Subcellular Localization of Inorganic Ions in Plant Cells by in vivo Precipitation

Abstract. *Uptake of iodide (as a possible tracer of chloride) by barley roots preloaded with thallium (as a tracer of potassium) resulted in in vivo precipitation of the almost insoluble yellow thallium iodide. Electron microscopic observation revealed in several cells a dense precipitate of thallium iodide within the cisternae of the endoplasmic reticulum, which suggests that this membrane system is involved in intracellular ion transport.*

Localization of nutrient ions at the subcellular level may contribute to our knowledge of the role of organelles in the ionic relations of the cell. However, study of the cytochemistry of ions suffers from two serious problems—the diffusibility and invisibility of the small water-soluble particles. Both can potentially be overcome if the ions can be precipitated during fixation by use of a reagent containing a heavy metal which provides contrast in the electron microscope, as proposed by Komnick (*1*). However, the specificity of this method has been questioned, especially for Na^+ precipitation with antimonate (*2*), although some authors demonstrated that Na was present in their precipitates (*3*). In this respect the precipitation of Cl^- with Ag^+, which has been less used, may be more successful (*4*). A more fundamental criticism of the Komnick method is that the ions may diffuse during penetration of the fixative and the reagent. Zadunaisky (*5*) reported even leakage of appreciable amounts of ^{22}Na from tissues during antimonate fixation. Whether antimonate penetrates adequately into the cells has also been questioned (*6*).

We tried to avoid these problems by allowing barley roots with a low salt content to form such precipitates in vivo by successive accumulation of the constituents of an electron-dense, almost insoluble salt.

For several reasons we chose Tl^+ and I^- for this procedure. First, kinetic experiments showed that Tl^+ can take the place of K^+ in our material and is rapidly absorbed (*7, 8*). This reflects the general ability of Tl^+ to serve as a "physiological isotope" for K^+ (*9*). Second, Tl^+ is an electron-dense ion (the average mass number is 204) which can be easily precipitated with several inorganic and organic reagents, such as I^-, CrO_4^-, S^{2-}, and thionalide. Third, I^- is taken up by plant roots in fairly large amounts from concentrations greater than 1 m*M*. Although it is uncertain whether I^- is taken up by the Cl^- uptake mechanism, the competition between I^- and Cl^- (*10*) resembles the pattern found for Tl^+ and Rb^+ (*7*). Fourth, the solubility of TlI in water (25°C) is only 2×10^{-4} *M*. Fifth, the reaction is completely specific in our material: we never found precipitates after accumulation of either Tl^+ or I^- alone. Finally, precipitation of the TlI in the tissue can be recognized directly by its bright yellow color.

By administering the two ions successively we hoped to mark any intracellular structures involved in ion transport. The salient advantage of this approach, compared with the Komnick method, is that both ions are brought together by cellular activity, so that unwanted diffusion is avoided. From the possible sequences of uptake of both ions, uptake times, and concentrations, we started with the following procedure.

Barley roots, which had been loaded with Tl^+ up to saturation of the cytoplasm (*7*), were allowed to absorb I^- (*11*). They started to show a yellow color within 5 minutes. After 30 minutes of I^- uptake, the roots were prepared for the electron microscope (*12*). In many cells we found small rounded and oblong particles of TlI, either dispersed in the cytoplasm or aggregated into clusters or sheets adjacent to the cell surface (Fig. 1A). The significance of their location is not yet clear. Some of them may have been formed during fixation (*13*).

In some cortical cells the precipitate was located unambiguously within the cisternae of the endoplasmic reticulum (Fig. 1, B to D). In several other cells evidence for the same location was circumstantial because the presence of endoplasmic reticulum around similar precipitates could not be established with certainty. There is little doubt that

