Characterization of Cadmium Uptake by Plant Tissue[1,2]

Jay M. Cutler and Donald W. Rains
Department of Agronomy and Range Science, University of California, Davis, California 95616

ABSTRACT

The uptake of cadmium by excised root tissue of barley (*Hordeum vulgare* L. cv. Arivat) was investigated with respect to kinetics, concentration, and interactions with various cations. The role of metabolism in Cd absorption was examined using a range of temperatures, anaerobic treatments, and chemical inhibitors. The uptake and distribution of Cd in intact barley plants was also determined. A large fraction of the Cd taken up by excised barley roots was apparently the result of exchange adsorption and was displaced by subsequent desorption with unlabeled Cd, Zn, Cu, or Hg. Another fraction of Cd which could not be displaced by desorption in unlabeled Cd was thought to result from strong irreversible binding of Cd, perhaps on sites of the cell wall. The fraction of the Cd taken up beyond that by exchange adsorption by fresh roots was a linear function of temperature, and inhibited by conditions of low oxygen and by the presence of 2,4-dinitrophenol. It was concluded that this fraction of Cd entered excised barley roots by diffusion. Diffusion, when followed by sequestering, probably accounts for the accumulation of Cd observed in intact barley plants.

It is a common observation that elements not known to be essential to plants are nevertheless accumulated by plants in appreciable quantities (2). The accumulation of Cd by plants from contaminated soils and nutrient solutions has been reported by several investigators (5, 7–9, 16, 21). The essentialness of Cd for either plants or animals has not yet been demonstrated and the accumulation of Cd at higher concentrations is reported to be toxic (6, 15, 16). Various biochemical roles have been reported for Cd in plants (22). However, little is yet known about the mechanisms involved in its uptake and the dependence of this uptake upon environmental factors. This study was carried out to characterize Cd uptake by plants.

MATERIALS AND METHODS

Seedlings of barley (*Hordeum vulgare* L. cv. Arivat) were grown for 6 days in 0.2 mM CaSO₄ in the dark as described by Epstein (1). In experiments using intact plants, the seedlings, after germination, were transferred to standard half strength Johnson's nutrient solution (9) and then transferred to a growth chamber. After a period of 1 week the solutions were renewed and 1 mg/l of labeled Cd was added. The plants were harvested at regular intervals and transferred for 2 hr to fresh nutrient solutions containing no Cd to desorb exchangeable Cd. The plants were then divided into roots and shoots and analyzed for content of labeled Cd. In experiments using excised roots, the roots, on the day of the experiment, were thoroughly rinsed with water and excised, and experiments were conducted as described by Epstein *et al.* (4) but with some minor modifications.

Samples of excised roots weighing 1 g (fresh weight) were kept for 30 min in an aerated holding solution containing 0.2 mM Ca (NO₃)₂ and then transferred to experimental solutions, usually maintained at 30 C, and containing Cd labeled with ¹¹⁵Cd and 0.2 mM Ca(NO₃)₂. Several experiments involved airdried roots grown in the same manner as fresh roots but allowed to desiccate in air for 24 hr before use. This tissue was allowed to rehydrate for 30 min in an aerated holding solution containing 0.2 mM Ca (NO₃)₂ previous to use in experiments. Such tissue was included to determine the extent of nonmetabolic uptake by a tissue similar in composition to fresh roots.

Since it is now well demonstrated that Ca is required to maintain the structural and functional integrity of plant cell membranes (1, 2, 12, 14, 18), Ca was included at 0.2 mM in all experimental solutions. The pH of absorption solutions, initially 5.5, did not vary by more than 0.2 units during the experiments. The absorption period, which varied from 2 to 120 min, was terminated by one of two methods, depending on whether or not that fraction of labeled Cd associated with the tissue in readily exchangeable form was to be removed. When it was desirable to desorb the labile fraction the absorption period was followed by a desorption period in a solution containing 0.2 mM Ca(NO₃)₂ and unlabeled Cd, or other ions. When it was not desirable to remove this labile fraction, the samples were rinsed in water after the absorption period. All experimental solutions were of sufficient volume that depletion did not exceed 10% of the initial Cd content.

