases loss of salt by diffusion from the vascular conducting elements to the environment is deemed to be prevented by the Casparian strips of the endodermis.

When Crafts and Broyer[1] first examined the question of salt movement into the xylem of roots, they proposed that ions leak into the xylem from contiguous living cells within the stele, and that the leaky condition of stelar cells is the consequence of diminished respiratory metabolism brought about by an unduly low O_2 and high CO_2 concentration thought to obtain in the center of the root. The latter inter-

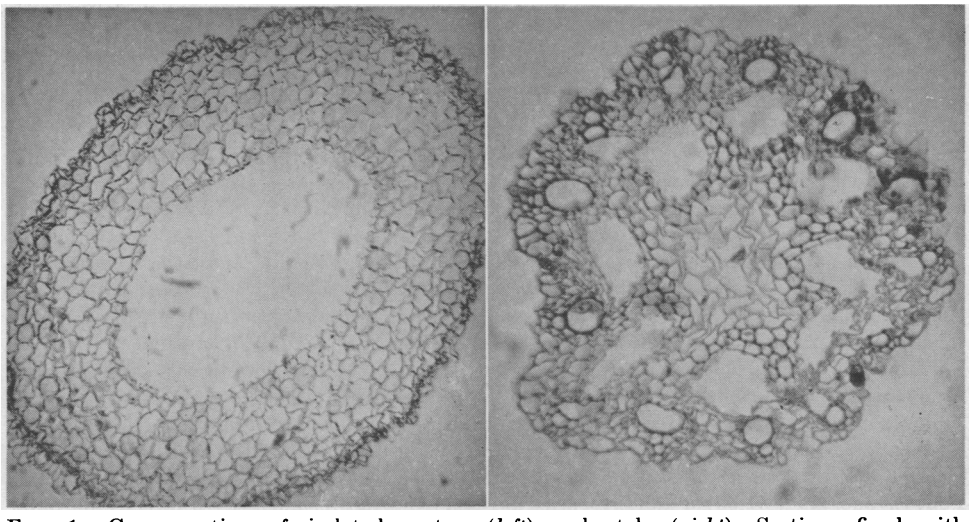

FIG. 1.—Cross section of isolated cortex (*left*) and stele (*right*). Sections fixed with formaldehyde-propionic acid-ethanol. Magnification: cortex, 80×; stele, 190×.

pretation closely resembled that put upon the behavior of storage-organ slices wherein it was early recognized that the surface cell layers display a markedly higher respiration rate than does the deeper-seated tissue, and that the surface cells are more effective in salt absorption.[13] More recently, a new interpretation has been assigned to the classical inverse relation between respiration rate and thickness in storage-organ discs.[14] It was noted first that thickness influences the *development* rather than the activity of a component of the total respiration which arises primarily at the disc surface in the hours after slicing; and secondly, that the foregoing component is qualitatively distinct from the initial, or basal, respiration. The regulatory agent responsible for the observed inverse relation between respiration rate and thickness was deemed to be an endogenously produced volatile metabolite other than CO_2, and the profound physiological changes attending the rise in respiration[15, 16] were considered to stem from the developed respiratory component which, for practical purposes, is confined to the disc surface.

On the presumption that the distinction between surface and internal cells observed in storage-organ slices reflects a fundamental aspect of tissue organization, it was considered that the internal parenchyma of the stele may bear the same relationship to the superficial cortical cells of fibrous roots as deep-seated cells of storage-organ slices bear to those of the surface. In this view it was anticipated that stelar cells should be both leaky and ineffective in salt absorption at physiological salt concentrations, and that stelar cells should develop a more pronounced differential permeability and an enhanced capacity for salt absorption following a period in the absence of the cortex. Both expectations were realized.

