whereas compliance due to tissue forces was not significantly different ($P > .05$). The calculated C_{surf} was very significantly lower, 37 percent ($P < .01$), in infected rats. Within control and infected groups, the inflation and deflation limbs of the air *P-V* curves had similar slopes. This indicates that opening pressure was not the explanation of the lower compliance.

Our evidence indicates that altered pulmonary function in rat lungs infected with *M. pulmonis* is due predominantly to an increase in surface forces (*13*), implying disruption of the surfactant system.

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References and Notes

1. E. M. Scarpelli, *The Surfactant System of the Lung* (Lea and Febiger, Philadelphia, 1968).
2. Since mycoplasmas are indigenous in most rat colonies, we purchased gnotobiotic rodents (CD-R stock males, Charles River) or we used CD-F derived rats which had been raised under gnotobiotic conditions at Ohio State. Rats were then housed individually in disposable plastic cages (Maryland Plastics) and raised in a conventional manner. Cages were covered with a filter frame and fiberglass-plastic filter (Isocap, Carworth). Protection against airborne cross-contamination was effective, since we could not culture mycoplasmas from throat swabs taken from animals before inoculation or from control animals. Rats were isolated under these conditions at least 1 week before the start of experiments. All appeared to be free from previous experience with *M. pulmonis*, since, by a fluorescence test, their serums did not show antibody against this organism.
3. Innovar Injection (McNeil Lab, Fort Washington, Pa.) contains, per milliliter, 0.05 mg of fentanyl, a narcotic analgesic, and 2.5 mg droperidol, a narcoleptic agent. Rats received 0.075 to 0.1 ml, depending on their age and weight.
4. *Mycoplasma pulmonis* strain N3 had been obtained from J. Nelson of Rockefeller University. In our laboratory, it was passaged through rats to enhance its virulence, and the isolate used in these experiments had been recovered from the lungs of one of the animals. The organism was cultured and concentrated according to the method described by N. L. Somerson, W. D. James, B. E. Walls, R. M. Chanock, *Ann. N.Y. Acad. Sci.* **143**, 348 (1967). Suspensions of organisms inoculated into rats contained at least 10^{10} mycoplasma colony-forming units.
5. J. D. Pollack, N. L. Somerson, L. B. Senterfit, *J. Bacteriol.* **97**, 612 (1969). The PPLO serum fraction was used at 3 percent final concentration.
6. The time of killing was arbitrarily selected. Infected animals were killed within a period of 8 to 49 days after inoculation.
7. Minced lung tissue was inoculated into roller-tube cultures of rat embryonic kidney, WI-38, HeLa cells, and primary green monkey kidney cells. Two cultures of each type of cell were incubated at 37°C, and one at 33°C. Cell cultures were held at least 7 days, examined for cytopathic effects, and subjected to two additional passages.
8. D. L. Beckman and H. S. Weiss, *J. Appl. Physiol.* **26**, 700 (1969).
9. All cardiac tissue had been removed from the lungs. Lungs were tested for leaks by inflating them with air while submerged in saline; small leaks were repaired with Eastman 910 adhesive.
10. Buoyancy in 0.9 percent saline after the air *P-V* curves was used to estimate trapped or retained gas.
11. Antibody to *M. pulmonis* was measured in a fluorescent test described by P. Y. Ertel, I. J. Ertel, N. L. Somerson, J. D. Pollack, *Proc. Soc. Exp. Biol. Med.* **134**, 44 (1970). Mycoplasmas were isolated from the lungs of one rat who did not show antibody. The animal had been killed 8 days after inoculation, probably before this antibody was produced.
12. The lack of microscopic pulmonary lesions contrasts with the findings of A. B. Organick and I. I. Lutsky [*J. Bacteriol.* **92**, 1154 (1966); *ibid.*, p. 1166], who studied *M. pulmonis* infection in both conventional and gnotobiotic mice. They reported consolidation with a polymorphonuclear cellular response in the acute stage of infection. In some cases they showed distension of alveolar spaces and disruption of mitochondria. Possibly the rats we used were more resistant than mice to *M. pulmonis*. However, D. F. Kohn and B. E. Kirk [in *Lab. Anim. Care* **19**, 321 (1969)] reported bronchiectasis in rats given *M. pulmonis*. Perhaps our strain was not as virulent as the one they used.
13. We recognize that, theoretically, bronchoconstriction could mimic an increase in surface tension [see E. P. Radford, Jr., and N. M. Lefcoe, *Amer. J. Physiol.* **180**, 479 (1955)]. However, there is no evidence that such a phenomenon occurs in mycoplasma-infected rats.
14. Supported by NIH grant AI 08587. We thank J. Pitt, K. Knore, and F. Elner for excellent technical assistance.

