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Penetration of Ions through Isolated Cuticles [1, 2, 3]

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Absorption of mineral nutrients by leaves of plants was demonstrated experimentally over 100 years ago (16). Since then numerous workers have studied the uptake of ions by aerial plant parts. In recent years it has been possible, by radioisotopic techniques, to assess accurately the extent of nonroot absorption, and subsequent transport of trace as well as the major nutrient elements (1, 2, 12).

Any study of the mechanism of foliar absorption must consider ion penetration of the cuticle. This is the first barrier to be penetrated before a chemical applied to a leaf can contact plant protoplasm. Although the permeability of the cuticular membrane to ions, herbicides, and pesticides has been discussed (3, 7, 9, 11) their mode of entry through the cuticle is still not well understood.

Stomatal pores may or may not be present in cuticular membranes, depending on the characteristics of the plant from which the cuticle is isolated. Stomata may serve as a portal of entry into the leaf, however, if entry is gained through the stomata it is not equivalent to absorption. Further, stomatal absorption does not preclude the necessity of cuticular penetration, since substances in the intercellular spaces still must pass through a layer of cutin (10, 15).

Nutrient losses from above ground plant parts by action of rain and dew are also significant. Tukey et al. (13, 14) found that some nutrients like Na and Mn were leached easily, Ca, Mg, S, K, Sr-Y, moderately, and Fe, Zn, P, Cl with difficulty. Studies thus far have been concerned either with foliar absorption or foliar leaching, but not both. In this report, the rate of cation and anion penetration from the outer to the inner surface and, from the inner to the outer surface of isolated cuticular membranes was studied. The permeability of a synthetic dialyzing membrane was compared with that of the biologically synthesized cuticular membranes.

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Materials and Methods

Preparation of Isolated Cuticles. Cuticular membranes were isolated enzymically from tomato (*Lycopersicon esculentum*) fruits and green onion (*Allium cepa*) leaves by Orgell's method (8) modified to include pectinase, cellulase, and hemicellulase (7). It was assumed that by this method of isolation the characteristics of the cuticular membranes were not altered.

Tomato fruit cuticle does not have stomatal pores while green onion leaf cuticle does, thus making cuticles from these plants desirable experimental material. Sections of fresh onion leaves and tomato fruit surfaces, 4 cm^2 were prepared and incubated in a solution consisting of 2 % (w/v) pectinase (Soluble Sclase: Sankyo Co., Tokyo) and 0.1 % (w/v) each of cellulase (Tokyo Kasei Co., Tokyo) and hemicellulase (Tokyo Kasei Co., Tokyo) in Walpole's acetate buffer (4) at pH 3.8 in the dark. Sodium ethylmercurithiosalicylate at a concentration of 1 mg per 10 ml was added as a disinfectant. Tissue and enzymes were incubated at 35° for approximately 24 hours. The incubation time was varied according to the plant material and degree of cuticularization. After the cuticular membrane was separated from the other wall constituents, the epidermal cell wall side was blotted with tissue paper to remove remains of cell constituents, washed several times in deionized water, dried, and refrigerated until used. A dialyzing membrane (No. 70160-1, Central Scientific Co.) was used as a control comparison, in which one side was arbitrarily designated as the outer and the other as the inner surface.

Apparatus for Penetration Studies. The apparatus used in the study of penetration of materials through isolated cuticular membranes is illustrated in figure 1. A large test tube (35 mm diam.) was suspended in a constant temperature (20°) water bath. This tube contained the outer solution which consisted of deionized water. Into this tube was suspended a small tube (15 mm inside diam.) with the cuticular membrane affixed over the tube opening by epoxy resin and a rubber band. The desired radioisotope solution; $Ca^{45}Cl_2$ (26 $\mu c/\mu mole$), $Rb^{86}Cl$ (500 $\mu c/\mu mole$), $FeS^{35}O_4$ (327 $\mu c/\mu mole$) or $RbCl^{36}$ (6×10^{-3} $\mu c/\mu mole$) was added to the small tube and immediately thereafter it was lowered into the solution in the large test tube. The meniscus of the labeled inner solution, in the small tube, was always adjusted to that of the outer solution to equalize the hydrostatic pressure on the cuticular membrane and prevented damage or rupture. Penetration of a given ion from what was originally the outer to the inner surfaces, or from the inner to the outer surfaces of the cuticular membranes was achieved by appropriate orientation of the membrane over the mouth of the inner tube. Use of dilute solutions (0.10mM) insured almost complete ionic dissociation. Three ml of solution was added to the inner small tube with 30 ml water in the outer large tube. The rate of ionic penetration through the membranes was determined by radioassay of 1 ml aliquots of the outer solution in the large tube using an end window G-M tube and standard scaler circuit.