Materials and Methods.—Grains of corn (*Zea mays*), Hy 2 × 07, were soaked and germinated as previously described.[17] Primary roots of 4-day-old seedlings were separated into stele and cortex by means of an electrical-wire insulation-stripper. The cutting edges of the instrument were adjusted to clear the stelar cylinder and then drawn firmly along the root from base to tip. In about half the roots the stele separated cleanly and easily from the surrounding tissue: somewhat less frequently the cortex was also obtained relatively intact, and free from stele elements except at the extreme tip. The apical 2 cm of stele and cortex were used in the experiments described

below. Figure 1 shows the anatomical structure typical of the two isolated tissue systems. Stele and cortex separate by shearing of the layer of small, regular endodermal cells,[18] and rupture of other cell walls is rare.

Experimental samples consisted of 20 sections of stele or 10 of cortex, having fresh weights of approximately 40 and 130 mg, respectively. Each cylinder of cortex was split lengthwise to ensure that the internal surface was exposed to the various bathing solutions. Material was either used within 2 hr of beginning dissection (fresh tissue), or transferred to 10^{-4} *M* $CaSO_4$ (5 ml/sample) in 50-ml Erlenmeyer flasks and gently shaken at 25°C for 22–24 hr to give aged tissue. For absorption of chloride, each sample was transferred to a 50-ml Erlenmeyer flask containing 5 ml of a solution of 5 mM phosphate buffer, pH 6.8–7.0, 0.5 mM $CaSO_4$, and either KCl or $CaCl_2$ labeled with Cl^{36}. The isotope (specific activity 0.347 mc/gm Cl) was neutralized with the hydroxide of the appropriate cation, diluted, and presented to the roots with or without addition of stable KCl or $CaCl_2$. Solutions so prepared contained a maximum of 70 μc/liter.

Flasks were gently shaken at 25°C in a waterbath shaker. At given intervals the contents of each flask were poured into a medium sintered-filter funnel, and the solution was rapidly drawn off by means of a suction pump. Root sections were then either removed from the filter and blotted dry, or washed in the filter with several changes (each 50–60 ml) of ice water, usually for 15 min. Each sample was then arranged on an aluminum planchet with 0.2 ml 3% polyvinyl alcohol (Du Pont Elvanol grade 51-05) as adhesive, dried at room temperature, and assayed directly for radioactivity with a gas-flow micromil window detector. Results are uncorrected for self-absorption, which was 5% or less.

The conductivity determinations shown in Figure 5 were made with a pipet-type conductivity cell and conductivity bridge (Industrial Instruments, Inc., model RC 16). The endogenous chloride content of stele and cortex was measured by means of an Aminco-Cotlove chloride titrator after extracting the chloride into boiling water. Untreated fresh and aged steles gave values of 2.5–3.2 μeq, and cortexes 7.3–7.4 μeq/gm fresh weight. For convenience, the terms "stele" and "cortex" will frequently be used in the singular to designate the tissue type, although experimentally, several sections of each type of tissue respectively were used as a sample.

Experimental Results.—Chloride uptake from dilute KCl solution by freshly isolated steles is markedly less than that of intact roots or freshly prepared cortex (Table 1). Since chloride absorption by fresh stele and cortex taken together essentially equals that of the intact root, little or no deleterious consequences of tissue separation are indicated. Following aging, absorption by stele and cortex is virtually identical on a fresh weight basis. However, while cortex absorption increases approximately twice on aging, stele absorption increases twentyfold. A more significant change in absorption characteristics attending aging is perceived in a comparison of absorption isotherms of fresh and aged steles (Fig. 2). Whereas the isotherm for chloride absorption by fresh steles is anomalous—specifically, exponential—the isotherm for aged steles is conventional, i.e., hyperbolic. The isotherm for chloride uptake by the cortex is hyperbolic in all cases, as is that for the intact root. The two types of isotherm imply distinctly different absorption

TABLE 1

CHLORIDE ABSORPTION BY TISSUES OF CORN ROOTS

Tissue	Number or weight of sections	Uptake, cpm		Uptake ratio, aged/fresh
		Fresh	Aged	
Stele	10	92	1,830	20
	100 mg fr wt	484	9,600	...
Cortex	10	4,880	12,230	2.5
	100 mg fr wt	3,840	9,620	...
Intact	10	5,180	8,430	...
Roots	100 mg fr wt	3,550	5,770	1.6

Absorption 3 hr at 25°C. KCl, 1.4 mM, pH 6.9. Tissue rinsed 15 min in ice water before counting. 10,000 cpm approximately equivalent to 1 μmole chloride. Each value average of two closely agreeing determinations.

