Plant Physiol. (1978) 61, 731–736

Sites of Absorption and Translocation of Iron in Barley Roots

TRACER AND MICROAUTORADIOGRAPHIC STUDIES

Received for publication July 6, 1977 and in revised form December 7, 1977

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ABSTRACT

Absorption and translocation of labeled Fe were measured at various locations along the length of intact seminal axes and lateral roots of iron-sufficient (+Fe) and iron-stressed (−Fe) barley (*Hordeum vulgare*) plants. In seminal axes of +Fe plants, rates of translocation were very much higher in a zone 1 to 4 cm from the root tip than elsewhere in the root. Lateral roots of high rates of translocation were also restricted to a narrow band of maturing or recently matured cells. In −Fe plants the patterns of uptake and translocation were essentially the same as in +Fe plants but the rates were 7- to 10-fold higher. The amount of labeled Fe bound to the root itself was not increased by Fe stress and its distribution along the root seemed inversely related to the ability to translocate Fe.

Microautoradiographic studies showed that most of the iron bound to roots was held in an extracellular peripheral band in which iron seemed to be precipitated. This process may be assisted by microbial colonies but did not depend on them since it was seen, although to a lesser extent, in sterile roots. In zones from which iron was translocated there was evidence that internal root tissues became labeled readily, but as translocation declined with distance from the root tip, radial penetration of Fe appeared to become progressively less. The results are discussed in relation to possible changes in the pH or redox potential of the surface of the root.

There is good evidence that Fe is reduced to the divalent ferrous state before entry into the cells of the plant root (5–7). It has also been suggested that the release of "reductants" or *in situ* reduction may be preferentially located in young tissues close to the expansion zone (1, 2, 5). Evidence in support of this has come from soybean and tomato roots where it was shown that *in situ* reduction of ferricyanide to form 'Turnbulls Blue' was particularly intense in this zone, especially in young lateral roots (1, 5) and in varieties which responded to Fe stress. Unfortunately these observations were not accompanied by direct measurements of the absorption and long distance transport of iron by the zones in question. In another species, *Zea mays*, these processes occurred rather uniformly along primary root axes of 18 cm in length (18); a further report also indicated that iron is both absorbed and translocated to the shoot from locations more than 30 cm from the root tip (14). This rather uniform pattern of iron translocation in corn (18) contrasts with the results presented for *Hordeum vulgare* (barley) in this paper. We show that most of the Fe reaching the shoot in a 24-hr period of both Fe-sufficient (+Fe) plants and iron-stressed (-Fe) plants is absorbed in parts of the root behind the extending zone of the axis.

MATERIALS AND METHODS

PLANT CULTURE

Seeds of barley (*H. vulgare* cv. Midas) were germinated on damp filter paper and transferred to a culture solution (10) with or without 10 μM FeEDDHA.[1] Plants were grown in these solutions for 15 to 18 days after germination in a greenhouse (17 C ± 2 C) in which supplementary illumination was used to extend the daylength to 16 hr. Twenty-four hr before the experiments began the plants were placed in one of two types of culture solution of the following composition (mM): (A) K^+, 0.51; Ca^{2+}, 0.15; NO_3^-, 0.3; Cl^-, 0.5; $H_2PO_4^-$, 0.01 and minor elements (10) with or without 10 μM FeEDDHA and with pH 6. (B) K^+, 0.51; Ca^{2+}, 5; NH_4^+, 2; Cl^-, 10.5; NO_3^-, 2; $H_2PO_4^-$, 0.01 with or without 10 μM Fe $(NO_3)_3$ and with pH 4. The high Ca concentration in solution B screened the roots from damaging effects of H ions. The plants were kept at 20 C and illuminated for 16 hr daily with a mixture of mercury vapor fluorescent and tungsten bulbs giving a light intensity at plant height of 25×10^3 lux.

EXPERIMENTAL TREATMENTS

Excised Root Systems. Shoots were cut from the plant to leave a small stump of leaf bases attached to the root system. The stump was sealed into a glass tube with wax. The xylem sap gathering in the tube was removed at intervals, weighed, and stored frozen prior to radioactive counting and chemical assay.