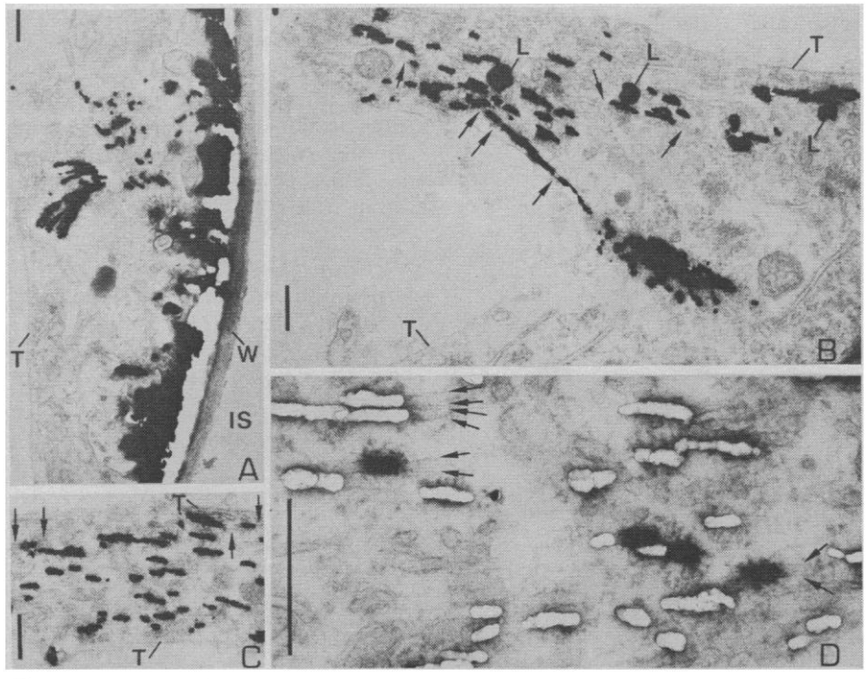

Fig. 1. Electron micrographs of ultrathin sections of barley roots which had been saturated with Tl^+ and then allowed to absorb I^-, showing precipitates of TlI (black). All sections shown were about 5 mm from the root tip and were unstained. The endoplasmic reticulum extending beyond the intracisternal precipitates can best be seen at the arrows. Abbreviations: *IS*, intercellular space; *L*, lipid body; *T*, tonoplast; *W*, cell wall. Scale bar, 0.5 μm. (A) Middle cortex cell with different types of precipitates; some of the large deposits along the cell wall came off, leaving white gaps. (B) Inner cortex cell; many cisternae of the endoplasmic reticulum contain precipitate. (C) Another section from the area shown in (B). (D) Area shown in (C) at higher magnification; most of the precipitate has now been melted and evaporated in the electron beam, leaving white holes.

these precipitates were formed in vivo during the uptake of I^- (*13*). As Tl, after saturation, can be presumed to be present all over the cytoplasm, the most obvious interpretation is that these precipitates mark the routes of I^- transport.

This first direct evidence for significant amounts of ions in the endoplasmic reticulum suggests that this membrane system plays a role in intracellular ion transport.

At present the role of compartmentation in ion transport in plant cells is being seriously discussed. Many authors have used the simple serial model advocated by Pitman (*14*), which implies that ions enter the vacuole via the bulk of the cytoplasmic content. However, kinetic evidence has suggested a more or less parallel relation between uptake into the cytoplasm and into the vacuole (*15*). Such a model requires a direct connection between plasmalemma and tonoplast, bypassing the cytoplasmic ion pool. It has been suggested that the endoplasmic reticulum and pinocytotic vesicles provide such a connection. Evidence for a close association between endoplasmic reticulum and tonoplast over large areas is of interest in this respect (*16*). Our findings lend support to the parallel model.