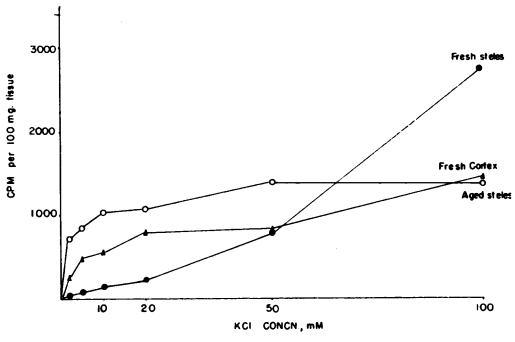

FIG. 2.—The relationship between chloride uptake and KCl concentration. Absorption period 3 hr at room temperature, followed by 15-min wash in ice water. Solutions 5 mM K phosphate, pH 6.9, 0.5 mM $CaSO_4$.

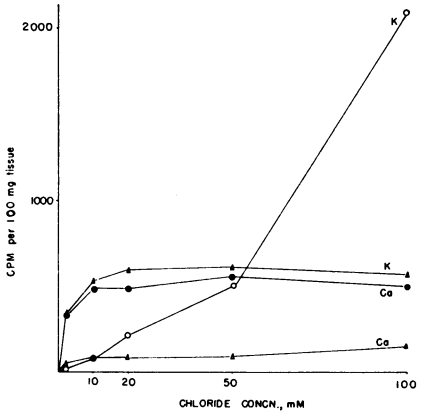

FIG. 3.—The influence of the cation on the absorption isotherm for chloride. Conditions as in Fig. 2. Open symbols, fresh tissue; solid symbols, aged. Excised steles.

mechanisms.[19] While a hyperbolic relation between absorption rate and concentration suggests a saturation phenomenon, that is, some type of carrier-mediated transport,[20] an exponentially increasing rate of uptake with rising concentration implies movement by free diffusion through a permeation barrier, where the permeability coefficient of the cation exceeds that of the anion.[19]

The surface membrane of plant cells is the seat of a bioelectric potential—presumably largely a diffusion potential[21]—which is negative when the cytoplasm is compared with the outside of the cell.[22] Since the rate of movement of an ion by diffusion is a function of its electrochemical potential gradient, a negative membrane potential will slow the passive entry of anions. When the cation of an externally presented salt has a higher permeability coefficient in the membrane than has its counter ion, diffusion will lead to a decrease in negativity of the membrane potential as the external salt concentration is raised. The decrease in electrical potential with increasing chemical potential means that the real diffusion driving force on the anion increases more rapidly than the external concentration,[19] hence the concave-upward isotherm, as observed with KCl. On the expectation that the permeativity of Ca^{++} should be considerably *less* than that of Cl^-, and on the possibility that Ca^{++} decreases membrane permeability to K^+ as well, the influence of Ca^{++} on chloride uptake from fresh steles was examined. Chloride absorption by fresh steles from $CaCl_2$ solutions is in the first place very much less than from comparable KCl solutions, and in the second place is a hyperbolic function of concentration (Fig. 3). Absorption of Cl^- by cortex, on the other hand, is indifferent to the nature of the counter ion. It appears, therefore, that whereas chloride uptake by cortex at all times has the characteristics of a carrier-mediated process, at least the rate-limiting step in absorption by excised steles changes from a diffusion-mediated to a carrier-mediated process with time. With respect to simple diffusive entry, steles become differentially permeable with aging.

