References and Notes

1. S. H. Socher and B. W. O'Malley, *Dev. Biol.* **30**, 411 (1973).
2. R. W. Turkington, *Developmental Aspects of the Cell Cycle*, I. L. Cameron, G. M. Padilla, A. M. Zimmerman, Eds. (Academic Press, New York, 1971), p. 315; I. S. Owens, B. K. Vonderhaar, Y. J. Topper, *J. Biol. Chem.* **248**, 472 (1972).
3. H. S. Grob, *Biol. Reprod.* **5**, 207 (1971).
4. K. L. Barker and J. M. Anderson, *Endocrinology* **83**, 585 (1968); R. H. Maurer and R. G. Chalkley, *J. Mol. Biol.* **27**, 431 (1967); G. Shyamala and J. Gorski, *J. Cell Biol.* **35**, 125A (1967); E. J. Sitko, thesis, University of Colorado (1969); T. W. Glenister, in *Methods in Mammalian Embryology*, J. C. Daniel, Jr., Ed. (W. H. Freeman, San Francisco, 1971), p. 320; D. Williams and J. Gorski, *Biochemistry* **12**, 297 (1973).
5. H. Kuramoto, S. Tamura, Y. Notake, *Am. J. Obstet. Gynecol.* **114**, 1012 (1973).
6. E. I. Kohorn and R. Tchao, *J. Endocrinol.* **45**, 401 (1969).
7. R. S. Krishnan and J. C. Daniel, Jr., *Science* **158**, 490 (1967).
8. J. C. Daniel, Jr., and R. S. Krishnan, *J. Exp. Zool.* **172**, 267 (1969).
9. A. A. El-Banna and J. C. Daniel, Jr., *Fertil. Steril.* **23**, 101 (1972).
10. M. A. Urzua, R. Stambaugh, G. Flickinger, L. Mastroianni, *ibid.* **21**, 860 (1970).
11. F. A. Murray, R. W. McGaughey, M. J. Yarus, *ibid.* **23**, 69 (1972).
12. M. H. Johnson, B. D. Cowan, J. C. Daniel, Jr., *ibid.*, p. 93.
13. C. Kirchner, *ibid.*, p. 131.

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The Casparian Strip as a Barrier to the Movement of Lanthanum in Corn Roots

Abstract. *The effectiveness of the Casparian strip as a barrier to apoplastic movement of solutes from cortex to stele of corn roots was investigated by using lanthanum in combination with electron microscopy. Lanthanum deposits were found only in cell walls and on the outside of the plasma membrane of epidermal, cortical, and endodermal cells up to the Casparian strip. Lanthanum was completely absent from the stele, indicating that the Casparian strip provides an effective barrier to apoplastic movement of solutes. Inhibitory effects of trivalent lanthanum ions on the absorption of potassium ions are discussed in relation to the nature of the lanthanum ion binding site on membranes.*

The endodermis of roots separates the cortex from the vascular tissue (stele). Endodermal cells have a chemically distinct band of hydrophobic wall material known as the Casparian strip which is presumed to act as a barrier to the diffusion of solutes through cell walls from cortex to stele. Electron microscopic studies of the uptake of uranyl ions by barley roots (*1*) and of the distribution of lead-ethylenediaminetetraacetic acid in wheat and carrot roots (*2*) provide evidence for this contention.

The cation La^{3+} does not penetrate cell membranes and can be visualized with the electron microscope. Because of these features, La^{3+} has been used to define extracellular channels in animals (*3*) and the cell wall continuum (apoplast) in plants (*4*). We report here the use of La^{3+} to examine the apoplast of corn roots with particular reference to the function of the Casparian strip.

Primary roots of 4-day-old corn seedlings were incubated in a solution containing La^{3+} (*p*H 5.7) and prepared for electron microscopy (*5*). Fixation at *p*H 7.3 or above resulted in localized deposition of La in cell walls and along the outer leaflet of the plasma membrane of epidermal (not shown) and cortical cells (Fig. 1, A and B). As expected, La^{3+} did not cross the plasma membrane into cells (Fig. 1, A–C).