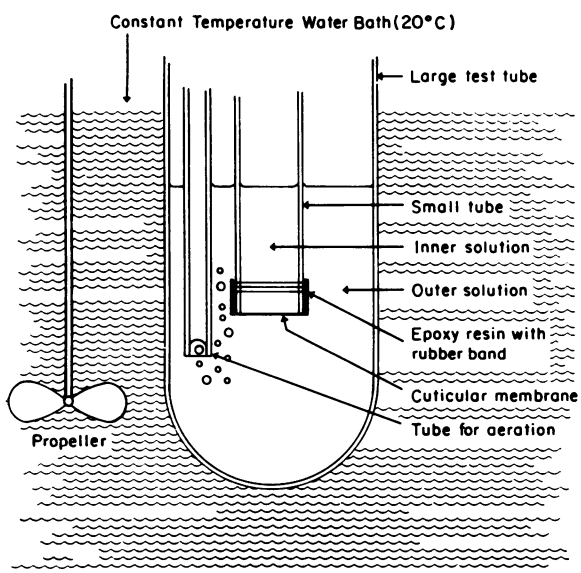


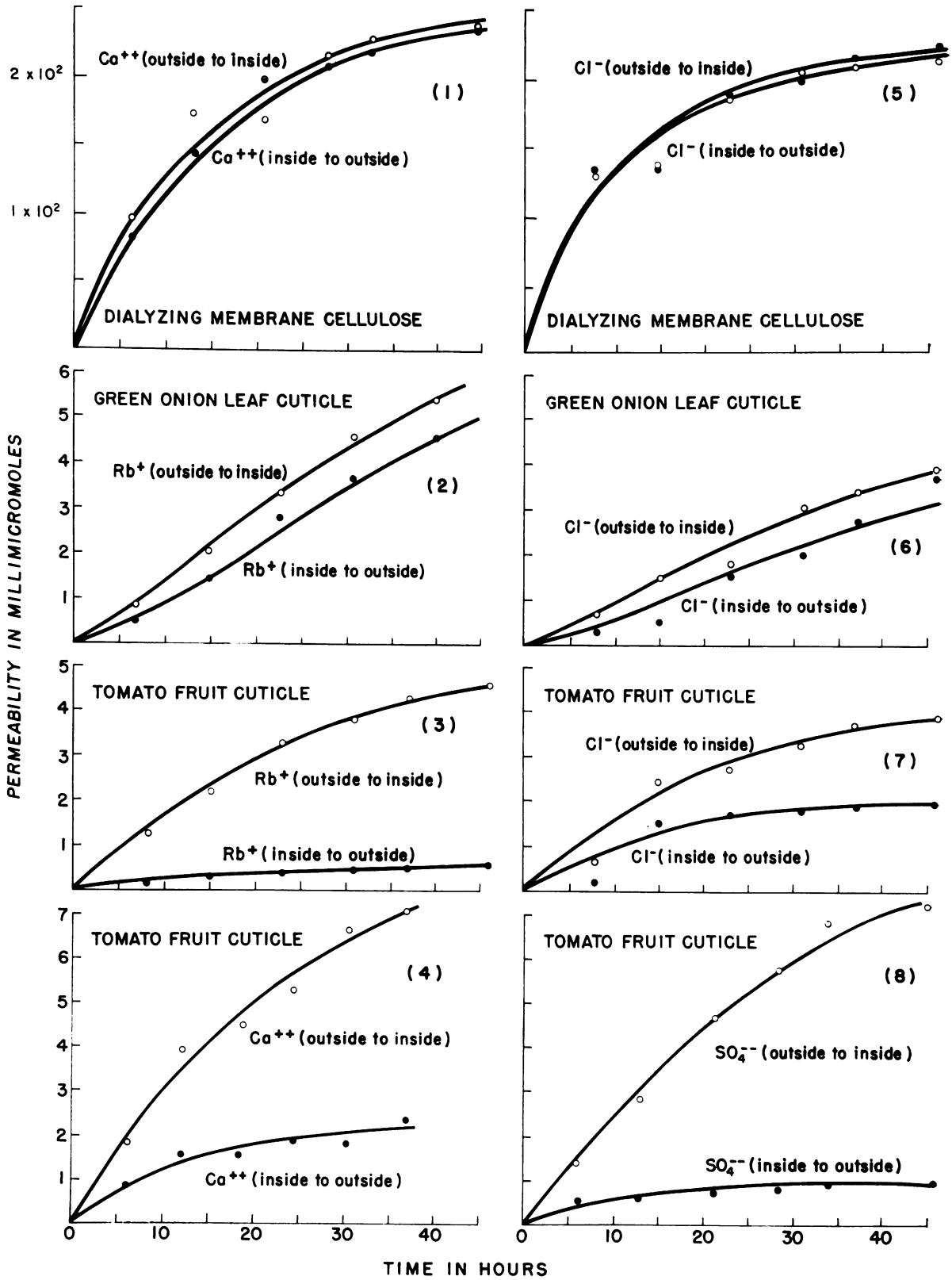
FIG. 1. Apparatus for measuring the permeability of a cuticular membrane to ions.