Analyses of roots, as described by Epstein *et al.* (3), were carried out on a Beckman Low Beta II Counter. All points plotted in the figures represent the mean of duplicate analyses on two or more root samples.

RESULTS AND DISCUSSION

Intact Plants. The Cd content of roots and shoots of intact barley plants grown in solution cultures containing 1 mg/l of labeled Cd is shown in Table I. The Cd content of both roots and shoots increased with time. On a fresh weight basis roots contained about 11 to 17 μg of Cd/g and shoots about 2 to 4 μg of Cd/g after 28 days. Such accumulation of Cd in excess of external concentration provides evidence that a mechanism of accumulation is found in barley. The lower concentration of Cd in the shoots of intact barley plants relative to the roots suggests that this element is retained in the root and lower por-

Table I. *Cd Content of Roots and Shoots*

Intact barley plants were grown up to 4 weeks in a half strength Johnson's nutrient solution to which 1 mg/1 of labeled Cd had been added. Roots were desorbed for 2 hr in fresh nutrient solution.

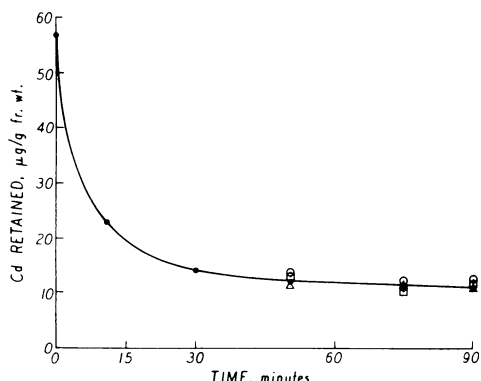| Time after Cd Addition	Tissue Cd Content	
	Roots	Shoots
days	*μg g dry wt*	
10	155.5	24.9
14	191.2	36.1
21	225.3	48.7
28	249.4	55.9

FIG. 1. Retention of ^{115}Cd by excised barley roots exposed to a solution of 50 mg/1 of unlabeled Cd (●), Zn (○), Cu (□), or Hg (△) after a 30-min period of exposure to a solution of 10 mg/1 of ^{115}Cd, 0.2 mM Ca (NO$_3$)$_2$.

tions of the stem although some movement does occur within the plant. In order to further elucidate the mechanisms responsible for the accumulation and retention of Cd by barley root tissue a number of experiments with excised barley roots were performed.

Exchangeable and Freely Diffusible Cd. Excised barley roots were exposed for 30 min to a 10 mg/1 of labeled Cd absorption solution containing 0.2 mM Ca(NO$_3$)$_2$. The root samples taken at time zero were thoroughly rinsed with water only; the other samples were transferred to desorption solutions containing 0.2 mM Ca(NO$_3$)$_2$ and 50 mg/1 of unlabeled Cd, Zn, Cu, or Hg for periods up to 90 min.

The results (Fig. 1) show that a large fraction of the labeled Cd had been desorbed from the excised roots within the initial 30 min in the desorption solution, with little further loss thereafter. This fraction, which was largely independent of the desorbing cation (as long as the cation was present in large excess) was considered to represent both freely diffusible and exchangeable ("outer space") Cd in the tissue, and accounted for the largest fraction of Cd uptake in this short term experiment. The extent of adsorption of labeled Cd is expected to vary with the nature and quantities of other solutes present in the absorption solution. In the absence of Ca, a divalent cation which can also occupy cell wall exchange sites, we found that the exchangeable fraction of Cd was greater than when Ca was present. However, since Ca is accepted as necessary for normal structure and function of plant cell membranes it was included in all experimental solutions.