Iron Uptake by Root Zones. The apparatus used to study the absorption of Fe was similar in most respects to that described in earlier papers (14). Segments (about 3.5 mm long) of root were isolated from the bulk of the root system by sealing them across the diameter of a polyethylene tube, using warm anhydrous lanolin and a silicone rubber compound (Silastic 9161 RTV, Hopkins and Williams, U.K.). Lateral roots were treated somewhat differently; apical portions of the root of varying length were totally enclosed in the flow cell. This was done because the linear distances over which the major changes in physiological development occur in lateral roots are much shorter (22) than the 3-mm diameter of the flow cell used. On longer laterals, 3-mm segments in the basal zone, where the anatomical development is generally complete, were labeled in the same way as segments from the main axes. Nutrient solution containing ^{59}Fe (usually 1–2 $\mu Ci \cdot ml^{-1}$), was pumped through the flow cell containing the root segments, while the remainder of the root system was kept in a nonradioactive solution of similar composition. A second plant was placed with its entire root system in the outer, unlabeled solution, as a check; it is easy to see how errors between replicate roots arise, particularly if they are elongating at different rates. This factor should be borne in mind if this technique is applied to very rapidly elongating roots such as sunflower or tomato. In all of the data presented the nominal segment position refers to the center point of about a 3.5-mm segment at the beginning of the experiment.

[1] Abbreviations: FeEDDHA: ferric diaminoethane-N,N-di-*o*-hydroxyphenyl acetic acid; CCCP: carbonyl cyanide *m*-chlorophenylhydrazone.

AUTORADIOGRAPHY

Frozen Section Technique. Roots were treated with solution similar in composition to the above containing ^{59}Fe or ^{55}Fe in chelated or ionic forms at higher specific radioactivity (2–4 $\mu Ci \cdot ml^{-1}$) than used in other experiments. At the end of the labeling period selected zones of the axis were taken, rapidly dipped in ice-cold 15% methyl cellulose, then frozen in contact with liquid N_2. All subsequent steps in block-making, section cutting, mounting, and exposure have been fully described elsewhere (24). The films used in the present work were AR 10 stripping film (Kodak) and G5 liquid emulsion (Ilford) to which frozen sections were exposed at −25 C and which were developed subsequently in D 19 (Kodak). The density of silver grains in autoradiographs was measured automatically using a microscope photometer (Leitz), operating in the reflectance mode, to which a motorized stage drive was attached for scanning. Signals from the photomultiplier were converted to digital voltages and printed out.

Electron Microscope Autoradiography. Root tissue, treated for 24 hr with ^{55}Fe at high specific radioactivity (7 $\mu Ci \cdot ml^{-1}$), was fixed in glutaraldehyde-OsO_4 by the procedure of Robards *et al.* (22) except that tissue washing in phosphate buffer after glutaraldehyde fixation was reduced to about 20 min. In comparable material labeled with ^{59}Fe, loss of tracer Fe during fixation and embedding amounted to about 40 to 50% of that originally present. Autoradiographs of ultrathin plastic-embedded sections were prepared as described by Parry and Blackett (20).

RESULTS

MOVEMENT OF TRACER AND NONTRACER IRON INTO THE XYLEM

The xylem sap from excised roots which had been pretreated at two temperatures was taken at intervals after the addition of labeled Fe (FeEDDHA) to the culture solution. Other published work showed that pretreatment at 10 C greatly increased the rate of sap flow (12) when roots were subsequently excised. After 16 hr a high specific radioactivity of labeled Fe was found in the exudate from both 10 and 20 C pretreated roots (Fig. 1). The approach to the situation where all of the Fe moving in the xylem sap was derived from the external solution was much more rapid in roots pretreated at 10 C, where the sap flow was greater. We concluded, therefore, that over a 24-hr period the movement of tracer Fe would represent a reasonable description of the Fe supply received by the shoot of intact plants where water flow in the xylem would be more rapid than in the excised roots.

IRON TRANSLOCATION FROM SEMINAL AXES

Figure 2 shows the translocation of iron from treated segments located at different distances along the seminal axis. Segments in the mature zones were located between, but did not include, lateral root branches. Translocation (about 50% of which was recovered in the shoot) was greatest in a zone 1 to 5 cm from the root tip; the mature portions of the axis supplied little Fe to other parts of the plant. This pattern was basically the same in +Fe and −Fe roots. Integration of the area under the curves in Figure 2 gives a ratio of −Fe to +Fe of approximately 7:1. This is somewhat less than the over-all response of the root system which is usually measured in the range of 10 to 100:1. This discrepancy may result from the lack of sufficient sampling points to define the curves over the whole range of root length.