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11. Excised roots of 8-day-old seedlings of barley (cultivar Herta) of low salt content were obtained as described in (*7*). After incubation (4 hours) in 0.1 m*M* Tl_2SO_4 + 0.1 m*M* $Ca(HCO_3)_2$ (aerated, *p*H 7) in the dark at 25°C and rinsing (1 minute) in demineralized water, roots were incubated for 30 minutes in 5 m*M* NH_4I, 0.1 m*M* $Ca(HCO_3)_2$, and 5 m*M* $CaSO_4$ (aerated, *p*H 7).
12. Specimens were fixed for 2½ hours in phosphate-buffered OsO_4 (1 percent, *p*H 7.4) at 4°C. They were dehydrated for 2½ hours in dry ethanol vapor as described → P. Sitte [*Naturwissenschaften* **17**, 402 (1962)]. The total fluid volume was less than 0.5 ml per 5 to 20 cm (length) of root. Specimens were embedded in ERL-4206 mixture [composition intermediate between A and B described in → A. R. Spurr, *J. Ultrastruct. Res.* **26**, 31 (1968)]. Except for the ERL all media contained 5 m*M* NH_4I to prevent solution of the precipitates. Ultrathin sections were collected on a saturated solution of TlI. Grids were quickly rinsed in isopentane to remove adhering solution.
13. In parallel experiments the amount of I^- taken up within 30 minutes appeared to be less than half the amount of Tl^+ absorbed in 4 hours. Therefore at the end of the I^- absorption period more than half the amount of Tl^+ present in the tissue must still have escaped precipitation. Loss of Tl^+ from root batches during subsequent fixation in the presence of 5 m*M* I^- appeared to be only about 8 percent, however. Consequently considerable amounts of Tl^+ must have been precipitated by the iodide in the fixative. Thus, the fixation we applied after the in vivo precipitation was a Komnick fixation. As stated in the introduction, results of the Komnick method are subject to fundamental criticism so we had to discriminate between precipitates formed in vivo and those formed during fixation. The latter were visualized by omission of the in vivo precipitation step from the whole procedure. Electron microscopic observation then revealed TlI particles in clusters near the cell wall or dispersed in the cytoplasm, but membrane-bound precipitates were never found.
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17. We thank G. G. J. Bange for his valuable suggestions during the experiments and the preparation of the report, and R. J. Helder, K. R. Libbenga, and A. Quispel for their comments on the manuscript.

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Male-Induced Pregnancy Termination in the Prairie Vole, Microtus ochrogaster

Abstract. *Postimplantation as well as preimplantation pregnancy in prairie voles can be terminated by replacing the original stud male with an unfamiliar male. The pregnancy is disrupted by the ensuing male-induced estrus. Females spontaneously abort their litters, become receptive, and successfully breed again 4 or 5 days after introduction of the new male.*

Olfactory, endocrine, and social factors involved in pregnancy block (the Bruce effect) have been extensively investigated (*1*). Preimplantation pregnancy block occurs in laboratory mice (*Mus musculus*) (*2*), wild house mice (*Mus musculus*) (*3*), prairie deer mice (*Peromyscus maniculatus bairdii*) (*4*), field voles (*Microtus agrestis*) (*5*), and meadow voles (*Microtus pennsylvanicus*) (*6*). Postimplantation pregnancy block in the prairie vole (*Microtus ochrogaster*) is described in this report; the known period of susceptibility to pregnancy block is thus extended to virtually all stages of pregnancy. This contrasts with the limited period for pregnancy block demonstrated in *Mus* (*7*). Other species in which block of pregnancy has been described have been investigated only during the preimplantation period.

Laboratory-born prairie voles were housed in 41 by 24 by 18 cm solid bottom cages supplied with wood shavings and straw and had free access to rabbit chow and water as described (*8*). All females were nulliparous and initially anestrous. Control females ($N = 28$) were paired with sexually inexperienced males of similar age (60 to 120 days). Experimental females ($N = 61$) were similarly paired but had their original mates replaced by unfamiliar males at intervals ranging from 5 to 19 days after first pairing. Pairing under the normal control breeding conditions results in a conception rate of 87 percent; hence, nearly all females were assumed to be pregnant. Gestation was judged to be the interval from pairing minus the 2 to 3 days required for estrus induction and breeding (*9*). Experimental females were thus divided among five groups assumed to be either 3 to 4, 8 to 9, 12 to 13, 14 to 15, or 16 to 17 days pregnant at introduction of the second male ($N = 10$, 14, 13, 12, and 12, respectively).

To verify actual breeding dates and to determine more precisely the duration of gestation before introduction of the second male, 36 percent of females were examined daily for a copulation plug or sperm in a vaginal lavage. The breeding performance and time to par-