In another experiment in which the concentration of unlabeled Cd in the desorption solution was varied, we found

that only with concentrations of Cd of 50 mg/1 or more was complete desorption observed in 30 min. To ensure complete desorption of exchangeable Cd in all subsequent experiments, the tissue, after the absorption period, was desorbed for 30 min in a solution containing 50 mg/1 of unlabeled Cd.

Kinetics and Concentration Dependence. Fresh and air-dried excised roots were exposed for up to 60 min to absorption solutions containing 10 mg/1 of labeled Cd. All tissue was subsequently desorbed in a solution containing 50 mg/1 of unlabeled Cd. Figure 2 shows the time course of Cd uptake by the roots. The nonmetabolizing, air-dried roots took up and retained labeled Cd up to 30 min. This nonmetabolic, nonexchangeable uptake of labeled Cd by air-dried roots suggested that irreversible sequestering of Cd took place in the excised barley roots. Desorption in 1 mM EDTA also failed to remove this nonmetabolically bound Cd. In this experiment the air-dried roots saturated in about 30 min, suggesting that the availability of binding sites was limited. Uptake by air-dried roots was about equal to that by fresh roots for the first 20 min of exposure time and thereafter was exceeded by the uptake by fresh roots. We were unable to determine the nature of these binding sites in our tissue-level studies, but work with zinc and copper, which are known to behave similarly to Cd in many biochemical associations (22), would suggest that certain cell wall materials have a large capacity for binding these heavy metals (17, 18) and perhaps Cd also. The uptake of Cd by air-dried roots is discussed further under the topic of metabolism.

Uptake of labeled Cd by fresh roots (Fig. 2), initially rapid, declined with time up to about 30 min, the time required to saturate the air-dried roots. Uptake after about 30 min was a nearly linear function of time up to 2 hr and had a rate of 8.6 μg/g fresh weight·hr.

The difference between the curves for fresh and air-dried tissue, which may represent the metabolic fraction of uptake, was a linear function of time after about 20 min. The similar nature of the two curves for the first 20 min of exposure would then suggest that nonmetabolic sequestering predominated in both types of tissue until a large fraction of binding sites had been filled, after which time a second mechanism or a greater
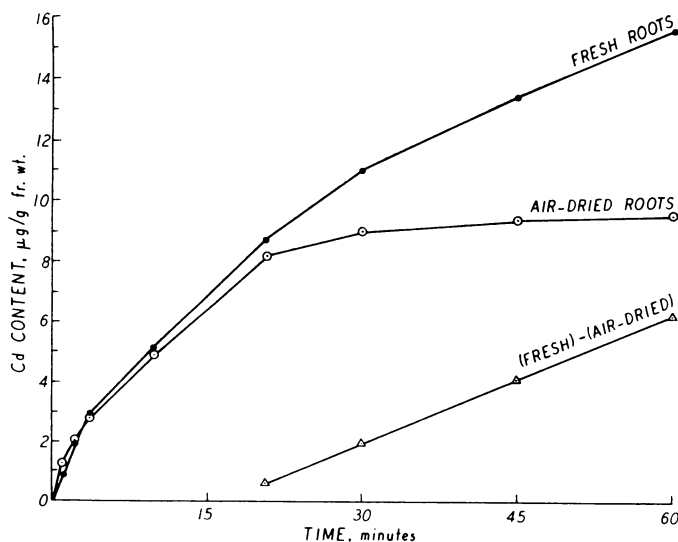

FIG. 2. Labeled Cd content as a function of absorption time for fresh and air-dried roots and the difference between these when exposed to 10 mg/1 ^{115}Cd, 0.2 mM Ca (NO$_3$)$_2$ with a subsequent 30-min period of desorption in 50 mg/1 of unlabeled Cd, 0.2 mM Ca (NO$_3$)$_2$.

binding capacity allowed further uptake in fresh roots. The failure of the difference between the curves to extrapolate to zero at time zero might result from a greater number of rapidly available binding sites in the air-dried tissue than in fresh tissue.