The amount of labeled Fe associated with treated segments was inversely related to the pattern of translocation, the amount increasing in the mature zones of the root in both pretreatments (Fig. 3). For reasons made clear in the section on microautoradiography, little physiological significance attaches to this accumulation because, in the mature zone, most of the iron was associated with the root periphery and was not within the root tissues.

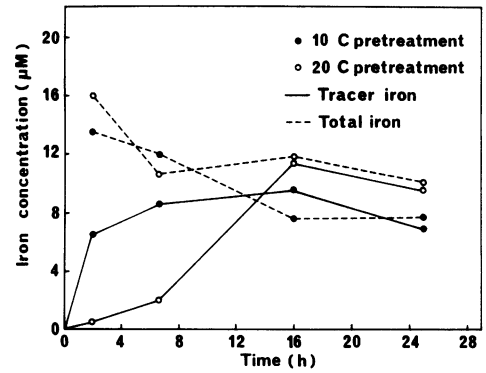

FIG. 1. Concentrations of tracer-labeled and unlabeled Fe in the xylem sap exuded at 20 C by detached barley root systems treated with labeled FeEDDHA (10 μM). During the pretreatment period plants had been kept for 5 days at the temperatures specified and grown in a solution containing 10 μM Fe.

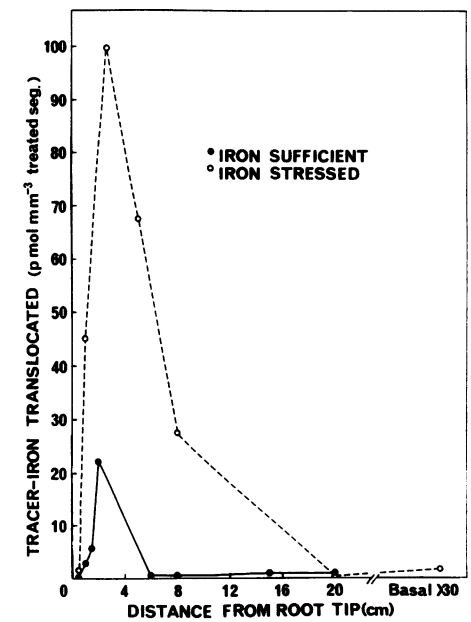

FIG. 2. Amount of labeled Fe translocated in 24 hr from treated segments located at various positions along the length of seminal root axes of barley. Plants had been pretreated in solutions with or without 10 μM Fe chelate and were supplied with 10 μM FeEDDHA + 1 μCi of ^{59}Fe ml^{-1} during the experiment.

TRANSLOCATION BY PRIMARY LATERAL ROOTS

As explained under "Materials and Methods," the performance of apical segments of lateral roots was tested by inserting the tip and an increasing length of developing root into the flow cell. Zones located at greater distances from the tip (>3.5 cm) were labeled in the way described for main axes. Lateral roots on −Fe plants were generally rather short and convoluted, very few of them being more than 2.5 to 3.5 cm in length. Figure 4, A and B shows the amounts of Fe translocated and bound, respectively, per unit volume of the segments. As with seminal axes, translocation was high in young zones of the root and relatively little in the more mature zones. Fe stress greatly increased the translocation from the apical zones without greatly increasing the amount of Fe bound to the segments. There was, again, an inverse relation between translocation and iron-binding by the treated segment.

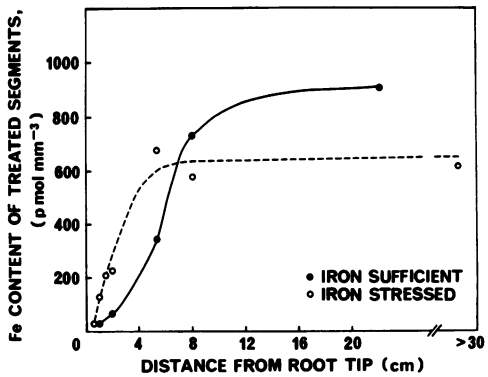

FIG. 3. Concentration of labeled Fe found after 24 hr in treated segments located at various positions along the length of seminal root axes of barley. Plants had been pretreated with or without 10 μM Fe chelate and were supplied with 10 μM FeEDDHA + 1 μCi of ^{59}Fe ml^{-1} during the experiments.