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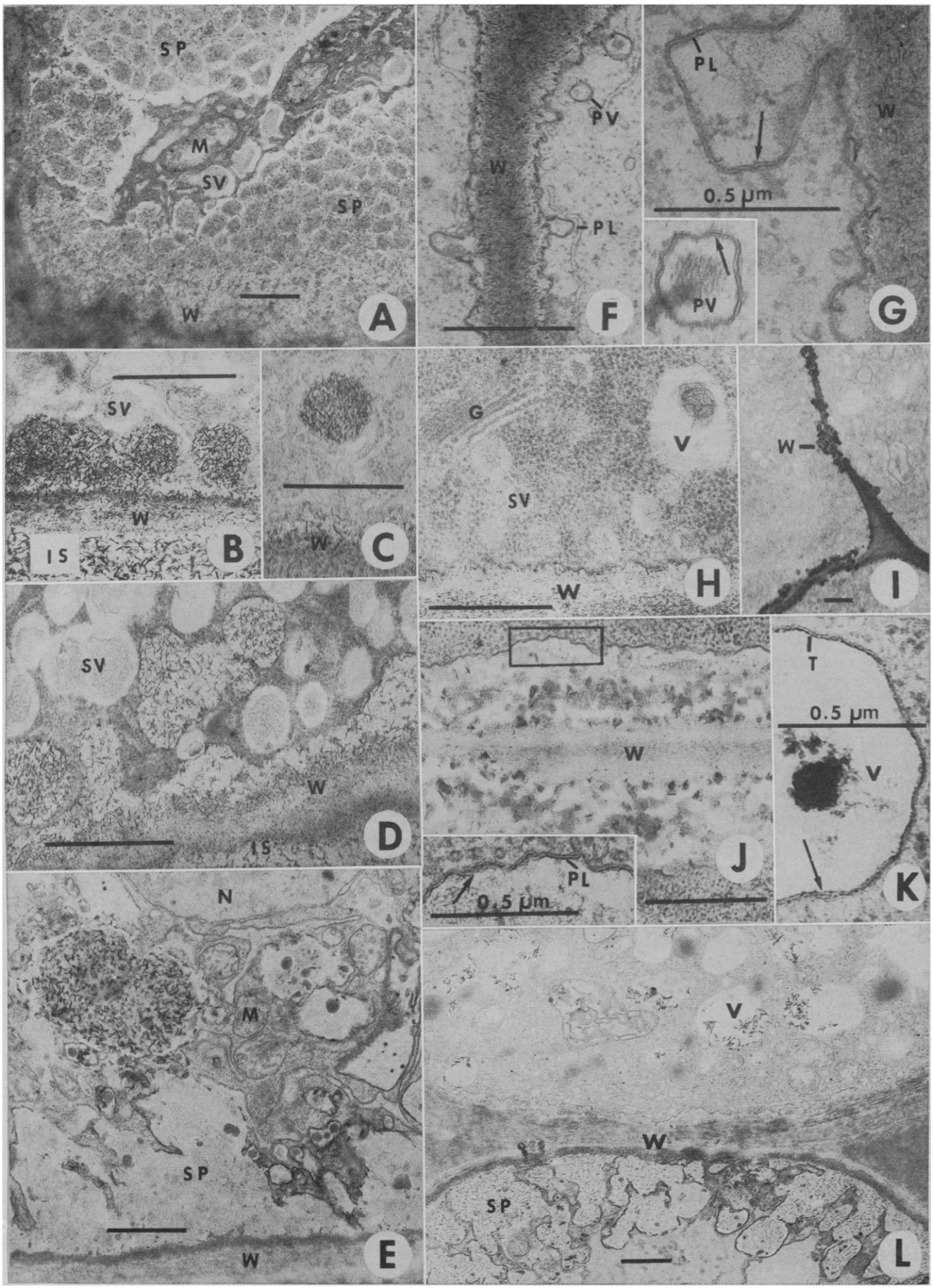
Pinocytosis and Membrane Dilation in Uranyl-Treated Plant Roots