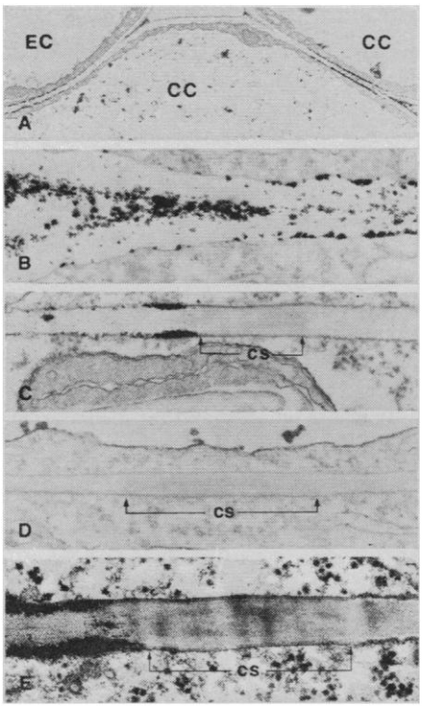

Fig. 1. (A) Lanthanum deposition between two cortical cells (*CC*) and between a cortical cell and an endodermal cell (*EC*) (unstained, × 41,000). (B) High magnification of lanthanum deposition between two cortical cells (unstained, × 27,500). (C) A portion of a radial wall between two endodermal cells showing lanthanum deposition up to the Casparian strip (*CS*). The Casparian strip region is the dark area of the wall designated by the arrows (unstained, × 33,500). (D) Untreated control of the Casparian strip (between arrows) (unstained, × 27,500). (E) Stained preparation showing lanthanum deposition up to the Casparian strip (between arrows) (× 69,000).

Lanthanum was also found deposited along the cortical side of endodermal cells up to the Casparian strip (Fig. 1, C and E). The stelar side of the Casparian strip and the cells in the stele were completely free of La deposits, as were those of untreated control tissue (Fig. 1, C and D).

High magnification of the cell wall region between two endodermal cells (Fig. 1, C and E) showed that La was deposited in the cell wall and along the plasma membrane up to the Casparian strip (dark area of cell wall). Plasma membrane associated with the Casparian strip had a tripartite structure and formed a smooth, tight junction with the wall material (Fig. 1E) (*6*).

Our results indicate that the Casparian strip of corn roots provide a barrier to the diffusion of La^{3+} (and presumably other solutes) in the apoplast from the cortex to the stele. Solutes entering vascular tissue of roots possessing an intact endodermis must do so by first being absorbed into epidermal, cortical, or endodermal cells and then moving through the cytoplasmic continuum (symplast) to the stele.

The association of lanthanum with the outside of the plasma membrane (Fig. 1, A and B) (*3*, *4*) suggests that La^{3+} binds to some site on the membrane. It has been proposed that La^{3+} binds to Ca^{2+} sites on membranes because of correlations between Ca^{2+} exchange induced by La^{3+} and several physiological effects (*7*). Further, in atomic absorption spectroscopy La^{3+} is routinely used to displace Ca^{2+} from complexes with phosphate (*8*). Hence, La^{3+} is believed to bind to, and (or) displace Ca^{2+} from, membrane sites normally occupied by Ca^{2+}.

In corn roots, Ca^{2+} (0.5 m*M* or less) has been shown to be an inhibitor of

K^+ absorption (*9*). We measured the rate of K^+ absorption by corn roots (*10*) at several concentrations of La^{3+} and found an inhibition similar to that reported for Ca^{2+} (for example, at 0, 0.05, 0.1, 0.25, and 0.5 m*M* $La(NO_3)_3$, K^+ absorption was 2.43, 1.81, 1.44, 1.31, and 1.26 μmole per gram fresh weight per hour, respectively). In the presence of Ca^{2+}, K^+ absorption was inhibited (0.25 m*M* Ca^{2+}; 1.03 μmole per gram fresh weight per hour) and addition of La^{3+} had no further inhibitory effect. These results are consistent with the proposal that the sites of La^{3+} deposition on membranes are, at least in part, sites which also bind Ca^{2+}.