Loss of salt by excised tissues: The change in permeability in steles deduced above might be expected to be reflected in the leakage behavior of the tissue. A cessation of leakage is a concomitant of aging in storage-organ slices.[23, 24] Figure 4 shows the

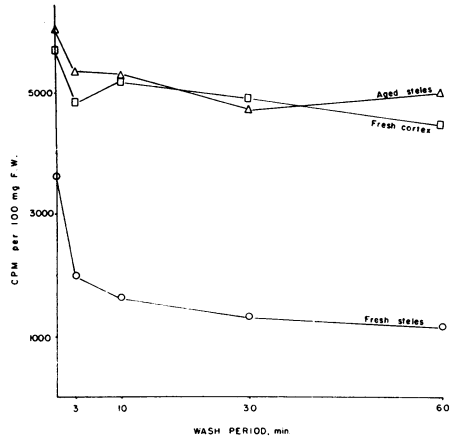

FIG. 4.—The loss of Cl^{36} from excised root tissue as a function of time. Preincubation for 3 hr at room temperature in KCl^{36} of the following concentration: fresh steles, 10 mM; cortex, 5 mM; aged steles, 0.5 mM. Medium as in Fig. 2. Roots were surface-dried and transferred to ice water for leakage measurements.

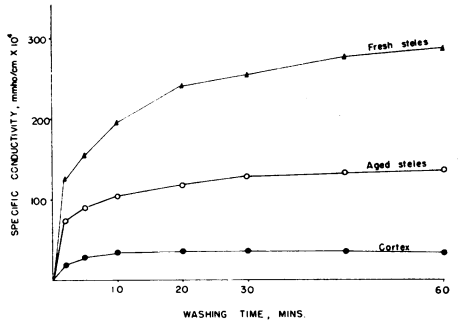

FIG. 5.—The loss of salt from excised root tissue as determined conductimetrically. Samples preincubated for 3 hr at room temperature in 50 mM KCl. Medium as in Fig. 2. Samples rinsed for 15 sec, and transferred to 5 ml distilled water, room temperature, in conductivity cell. Ordinate corrected for water conductivity. Details in text.

loss of radioactivity from stele and cortex previously incubated for 3 hr in KCl solutions labeled with Cl^{36}. Chloride concentrations were chosen to achieve essentially the same specific activity in each of the tissues during preincubation. Loss of label from the free space is virtually complete in 3 min. Loss of label from fresh stele in the subsequent hour was almost 5 times as great as that from either fresh cortex or aged stele. Loss in each case was unchanged in 20 mM KCl, 5 mM KCN, or in water at room temperature, indicating the low rate of loss from aged stele and from cortex is not the result of reabsorption.

Salt loss was also followed conductimetrically. Tissues were first incubated for 3 hr in 50 mM KCl from which absorption is essentially the same by fresh and aged steles and by cortex (Fig. 2). Thereafter, tissue was rinsed, transferred to distilled water, and the conductivity of the latter measured as a function of time. In 1 hr the conductivity change in the solution bathing fresh steles was 10 times that in the medium bathing cortex, and more than twice that in the water around aged steles (Fig. 5). The change in leakage behavior of excised stelar tissue with time is consistent with the change in absorption kinetics.

The effect of an uncoupling agent on chloride absorption as a function of tissue condition: Chloride absorption by fresh and aged steles alike, as well as by cortex, is markedly inhibited by an uncoupler of oxidative phosphorylation, carbonyl cyanide m-chlorophenylhydrazone (m-Cl-CCP)[25] (Table 2). The obvious sensitivity of chloride uptake by fresh steles to m-Cl-CCP is in apparent contradiction to the contention that the rate-limiting step governing absorption in fresh steles is a free-diffusion process. The bulk of ions which are absorbed and retained in plant cells are to be found in the large central vacuoles.[1, 6–8] To enter the vacuole, ions must traverse two membranes—the surface plasma membrane, and the tonoplast which surrounds the vacuole. It is suggested that it is the plasma membrane whose characteristics change so markedly with aging in excised steles, and that

transfer of chloride into the vacuole is at all times a metabolically implemented process. Thus, in relatively long periods (hours), much of the chloride absorbed is passed on to the vacuole, which explains the sensitivity of absorption to an uncoupling agent at the same time that the rate-limiting step of absorption—passage through the plasma membrane—is passive.