Abstract. *Electron-dense crystals formed in plant roots exposed to uranyl acetate have been used to identify binding sites and to follow the pinocytotic uptake of uranyl in the oat rootcap. Before uranyl enters the protoplast, the plasmalemma is greatly dilated. After uranyl is sequestered in vacuoles, the tonoplast is similarly dilated.*

When oat roots were placed in solutions of uranyl acetate, then fixed in phosphate-buffered glutaraldehyde, fixed in osmium tetroxide, and stained with lead citrate (*1*), very dense, needle-shaped crystals, easily visible in electron micrographs of thin sections, were formed. The crystals also formed in material fixed only in phosphate-buffered glutaraldehyde without additional fixation or staining. In view of this and their very high electron density, we conclude that they represent some type of uranium complex. They are probably not simply uranyl phosphate crystals since they were not found when aqueous or phosphate-buffered potassium permanganate was used as a fixative or when uranyl acetate was mixed in a variety of concentrations with phosphate buffer and deposited an collodion-coated grids. We have used these crystals to follow the binding and uptake of uranyl in cells of the oat rootcap (*2*).

The number and distribution of uranyl crystals in outer secretory cells of the oat rootcap varied with the concentration of uranyl solutions used and the length of time the roots were exposed. In one test, roots were exposed to 1 m*M* uranyl acetate for 5, 10, 20, and 30 minutes, then removed and rinsed in three changes of distilled water for a total desorption time of 30 minutes. Although the number of crystals increased as exposure to uranyl increased, uranyl crystals were, in all cases, sharply localized in the cell walls, intercellular spaces, and in secretory products in direct contact with cell

Fig. 1. Localization of uranyl crystals in cells of the oat rootcap. (A–E) Portions of cells in outer secretory layers of the rootcap from roots exposed for 10 minutes (A) or 1 hour (B–D) to 1 m*M* uranyl acetate. Secretory vesicles (*sv*) are common and in (A) secretory products have accumulated between the plasmalemma (*pl*) and the cell wall. Uranyl crystals are abundant in cell walls (*w*), intercellular spaces (*is*), and secretory products (*sp*). (E) Outer secretory cell exposed to 0.1 m*M* uranyl acetate for 20 hours. Note the normal appearance of the nucleus (*n*) and mitochondria (*m*). (F–I) Portions of normally nonsecretory cells in the cap interior of oat roots exposed to 1 m*M* uranyl acetate for 1 (F and G), 2 (H), or 6 (I) hours. Note invaginations of the plasmalemma (*pl*) and presence of pinocytotic vesicles (*pv*) in (F) and transitions in membrane structure (arrows) in (G). In (H), the vacuole (*v*) contains a dense inclusion, many secretory vesicles (*sv*) are present, and Golgi dictyosomes (*g*) are active. (I) Massive deposits of uranyl crystals in walls of cells just beneath the outer secretory layers. (J–L) Portions of interior cap cells from oat roots exposed to 0.1 m*M* uranyl acetate for 10 (J and K) or 20 (L) hours. (J and K) Swollen cell wall (J) and part of vacuole with a dense deposit (K) in interior root cap cells. Arrows (K and enlarged area of J) indicate points of apparent membrane overlap in tonoplast (*t*) and plasmalemma (*pl*). (L) Cell below with uranyl crystals localized in secretory products (*sp*) is in the outer secretory region of the rootcap. Contrast with the normally nonsecretory cell above with crystals in vacuoles (*v*). All roots were desorbed after exposure to uranyl for 30 minutes in distilled water, except for that in (D) which was desorbed 2 hours. Unless otherwise indicated, scale equals 1 μm.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walls. Except for an unusually massive accumulation of secretory products along the cell wall, Fig. 1A is typical of the results of this test. To check the possibility that changes in the localization of uranyl occurred during desorption, the test was repeated with desorption time reduced to 30 seconds. Results were entirely similar except that, in addition to crystals, cell walls and intercellular spaces contained dense, noncrystalline deposits.