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References and Notes

1. A. W. Robards and M. E. Robb, *Science* **178**, 980 (1972).
2. T. W. Tanton and S. H. Crowdy, *J. Exp. Bot.* **23**, 600 (1972).
3. J. P. Revel and M. J. Karnovsky, *J. Cell Biol.* **33**, c7 (1967); R. Ritch and C. W. Philpott, *Exp. Cell Res.* **55**, 17 (19(→ T. M. Forte and J. G. Forte, *J. Cell Biol.* **47**, 782 (19' → P. R. Garant, *J. Ultrastruct. Res.* **40**, 333 (19' → T. E. Machen, D. Erlij, F. B. P. Wooding, *J. Cell Biol.* **54**, 302 (1972).
4. B. E. S. Gunning and J. S. Pate, *Protoplasma* **68**, 107 (1969); W. W. Thomson, K. A. Platt, N. Campbell, *Cytobios*, in press; N. Campbell, W. W. Thomson, K. Platt, *J. Exp. Bot.*, in press.
5. Corn seedlings (*Zea mays* L., WF 9 X M 14) were obtained by allowing seeds to germinate on paper towels saturated with 0.1 m*M* $CaCl_2$ at 26°C for 4 days in the dark [R. T. Leonard and J. B. Hanson, *Plant Physiol.* **49**, 430 (1972)]. The primary roots were excised and rinsed three times in cold distilled water. Roots were incubated for 1 hour with aeration at 30°C in 0.25 m*M* KCl plus 1 percent $La(NO_3)_3$ such that the "opened" end of the excised root did not come into contact with the incubation medium. After incubation, the apical 2 cm of the root was excised and fixed for 1.5 hours in 2.5 percent glutaraldehyde in 0.1*M* KH_2PO_4 adjusted to *p*H 7.5 with NaOH. The tissue was then fixed in 2 percent OsO_4 for 1 hour, and then dehydrated in a graded acetone series before being embedded in Spurr's epoxy plas → [A. R. Spurr, *J. Ultrastruct. Res.* **26**, 31 (1969)]. Thin sections were cut 1.5 cm from the root apex on a Porter-Blum MT-2 microtome and were either stained in 1 percent aqueous uranyl acetate and lead citr → [E. S. Reynolds, *J. Cell Biol.* **17**, 208 (1963)] or viewed unstained at 60 kv with a Philips 300 electron microscope.
6. H. T. Bonnett, Jr. [*J. Cell Biol.* **37**, 199 (1968)] plasmolyzed roots to demonstrate that the Casparian strip forms a tight junction with the plasma membrane.
7. J. Y. Lettvin, W. F. Pickard, W. S. McCulloch, W. Pitts, *Nature (Lond.)* **202**, 1338 (1964); C. F. Doggenweiler and S. Frenk, *Proc. Natl. Acad. Sci. U.S.A.* **53**, 425 (1965); M. Takata, W. F. Pickard, J. Y. Lettvin, J. W. Moore, *J. Gen. Physiol.* **50**, 461 (1966); G. B. Weiss, *J. Pharmacol. Exp. Ther.* **174**, 517 (1970); W. G. Sanborn and G. A. Langer, *J. Gen. Physiol.* **56**, 191 (19' → G. A. Langer and J. S. Frank, *J. Cell Biol.* **54**, 441 (1972).
8. J. B. Willis, *Anal. Chem.* **33**, 556 (1961).
9. O. E. Elzam and T. K. Hodges, *Plant Physiol.* **42**, 1483 (1967).
10. Primary roots were excised, rinsed three times in cold distilled water, and cut into 2-cm sections. Batches of 16 sections (a random selection of 4 apical and 12 basal, about 250 mg fresh weight) were incubated with aeration at 30°C in 35 ml of 0.25 m*M* KCl labeled with ^{86}Rb. After 10 minutes, the tissue was removed from the solution, rinsed with 25 ml of 1 m*M* KCl plus 0.25 m*M* $CaCl_2$, and then placed for 5 minutes in the same solution for exchange at 22°C. Lanthanum nitrate and $CaCl_2$ were added as indicated in the text. Blotted and weighed sections were counted in scintillation fluid [G. S. Bruno and J. E. Christian, *Anal. Chem.* **33**, 1216 (1961)] containing a thixotrophic gel.
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Diphenylhydantoin: Effects on Calcium Metabolism in the Chick