The rate of uptake of labeled Cd by fresh and air-dried root tissue as a function of the concentration of labeled Cd in the absorption solution is illustrated in Figure 3. The tissue was exposed for 30 min to an absorption solution and then desorbed. The saturation kinetics observed for fresh roots are typical of the uptake of numerous ions by many different kinds of plant tissue. Aid-dried roots also showed saturation kinetics, as to be expected if the Cd content of this tissue reflects binding at a limited number of sites.

Selectivity. The selectivity of the Cd absorption process was examined in several experiments. Excised barley roots were exposed for 30 min to absorption solutions containing 5 to 20 mg/l of labeled Cd and 0.18 meq/l of Zn, Cu, or nonradioactive Cd. All samples were desorbed for 30 min in 50 mg/l of unlabeled Cd following the absorption period. The results of these experiments are illustrated in Table II. The inhibitory effect of Cu was somewhat greater than that of nonradioactive Cd, and that of Zn was somewhat less but all had qualitatively similar effects. Schmid et al. (20) found that absorption of labeled Zn was also inhibited more severely by Cu than by unlabeled Zn. They attributed this to competitive inhibition of Zn uptake by Cu, but it is also possible that at the levels of Cu added the absorption process may haye been metabolically impaired by toxic levels of Cu. Lineweaver-Burk plots of our data aid little in further interpreting the nature of these interactions. In these, Cd and Zn appear to be competitive whereas the interaction between Cd and Cu appeared more complex and could not be interpreted in a straight forward manner. Further comparison of our data with that of Schmid et al. suggests that the uptake of Cd and Zn differ. Most notable of these differences is that whereas the uptake of Zn was a linear function of time from about time zero (their Fig. 2) that of Cd was not (Fig. 2).

Role of Metabolism. The accumulation of Cd by fresh excised barley roots in concentrations in excess of those of the absorption solution (Table I), as well as the kinetics (Fig. 2) and concentration response of this uptake (Fig. 3), led us to suspect that Cd might be actively absorbed by excised root tissue. The
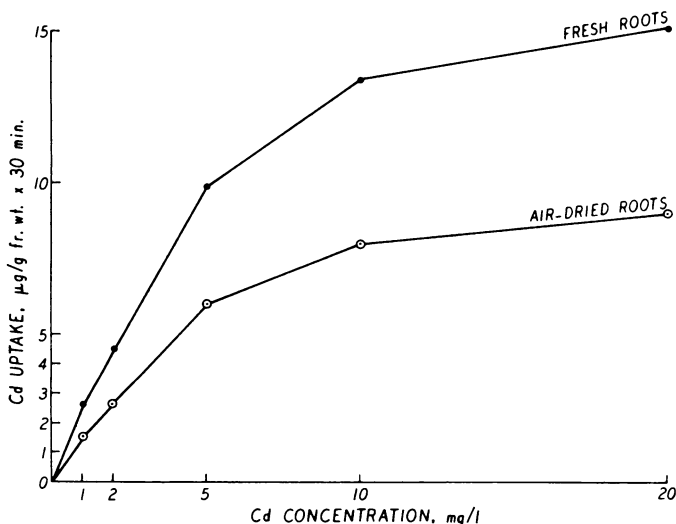
FIG. 3. Rate of uptake of ^{115}Cd by fresh and air-dried roots as a function of the concentration of ^{115}Cd in the absorption solution. Tissue was desorbed as indicated in the legend for Figure 2.

Table II. *Rate of Absorption of* ^{115}*Cd as a Function of the Concentration of* ^{115}*Cd and the Effects of Zn, Cu, and Unlabeled Cd*

Tissue was desorbed for 30 min in 50 mg/l Cd.