More massive depositions of crystals were found in secretory cells of roots exposed for 1 hour to 1 m*M* uranyl. Most crystals were confined to the wall and structures in direct contact with it (Fig. 1B) but some packets of crystals were within the protoplast (Fig. 1C). To determine how firmly uranyl was bound, desorption time was increased to 2 hours. Although reduced in number, many crystals were still present and more packets were free in the cytoplasm (Fig. 1D). Since exposures of 2 hours or more to 1 m*M* uranyl severely disrupted many exterior cells, roots were treated for 10 and 20 hours in 0.1 m*M* solutions. After 10 hours, the distribution of uranyl crystals was similar to that shown in Fig. 1D. After 20 hours, crystals were concentrated inside the protoplast in distinct packets (Fig. 1E) which, by serial sections, were shown to be surrounded by cytoplasm.

In general, results with secretory outer rootcap cells indicated rapid initial binding of UO_2^{2+} in cell walls, in secretory products in contact with the walls, and in intercellular slime. Later, masses of secretory products which contained uranyl crystals appeared to separate from the wall and migrate into the protoplast pinocytotically (*3*).

In roots exposed to 1 m*M* uranyl for 30 minutes or less a few crystals were found in the walls of nonsecretory cells immediately beneath the secretory region. When exposure was increased to 1 hour, many uranyl crystals were present in cell walls in this area (six to eight layers of cells beneath the root surface). The surface contours of these cells were extremely irregular with numerous invaginations of the plasmalemma (Fig. 1, F and G). Vesicles which appeared to have arisen pinocytotically from invaginations of the plasmalemma were common (Fig. 1F) and some were shown by serial sections to be free in the cytoplasm. Otherwise the cells were normal with no evidence of secretory activity and with no uranyl crystals other than those in the cell walls.

Cells in the interior of rootcaps exposed to 1 m*M* uranyl for 2 hours had hypersecretory dictyosomes, large numbers of secretory vesicles, and dense amorphous or membranous structures in vacuoles (Fig. 1H). After exposures for 4 to 6 hours, cell walls in the area just beneath the secretory layers were packed with uranyl and had many wartlike protrusions (Fig. 1I).

Cell walls in the interior of roots exposed to 0.1 m*M* uranyl for 10 hours were swollen and contained, in addition to uranyl crystals, a variety of membranous, vesicular, and tubular elements (Fig. 1J). Uranyl crystals were found in some vacuoles but most vacuoles contained dense, noncrystalline material (Fig. 1K). Results were similar in roots exposed to 0.1 m*M* uranyl for 20 hours except that fewer crystals were present in cell walls and more were found in vacuoles. Also, the difference in response of secretory outer cells of the rootcap and those in the interior was clearly evident. Secretory cells with uranyl crystals confined to secretory products were often adjacent to cells with few or no secretory products and in which uranyl crystals were concentrated in vacuoles (Fig. 1L).

In cells of the rootcap interior, uranyl is bound initially in cell walls. If exposure to 1 m*M* uranyl is extended beyond 1 hour, secretory activity is induced and dense material (which may contain uranyl) appears in vacuoles. With prolonged exposure to 1 m*M* uranyl, the cell walls become overloaded. Long exposures to tenfold less uranyl allow the cells to respond and to sequester uranyl in vacuoles. How uranyl is transferred from walls to vacuoles is uncertain, but pinocytotic vesicles and Golgi secretory products could serve as vehicles.

In higher plants the plasmalemma appears as two dark lines separated by an electron-lucid area with an overall width of 70 to 100 Å (*4*). In untreated oat roots, the plasmalemma averages 90 Å. In roots exposed to 1 m*M* uranyl for 1 hour, the plasmalemma of cells in the root interior was greatly dilated; it ranged in overall width from 150 to 200 Å (Fig. 1G). Cells with dilated plasmalemmas also contained vesicles with dilated membranes (Fig. 1F), some of which were shown by serial sections to be free in the cytoplasm. In the invaginations of the plasmalemma there were abrupt transitions from a normal tripartite structure to one with two dense outer lines, two adjacent clear lines, and a dense center line about twice as wide as the outer two (Fig. 1G, arrow). Similar transitions in structure were found in dilated vesicular membranes (Fig. 1G, inset). Membranes other than the plasmalemma and vesicles apparently derived from it showed no changes in roots treated with 1 m*M* uranyl for 1 hour.