Abstract. *Rickets, hypocalcemia, decreased duodenal calcium transport, and reduction of calcium binding protein have been produced in chicks treated with diphenylhydantoin. These effects are directly related to diphenylhydantoin dose and inversely related to the intake of vitamin D_3 (cholecalciferol).*

Hypocalcemia, rickets, and osteomalacia develop with variable frequency in patients on long-term anticonvulsant therapy (*1*). Both phenobarbital (PB) and diphenylhydantoin (DPH) have been implicated; several reports suggest that these agents, by altering calciferol metabolism, may produce a state of functional vitamin D deficiency. Our studies in chicks demonstrate that DPH causes a decrease in intestinal calcium absorption, intestinal calcium binding protein (CaBP), serum calcium, and bone ash. Radiographic and histologic evidence of rickets was also seen. These effects were directly related to the DPH dose and inversely related to the amount of cholecalciferol, that is, vitamin D_3 (D_3), given concomitantly.

Cholecalciferol is hydroxylated in the liver to 25-dihydroxycholecalciferol [25-(OH)D_3] and in the kidney to 1,25-$(OH)_2$-dihydroxycholecalciferol [1,25-$(OH)_2D_3$] (*2*). Each step increases polarity and biological activity. The same sequence obtains for ergocalciferol (D_2), which is the form commonly used for dietary supplementation. Hahn *et al.* (*3*) found decreased serum concentrations of 25-(OH)D_3 in patients receiving either PB or DPH. Concentrations of 25-(OH)D_3 correlated directly with serum calcium and with calciferol intake. Hahn *et al.* (*4*) also reported that the half-life of D_3 in the plasma was shortened in subjects receiving PB and described increased conversion of D_3 and 25-(OH)D_3 to more polar metabolites by hepatic microsomes from PB-treated rats.

Tolman *et al.* (*5*) reported accelerated plasma disappearance rates and increased urinary excretion of both D_3 and 25-(OH)D_3 in patients who received either PB or DPH. In contrast, Schaefer *et al.* (*6*) and Silver *et al.* (*7*) found increased serum 25-(OH)D_3 under similar circumstances. Healing of bone lesions has been reported after administration of large doses of D_3 (*1*) or small doses of 25-(OH)D_3 (*8*). These findings have led to the view that anticonvulsants act as calciferol antagonists by inducing liver enzymes that convert D_3 to inactive or rapidly excreted metabolites (or both).

In the rat, both Koch *et al.* (*9*) and Caspary (*10*) found that absorption of calcium was depressed by DPH and PB. Neither found any significant change in intestinal calcium binding activity, and concluded that the synthesis of the CaBP was not affected. Since CaBP has an absolute dependence on vitamin D (or metabolite or vitamin D active steroid) for its synthesis and maintenance (*11*), the negative findings of Koch and Caspary would have important implications with respect to the pathogenesis of osteomalacia and rickets accompanying anticonvulsant therapy, if verified. Also, Clark *et al.* (*12*) saw no radiographic evidence of rickets in DPH-treated rats.

White Leghorn cockerels (1 day old) were offered a calciferol-free but otherwise adequate diet (*13*). After 5 days they were divided into seven groups whose D_3 and DPH intakes are given in Table 1. Crystalline D_3 was dissolved in sesame oil to give 3 or 6 international units (I.U.) per 0.1 ml, which was administered orally. Group 1 received sesame oil alone. The DPH (diphenylhydantoin, Eastman) was added to the diet to give 1 or 2.5 g per kilogram of diet, and DPH intake was calculated from weekly averages of food consumption and body weight (*W*). The body weight was converted to surface area