TABLE 2

THE EFFECT OF AN UNCOUPLER (m-Cl-CCP) ON CHLORIDE UPTAKE BY CORN ROOT TISSUES

	Absorption as Per Cent of Control				
Tissue	1.4	5.0	20	50	100 mM KCl
Fresh stele	14	...	35	42	40
Aged stele	3	...	19	32	43
Fresh cortex	...	4	12	...	35

Carbonyl cyanide m-chlorophenylhydrazone (m-Cl-CCP), 10^{-5} *M*. Absorption 3 hr at 25°C, pH 7.0. Sections washed 15 min in ice water before counting.

The foregoing presumption was tested by examining the sensitivity of chloride uptake by fresh steles to m-Cl-CCP in a short period (20 min), in which time it might be expected that absorption would be more extensively into the cytoplasm than into the vacuole. Figure 6 reveals that short-term chloride absorption by fresh steles is largely insensitive to m-Cl-CCP, and that the isotherm for such short-term absorption is exponential. The modicum of sensitivity noted is ascribed to the fact that there is some vacuolar absorption at all times. In this view the absorption step which imparts the peculiar isotherm to the uptake of chloride by excised fresh steles involves the diffusive permeation of the plasma membrane.

Discussion.—There are at least two conditions which must be met if salts are to be delivered to the xylem through the symplasm of the root and be found there at concentrations in excess of the external medium. On the one hand, accumulation in the thermodynamic sense must occur in the *cytoplasm* of the epidermal and cortical cells which act as gathering and concentrating agents for the salts which ultimately appear in the vessels. On the other hand, salts must leak from the symplasm into the xylem somewhere within the stele. Since the cell vacuoles do not enter into this picture of salt transport across the root, it must be the plasma membrane which in the intact root changes its characteristics with cell location, being markedly differentially permeable in the cells of the epidermis and cortex, and being in effect porous to ions somewhere inside of the endodermal cylinder.

Evidence presented above indicates that cells within the stele are indeed normally leaky. Not only is the loss of salts from freshly excised steles well in excess of that from the cortex, but the absorption isotherm for chloride in fresh steles, being exponential in form, bespeaks diffusive entry of chloride, while the hyperbolic isotherm typifying absorption by the cortex strongly implies carrier-mediated transport. If the significance of the exponential isotherm should be that imputed to it, the characterization of the absorption isotherm under selected conditions should provide a sensitive means to investigate the permeability characteristics of cell membranes. Actual measurements of plasma membrane potentials in higher plant cells in relation to the ionic composition of the external solution have borne out the presumptions underlying the theoretical basis for the exponential isotherm.[22]

When excised steles are aged, not only is their leakiness diminished, but the anomalous isotherm typical of fresh steles is transformed to a conventional hyperbolic isotherm. A carrier-mediated transport of chloride apparently assumes kinetic control in lieu of a diffusion-mediated process. In fresh steles it must be the plasma membrane which is relatively porous and which is responsible for the exponential isotherm. For one thing, the chloride content of untreated fresh and

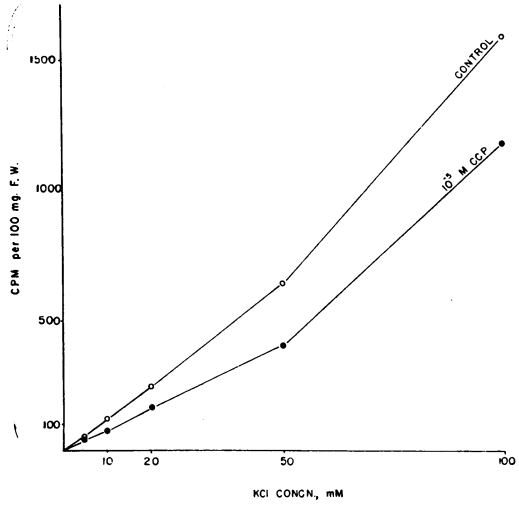

FIG. 6.—The influence of the uncoupler m-Cl-CCP on short-term chloride absorption by freshly isolated steles. Carbonyl cyanide m-chlorophenylhydrazone (m-Cl-CCP), 10^{-5} *M*. K phosphate, 5 mM, pH 7.1, $CaSO_4$, 0.5 mM. Absorption period, 20 min, room temperature. 15-sec wash in ice water preceding determination of tissue radioactivity.