The plasmalemma was also dilated in roots exposed to 0.1 m*M* uranyl for 10 to 20 hours (Fig. 1J, inset). It was also often asymmetric with the dark line next to the cell wall more densely stained than the one adjacent to the cytoplasm. Clear transitions in structure were not found, but in many areas two or more overlapping layers were present (Fig. 1J, arrow). Similar dilation with more pronounced asymmetry and areas of membrane overlap was characteristic of tonoplasts surrounding vacuoles which contained uranyl crystals or dense, noncrystalline deposits (Fig. 1K).

Although we have demonstrated uranyl crystals inside protoplasts, we believe our results support rather than contradict Rothstein's (*5*) conclusion that uranyl ions as such cannot readily penetrate to the interior of intact, isolated cells. After short exposures to uranyl, crystals were found only in cell walls and in secretory products in direct contact with walls. Even after prolonged exposures, mitochondria and nuclei adjacent to packets of uranyl crystals appeared normal (Fig. 1E). This suggests that few, if any, free uranyl ions move into or through the protoplast. Failure to find dilation of any membrane other than the plasmalemma in roots exposed to uranyl for 1 hour supports the same suggestion. On the other hand our results do not support the conclusion that the initial binding sites for uranyl ions are restricted to the outer surface of the plasmalemma. Instead, large quantities of uranyl are bound in the cell wall and in secretory products along its surface. Slime secreted by the rootcap may function as a lubricant to facilitate growth of the root through soil (*6*). The ability of this material to bind uranyl suggests that secretory products may also serve to protect the root from influxes of toxic cations.

Evidence for pinocytosis in higher plants has been, except for work with isolated protoplasts (*7*), circumstantial (*4, 8*). Although many electron micrographs suggest that pinocytosis occurs in plant cells, lack of markers by which the process could be followed has precluded firm conclusions. Crystals and

changes in membrane structure in uranyl-treated roots provide such markers. Because uranyl is toxic, our results do not establish pinocytosis as a normal mechanism of intake of material by plants. However, the best evidence of pinocytosis was with exposures to 1 m*M* uranyl for 1 hour (Fig. 1, F and G) or to 0.1 m*M* for 20 hours (Fig. 1E). Roots were not killed by these treatments; they grew at 30 to 50 percent of the normal rate when transferred to water.

In animals, salts and proteins induce pinocytosis (*8*), and this may account for active uptake of soluble macromolecular substances (*9*). In plants, a role for pinocytosis in active ion uptake has been postulated (*10*), and an increase in numbers of pinocytotic vesicles in roots exposed to salts has been reported (*11*). Chemically characterized plant-root secretions include acidic polysaccharides and protein-polysaccharide complexes of high viscosity (*12*). Such anionic polymers would not only bind UO_2^{2+} and other cations but also could account for cell wall swelling and invaginations of the plasmalemma. Released pinocytotically they could transport bound uranyl to vacuoles. Freed of uranyl, the carriers could be reused in a cyclic system of uptake. The driving force for such a process could be the creation of complex macromolecules (*13*). In view of evidence that uranyl and calcium ions bind to similar sites (*5*), our results may represent an exaggerated and abnormal picture of a transport system which functions less conspicuously in normal plants.