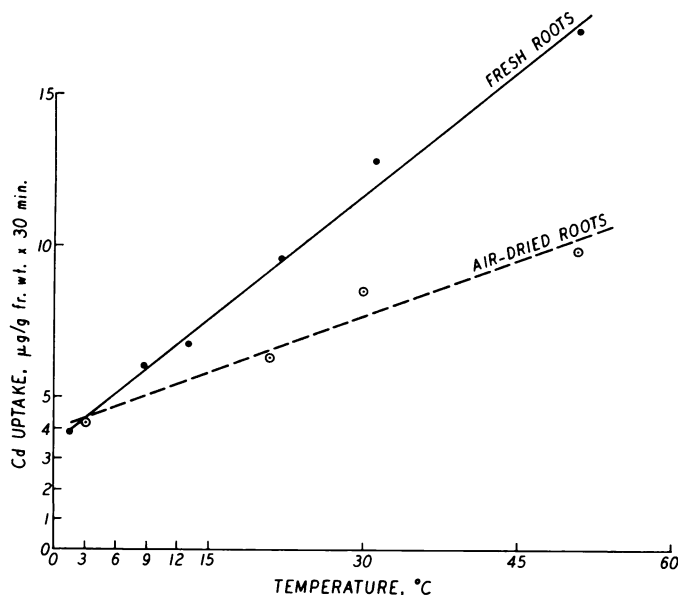| Concn of ^{115}Cd	Rate of ^{115}Cd Absorption at 10 mg/l			
	Control	Zn	Cu	Cd
mg/l	μg/g fresh wt × 30 min			
5	6.8	4.7	3.7	4.3
10	10.1	7.3	7.0	7.2
20	13.8	11.1	11.3	10.7

FIG. 4. Absorption of ^{115}Cd by fresh and air-dried roots as a function of temperature when the tissue was subsequently desorbed for 30-min in a solution of 50 mg/l of unlabeled Cd, 0.2 mM Ca(NO$_3$)$_2$.

role of metabolism in the absorption of Cd was investigated in several experiments.

Fresh and air-dried roots were exposed for 30 min to absorption solutions containing 10 mg/l of labeled Cd at various temperatures. Following the absorption period the samples were desorbed for 30 min in a solution containing 50 mg/l of unlabeled Cd and maintained at 30 C. Figure 4 shows the interesting effect of temperature upon the uptake of Cd. Within the range of 3 to 50 C the Cd uptake by both fresh and air-dried roots was a nearly linear function of temperature.

Linearity of response over the temperature range investigated is characteristic of some physical processes, notably diffusion, but it is not characteristic of responses dependent upon metabolism. This evidence suggests that the uptake of Cd is not directly dependent upon metabolism and thus that physical processes, most likely diffusion, were predominant. Diffusion, coupled with sequestering, might account for the accumulation of Cd in barley tissue. The observation that fresh tissue accumulates more Cd than air-dried tissue might be explained by the availability of more binding sites in this tissue and/or by the compartmentation and resistance to leakage afforded by intact, functional cell membranes. The convergence of the two plots at low temperature suggested that under these conditions, uptake by fresh roots is quite similar in magnitude to that of
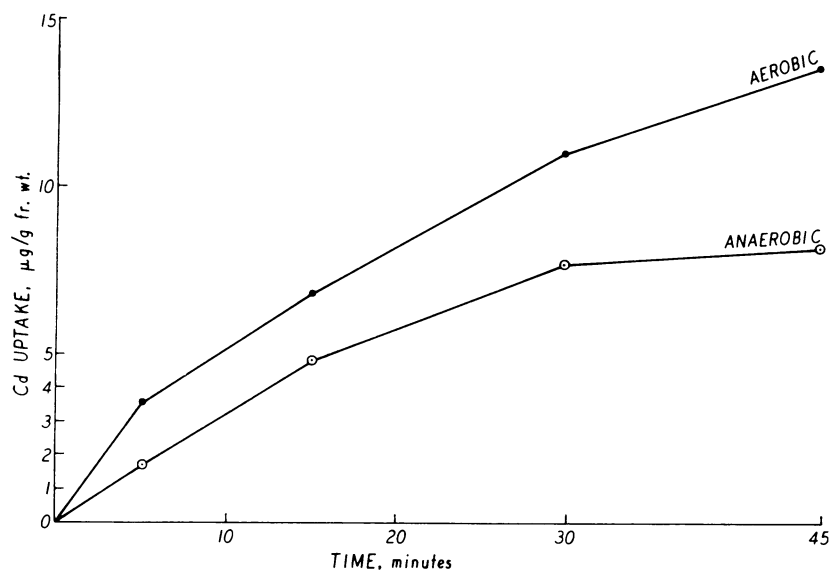
FIG. 5. Uptake of [115]Cd as a function of time from 10 mg/l [115]Cd solutions under aerobic and anaerobic conditions. The tissue was desorbed for 30-min in 50 mg/l of unlabeled Cd, 0.2 mM Ca (NO₃)₂.