aged steles is essentially the same—2.5–3.2 μeq/gm fresh weight—indicating there is little or no loss of chloride from the vacuole in 24 hr. Secondly, an absorption step which is uncoupler-insensitive and an exponential function of concentration can in effect be isolated by shortening the experimental period to the point where the uncoupler-sensitive vacuolar absorption is comparatively inconsequential (Fig. 6). Since the plasma membrane and tonoplast are in series with respect to the passage of ions into the vacuole, the stage in absorption which can be accentuated by shortening the time must relate to the plasma membrane.

If the plasma membrane were to become considerably more permeable with aging, it is conceivable that ion passage through the tonoplast would then become rate-limiting, and that the hyperbolic isotherm in aged steles would reflect carrier-mediated transport across the tonoplast. The evidence favors the opposite view, namely, that the plasma membrane becomes less permeable, and that the change in isotherm characteristics reflects the change in the plasma membrane. Aged steles are less, and not more, leaky than fresh steles. The time course of salt loss from fresh steles preincubated in KCl (Fig. 5), as well as the constancy of chloride concentration in untreated steles with time, indicate that leakage occurs from the cytoplasm and not from the vacuole. Thus a decrease in leakiness on aging indicates it is the plasma membrane whose permeability is diminished (see ref. 26).

The transformation in plasma membrane characteristics which takes place with time in excised steles must involve two changes. First, the permeability to freely diffusing ions is sharply diminished. Secondly, an ion carrier system arises or is activated, as indicated by the hyperbolic isotherm for chloride absorption. To fulfill the needs of the hypothesis as initially presented by Crafts and Broyer and as set out in the beginning of this section, the carrier for chloride transport into the cytoplasm must potentially be a pump, so that the concentration in the cytoplasm may exceed that in the external solution. The twentyfold enhancement of chloride uptake by steles on aging represents an increase in carrier-mediated transport. In aged steles virtually all transport is uncoupler-sensitive at low to moderate external salt concentrations. At unduly high concentrations uncoupler sensitivity decreases in all types of tissue (Table 2), suggesting some metabolism-independent entry. Nonmetabolic entry may explain the exceptionally high absorption rates in fresh stele at unnaturally high external concentrations (Fig. 3).

The cortex at all times displays uncoupler-sensitive absorption with a hyperbolic isotherm. Tissue characteristics therefore fit the requirements proposed by Crafts

and Broyer in which peripheral tissue of the root must be capable of active salt uptake, while tissue within the stele must be leaky. The endodermis need not be an absolute barrier to the back diffusion of salts from the vessels, through the free space, to the external solution. It need only be a kinetic barrier, slowing up back diffusion considerably. In fact there are gaps in the endodermis, and the endodermis is not fully formed for at least several millimeters behind the root tip.[2, 12]

Considering the fineness of the primary root of corn seedlings, the loose packing of cortical cells, and the moderate root respiration rate, it seems highly unlikely that an oxygen deficiency exists even in the vascular cylinder. In denser and thicker potato slices, neither the oxygen nor carbon dioxide tension is responsible for the influence of thickness on the physiological characteristics of the tissue. Nevertheless, a volatile metabolic product is implicated.[14] In any event, the influence of thickness on potato slices has been shown to be on the development of respiratory capacity—both with respect to magnitude and quality—and not on the control of respiration rate *per se*. The physiological transformation in excised steles resembles that in potato slices in respect to the time requirement, temperature sensitivity, dependence on metabolism, and loss of leakiness. As with potato slices, chloral, an analogue of acetaldehyde, prevents the transformation.[16] Preliminary experiments have indicated that the changes in permeability and absorption characteristics which attend aging in excised steles are accompanied by a profound alteration of the respiratory metabolism.

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