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References and Notes

1. Intact germinated oat (*Avena sativa* L.) seedlings with roots 5 to 7 mm long were placed for various periods of time in solutions of uranyl acetate. After removal and desorption in three changes of distilled water, the apical 3 mm of the root was cut off and immediately fixed at 23° to 25°C in 6 percent glutaraldehyde buffered with 0.03*M* potassium phosphate (*p*H 7.4) for 1 hour, then washed twice in buffer and fixed in 2 percent osmium tetroxide in the same buffer for 2 hours. After dehydration in a graded ethanol series, the root tips were embedded in Epo→[J. H. Luft, *J. Biophys. Biochem. Cytol.* **9**, 409 (1961)]. Thin sections were mounted on noncoated copper grids and stained for 5 minutes in 2 percent lead citrate [E. S. Reynolds, *J. Cell Biol.* **18**, 208 (1963)]. The magnification standard was a 28,000-line diffraction-grating replica. At least two and in most cases three or more roots of each of two oat cultivars, Compact (CI 8280) and a Victorgrain mutant (CI 7418), were examined for each treatment, and the illustrations are typical of results with these two varieties. Preliminary work with bean, barley, maize, and wheat roots has yielded results similar to those with oats, but are not extensive enough to justify conclusions about possible differences among species. The procedure for germinating oat seedlings has been published [H. H. Luke and H. Wheeler, *Phytopathology* **45**, 453 (1955)].
2. The ultrastructure of the cap region of untreated oat roots has been described (*14*) and is very similar to that of maize and wheat (*15*). The outer three to five layers of cells of the oat rootcap exhibit high secretory activity and, in this area, dictyosomes are hypersecretory, secretory vesicles are common in the cytoplasm, and intercellular spaces are filled with slime. Occasionally, accumulations of secretory products are found between the cell wall and plasmalemma in secretory cells of untreated oat roots. When treated with uranyl acetate, outer rootcap secretory cells undergo changes quite different from those in cells in the cap interior which normally do not exhibit secretory activity. Because of this, the effects of UO_2^{2+} on cells in these two regions will be discussed in separate sections. The rootcap was chosen for this study partly because its ultrastructural features were well known and partly because it represents a mass of cells isolated from the vascular system of the plant. We emphasize that the course of events found in the rootcap may not apply to other regions of the root. In preliminary work, crystals have been found localized in vacuoles in the root hair region of roots exposed to uranyl for as little as 10 minutes. The fragility of root hairs and their tendency to rupture when placed in hypotonic solutions preclude any conclusion as to how such localization occurred.
3. We will use pinocytosis as a general term for the intake of water or solids by invagination of the plasmalemma as defined by F. A. L. Clowes and B. E. Juniper [*Plant Cells* (Blackwell, Oxford, 1968), p. 490].
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16. Kentucky Agricultural Experiment Station Journal Series Paper No. 70-11-114. Supported in part by PHS research grant ES 00319. We thank S. Maggard and R. Wells for technical assistance, and our colleagues S. Diachun, J. W. Hendrix, A. J. Hiatt, and J. E. Leggett for helpful discussions.

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Developmental Variation in the Isoenzymes of Human Liver and Gastric Alcohol Dehydrogenase

Abstract. *The isoenzyme patterns of alcohol dehydrogenase from human liver and stomach are different in the fetal, newborn, childhood, and adult periods of life. This provides additional evidence that the fetal and adult alcohol dehydrogenases are qualitatively different. Gastric and hepatic isoenzyme patterns also differed from each other.*

Our understanding of the mechanism of differentiation may be advanced by studies which use specific developmental markers such as proteins that are present in the wild type of a species during a restricted period of development. Developmental markers already known in man include embryonic and fetal hemoglobins (*1*), the lactate dehydrogenases (*2*), the esterases (*3*), and creatine phosphokinases (*4*).

The activity of liver alcohol dehydrogenase (LADH) increases with fetal development in rats, guinea pigs, and man (*5*, *6*). Values of the Michaelis constant (K_m) and optimum values of *p*H differ between the human fetal and adult forms of this enzyme (*6*), and developmental variation in the isoenzymes of human LADH has also been reported (*7*). This study shows that the developmental variation of human LADH and human alcohol dehydrogenase from gastric mucosa (GADH) continues up to puberty.

Tissues were obtained 12 to 36 hours after death. Specimens from spontaneously aborted fetuses were obtained 12 to 24 hours after passage. An occasional fetal specimen was obtained by hysterotomy. All fetuses used had no visible external or internal malformations and tissues were analyzed immediately or stored at −20° to −25°C.

Tissues were homogenized at 0° to 4°C. The homogenate was diluted 1 : 5 with buffer prior to enzyme assay (*8*) and was applied directly to agarose gel for electrophoresis. Agar and agarose gel electrophoresis was performed according to a modification of the method described by Ursprung (*9*), and protein concentration was measured by a micromodification of the Lowry method (*10*).