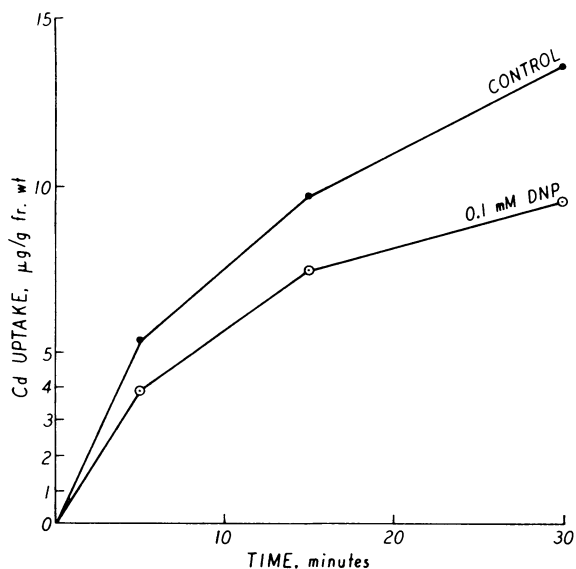

FIG. 6. Uptake of [115]Cd as a function of time from a 10 mg/l of [115]Cd solution as influenced by the presence of 0.1 mM DNP. The roots were subsequently desorbed as described in the legend for Figure 2.

air-dried roots and possibly by a similar process of diffusion and sequestering. Such purely physical binding would occur even at quite low temperatures, which would account for the observation that the plot for fresh roots does not extrapolate to zero rate at 0 C.

Air-dried roots at 30 C saturated in 30 min (Fig. 2). Variation in temperature would be expected to affect the rate of attaining saturation level and not the final saturation level. However, as illustrated in Figure 4, the Cd content (thought to represent a saturation level) of air-dried roots increased linearly with temperature. Such evidence suggests that the number of binding sites in this tissue must increase with increasing temperature. Macromolecular structures are known to be altered as temperature varies beyond certain ranges. Such disruption of macromolecules, especially at higher temperatures, might increase the availability of Cd-binding sites in the air-dried tissue. This might also explain, in part, the linearity of the temperature response of fresh roots. However, neither the nature of binding sites nor the changes attendant to dessication and subsequent rehydration of the root tissue were determined in this study and hence these conclusions are necessarily tentative. Decreased diffusional resistance is probably a factor in the temperature response curves of both tissue types.

To further study the nature of Cd uptake and its dependence upon metabolism the effect of anaerobiosis upon uptake was in-
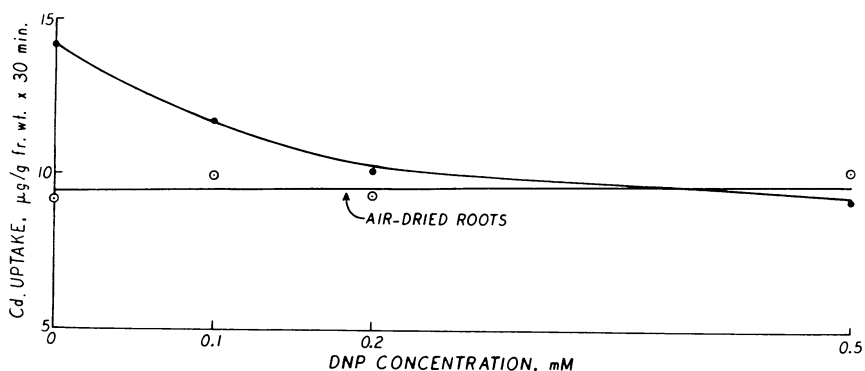