Results and Discussion

Nature of Isolated Cuticles. Enzymically separated cuticular membranes from green onion leaves were thin and translucent, while those from the tomato fruit were relatively thick and yellowish. Microscopic observation confirmed that cuticular membranes isolated from tomato fruit had no stomatal pores, while they were present on those from green onion leaves.

Rate of Ionic Penetration. The data on permeability of the membranes to Ca^{++}, Rb^{+}, SO_4^{--}, and Cl^{-} are summarized in figure 2. The results indicate, first, both cations and anions can penetrate cuticular membranes irrespective of the presence of stomatal pores. Secondly, the rate of penetration for cations and anions through both tomato fruit and green onion leaf cuticles was greater from the outer to inner surface than from the inner to the outer surface, irrespective of the presence of stomatal pores. The differences in rates of penetration in an inward and outward direction were smaller in the case of the onion leaf cuticle which contain stomatal pores. Stomata increase the absorbing surface over a given area and the cuticle associated with stomatal pores is thinner and perhaps of a different composition. These factors may favor equal bidirectional movement. Movement of ions through a dialyzing membrane was the same from either direction.

Ion Binding by Cuticular Surfaces. Since ionic penetration occurred preferentially from the outside to the inside, the comparative capacity of cuticular membranes to hold ions in a bound condition on the


Ca++ (outside to inside)
Ca++ (inside to outside)
(1)
2 x 10²
1 x 10²
DIALYZING MEMBRANE CELLULOSE
Cl⁻ (outside to inside)
Cl⁻ (inside to outside)
(5)
DIALYZING MEMBRANE CELLULOSE
GREEN ONION LEAF CUTICLE
Rb+ (outside to inside)
Rb+ (inside to outside)
(2)
GREEN ONION LEAF CUTICLE
Cl⁻ (outside to inside)
Cl⁻ (inside to outside)
(6)
TOMATO FRUIT CUTICLE
Rb+ (outside to inside)
Rb+ (inside to outside)
(3)
TOMATO FRUIT CUTICLE
Cl⁻ (outside to inside)
Cl⁻ (inside to outside)
(7)
TOMATO FRUIT CUTICLE
Ca++ (outside to inside)
Ca++ (inside to outside)
(4)
TOMATO FRUIT CUTICLE
SO4-- (outside to inside)
SO4-- (inside to outside)
(8)
PERMEABILITY IN MILLIMICROMOLES
TIME IN HOURS
0
10
20
30
40

2 surfaces was examined next. Segments of isolated cuticles were floated on 0.1mM solutions of $Ca^{45}Cl_2$, $Rb^{86}Cl$, $FeS^{35}O_4$ or $RbCl^{36}$ with either the inner or outer surface in contact with the labeled solution for 5 seconds. The exposed surface of the cuticular membrane was then blotted with tissue paper and 10 mm^2 sections were assayed for radioactivity. The bound ions retained (10^{-2} mμmole/cm^2) on the various cuticular surfaces are indicated in table I. Both cations and anions were retained on the inner surfaces of cuticles to a much greater extent than on the outer surfaces. No such differences between the 2 surfaces occurred with dialyzing membranes. Similar relationships were obtained when the exposed surfaces were washed with deionized water and blotted.

The total exchange capacities of the cuticular membrane surfaces may be much higher than those herein obtained. Five seconds contact with the 0.10mM solutions was probably not enough for equilibration. Thus, the values given for ion binding on cuticular surfaces were obtained under arbitrarily chosen conditions, and do not represent total exchange capacities, but are important for comparative purposes under the selected conditions.

Ionic Penetration through Different Membranes. There appeared to be a direct relationship between ion penetration from one surface to the other, and the ion binding capacity of the opposite side; with the rate of movement of ions through the membrane related to differences between the bound ion number of the outer and inner surfaces. With tomato fruit cuticle, approximately 3 times more calcium ions were bound on the inner surface (7.89 mμmole/cm^2) than on the outer surface (2.73 mμmole/cm^2) (table I). These data parallel the differences in the rates of calcium ion penetration from outside to inside and from inside to the outside surface of the same cuticles (fig 2). Differences in kind, size, and polarity of ions undoubtedly affects their penetration through cuticular membranes. It is significant, first, that movement of ions from the inner to the outer surface, and, secondly, the ion binding capacity of the outer surface were both less.