FIG. 7. The effect of increasing concentrations of DNP upon the absorption of [115]Cd from a 10 mg/l of [115]Cd solution by fresh and air-dried roots. The tissues were desorbed as described in the legend for Figure 2.

vestigated. Nitrogen gas was bubbled through the experimental solutions containing 10 mg/l of labeled Cd for 6 hr previous to the beginning of the experiments, and continued throughout the exposure periods. Cadmium uptake was lower under anaerobic conditions (Fig. 5) than in the control (air bubbled through the solutions). The effect of anaerobiosis may operate chiefly through decreased permeability of cell membranes to Cd under conditions of low oxygen as has been found to be the case for water transport (10). This response to anaerobiosis is consistent with the hypothesis that Cd moves into the tissue by diffusion.

The role of metabolism was also studied in several experiments in which DNP[3] was included in the experimental solutions. The presence of 0.1 mM DNP inhibited the uptake of Cd by fresh roots (Fig. 6), though not as severely as did conditions of low oxygen (Fig. 5). This observation can also be explained as resulting from a decreased permeability of cell membranes. Membrane permeability to passive water flux is known to be decreased by a variety of metabolic inhibitors (13) and permeability to Cd may be similarly affected. As illustrated in Figure 7, with increasing concentrations of DNP in the absorption solution up to 0.5 mM, Cd uptake decreased and converged with that by air-dried roots, for which uptake was unaffected by DNP. This convergence suggests that in short-term experiments Cd uptake by fresh roots with decreased permeability and impaired metabolism and by air-dried roots occurs by a similar process of diffusion and nonmetabolic binding, perhaps largely restricted to areas external to the cell membranes.

CONCLUSIONS

The accumulation of Cd by excised barley roots apparently involves three mechanisms.

The first mechanism can be characterized as exchange adsorption. This reversibly bound fraction of Cd associated with the roots is readily exchanged when the tissue is exposed to desorption solutions, and accounts for the largest amount of Cd taken up by roots in short term experiments (Fig. 1). These exchange sites were filled non-selectively with the transition type metals Zn, Cd, Cu, and Hg.

Another mechanism involved in Cd accumulation appears to involve a nonmetabolic binding of this element. This is apparently the primary accumulation mechanism operating in both air-dried roots and in fresh tissue subject to thorough desorption of exchangeable Cd. This uptake was thought to involve the irreversible sequestering of Cd to a fixed number of binding sites. Such sites may be present on cell wall constituents (17) or other macromolecules within the cell. Such binding can allow for the maintenance of a concentration gradient of Cd into the cell, thus allowing accumulation of this element by diffusion. Such binding may also serve, to a limited extent, to prevent entry of Cd into the cell cytoplasm, thus reducing the known toxic effects. Sequestering may also explain the relatively effective exclusion of Cd from the shoots of intact barley plants.

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[3] Abbreviation: DNP: 2,4-dinitrophenol.

The observation that Cd is transported to the shoots of intact plants indicates that Cd must at some point follow a symplastic pathway (i.e., cross cell membranes). Diffusion can account for the movement of Cd into barley root cells. Such a mechanism is consistent with the linear temperature response of sorption (Fig. 4), and the failure of sorption to respond to metabolic derangement at higher temperatures. Active metabolic uptake is not consistent with this response. The response to anaerobiosis and the presence of DNP can be reconciled with this model since both are known to reduce membrane permeability. Diffusion, coupled with sequestration, thus largely accounts for the accumulation and distribution of Cd found in barley.

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