Differences in ion penetration in the 2 directions were markedly greater for tomato fruit than for the green onion leaf cuticles. Stomatal pores were present in the green onion leaf cuticle, and this may have accounted for the lesser difference in penetration from the 2 surfaces. It is not likely, however, that the stomatal pores offered free passage to ions. There are at least 2 possible explanations. First, the cuticular membrane may have been removed with associated cell fragments that precluded free movement of ions. Secondly, the stomatal cavities themselves are lined with an internal cuticle which may have been isolated intact in the enzymatic separation and as a continuum with that on the leaf surface. Previous results (17) have shown that the size of stomatal pores in the onion leaf cuticle was large enough to permit free flow of ions into them but a cuticular barrier must still be traversed. There was no difference in passage of ions originating from either direction through the dialyzing membrane. Likewise ion binding was the same on either side, Ion penetration rates, however, were 40 to 400-fold greater than through the plant derived membranes (fig 2, table I). About 80 % of the cations and anions added to the inner solution penetrated through the dialyzing membrane after 40 hours, whereas approximately 0.2 to 2 % were observed for the isolated cuticles. No equilibrium in ionic penetration was reached or even approached during the 40 hour experimental period with the green onion leaf cuticle. Similarly no equilibrium was attained with the tomato cuticles when penetration was from the outside to the inside. However, an equilibrium was approached when the penetration was from the inside to the outside. Irrespective of cuticular membrane orientation, the total number of ions found in the outer solution (fig 1) was far lower than would be expected as a result of simple diffusion, as was the case with the dialyzing membrane. This may be explained by differences in the exchange capacities of the outer and inner surfaces of the cuticles.

Table I

Ion Binding on Cuticular Surfaces

Cuticle	Surface*	Ca^{++}	Rb^{+}	SO_4^{--}	Cl^{-}
		(mμ mole per cm^2 × 10^2)			
Tomato fruit	Outer	273	1.8	1.4	0.6
	Inner	789	122	17	21
Onion leaf	Outer	...	0.9	...	0.2
	Inner	...	6.5	...	0.4
Dialyzing membrane	Outer	141	...	...	36
	Inner	142	...	...	35

* Outer refers to the external cuticular surface exposed to the air. Inner designates the inside cuticular surface adjacent to the epidermal cells.

Schieferstein et al. (9) tested cuticular membranes isolated from the upper, nonstomatous surface of leaves of *Hedera helix* for permeability to water. They found that permeability in the inward direction was greater than that in the outward direction. Water flux may also affect ion penetration. Goodman and Addy (5) observed a significant unidirectional movement of urea, benzoic acid, glucose, maleic hydrazide, simazine, and atrazine. There was greater penetration from the mesophyll cell side (in-

FIG. 2. Rate of undirectional penetration of cations and anions through cuticular and dialyzing membranes: (1) Ca^{++} and a dialyzing membrane, (2) Rb^{+} and green onion leaf cuticle, (3) Rb^{+} and tomato fruit cuticle, (4) Ca^{++} and tomato fruit cuticle, (5) Cl^{-} and a dialyzing membrane, (6) Cl^{-} and green onion leaf cuticle, (7) Cl^{-} and tomato fruit cuticle, and (8) SO_4^{--} and tomato fruit cuticle.

ner) to the air side (outer) of both the upper and lower cuticular membranes. They concluded that molecular size, electrokinetic charge, and spatial configuration did not seem to influence penetrability. Goodman and Addy (5, 6), however, removed the upper and lower leaf cuticles with ammonium oxalate and oxalic acid. Such chemically separated cuticular membranes might be different from those isolated enzymically. Furthermore, the permeability to organic compounds may be different from that of inorganic ions.

Relation to Foliar Absorption and Leaching. The results presented herein relate to foliar absorption and leaching of mineral nutrients. Cuticular membranes, the first barriers in nutrient uptake by leaves appear highly permeable to monovalent and divalent cations and anions. It is significant that penetration occurs equally well through cuticles from plant surfaces with or without stomata. Furthermore, absorption (penetration from the outside surface inward) occurs more readily than leaching (penetration from the inside surface outward). These data suggest that uptake dominates loss of nutrients through foliar surfaces.

Summary

Penetration of radioactive cations (Ca^{45}, Rb^{86}) and anions ($S^{35}O_4$, Cl^{36}) through enzymically isolated cuticles of tomato fruit, having no stomatal pores, and green onion leaves, with stomatal pores, was studied. The permeability of these cuticular membranes from the outer surface to the inner surface, as compared to movement from the inner to outer surface was much greater for Ca^{++}, Rb^{+}, SO_4^{--} and Cl^{-}. These differences were greater in the tomato fruit cuticle, which lacks stomata, than in the cuticle of the green onion leaf, which possesses stomata. A dialyzing membrane showed no differences in permeability for ions originating at the two surfaces. Rate of penetration through different cuticular surfaces was directly related to the extent of ion binding on the surface which was opposite the site of initial entry. It is possible that the greater ion binding on the inside, compared to the outside, of isolated cuticular membranes facilitates foliar absorption.

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