MICROAUTORADIOGRAPHY

A number of approaches to the distribution to Fe in root tissues have been made as part of a wider program to be reported elsewhere. The results presented here bear only on the distribution of labeled Fe in mature, nontranslocating parts of the seminal root axis, and in the zone characterized by high rates of translocation.

Iron Bound at the Root Periphery. In both the mature and the translocating zones of the axis a large accumulation of labeled Fe was found at the root periphery. At a distance of more than 20 cm from the tip, labeled Fe was found in an intense continuous band at the root surface. Nearer the root tip, the peripheral band was generally less intensely labeled but localized "hot" spots were clearly evident (Fig. 5A). Closer inspection (Fig. 5B) clearly indicates that the accumulation was on the outside surface of the epidermis. Examination of fixed, araldite-embedded material in the electron microscope revealed that hot spots were frequently associated with clumps of bacteria (Fig. 5C) which exist in, or on, the polysaccharide material at the root surface (mucigel). Such images suggest that Fe may be precipitated in foci which are predominantly on the outer surface of the bacterial colonies (Fig. 5D). A comparison of surface-labeling in a zone 1 cm from the root tip in sterile and nonsterile roots showed that high peripheral accumulation was present in both (Table I), but that the frequency of hot spots was somewhat reduced in the sterile roots (Fig. 5E).

Iron Penetration into Root Tissues. Cross-fire of β particles emitted from ^{59}Fe at the root periphery can cause reduction of silver grains in the emulsion lying over other tissues, thus giving a spurious impression of their radioactivity. This problem was largely overcome by using ^{55}Fe, the Auger electrons of which have a much lower energy (5–6 kev) and shorter range in tissue and emulsion.

In sections taken 1 to 2 cm from the root apex it was clear that during 24-hr labeling, the various cell types in the root all became radioactive with a distinct tendency for accumulation in the vicinity of the endodermis and pericycle (Figs. 5E and 6). This tendency was first discernible when the Casparian band was laid down in the endodermis (unpublished observations; see also ref. 2). In the 4- to 5-cm zone the Fe was distributed differently: there appeared to be a continuous decline in radioactivity across the cortex so that labeling in the stelar tissues was only slightly above background (Fig. 6). In older parts of the axis, autoradiographs from a different experiment showed that radial penetration of Fe was decreased to a still greater extent.

EFFECT OF ROOT LENGTH ON IRON MOVEMENT INTO XYLEM SAP

As an additional test of the conclusions from segment labeling experiments and from autoradiography, unbranched portions (1.5–7.5 cm in length) were excised from roots of 14-day-old −Fe plants. The proximal ends were sealed into glass tubes and the exudate gathered over a period of 24 hr while the apical portions of varying length were treated with labeled FeEDDHA. Table II shows that there was significantly ($P < 0.01$) more ^{59}Fe recovered in xylem sap when the length of root bathed by tracer was increased from 1.5 to 4.5 cm, but that a further increase in the length of root exposed did not significantly increase the content of tracer Fe in the sap.

EFFECT OF CCCP AND L-ASCORBIC ACID ON IRON BINDING AND LONG DISTANCE TRANSPORT

In an attempt to relate iron-binding by roots to metabolism, the uptake of labeled iron by −Fe plants was studied in the presence of a respiratory uncoupler (CCCP) and a reducing agent (ascorbic acid). Table III shows that 3 μM CCCP inhibited transport to the shoot by 93% although the Fe associated with the root was not affected significantly. By contrast, roots bound far less Fe in the presence of 100 μM L-ascorbic acid but transport to the shoot was not reduced significantly. Attempts to desorb labeled Fe from control roots using unlabeled 100 μM $Fe(NO_3)_3$ at 1 C removed only a minor fraction (about 20–25% of the total) over 24 hr. We concluded that much of the Fe was in a nonexchangeable or slowly exchangeable form, suggesting that it was precipitated.

DISCUSSION

Several observations in the present work with barley suggest that Fe translocated to shoots is absorbed largely in the apical regions of root axes and lateral branches. This restriction cannot be explained readily in terms of the anatomical development of the root. With Ca and Mg ions (15, 22) there appeared to be a good correlation between the decline in their entry into the xylem and the formation of suberized lamellae in the walls of endodermal cells (22). This process begins in barley plants at 8 to 12 cm from the root tip and is concluded at around 20 cm when all cells in the endodermis become suberized. The decline in Fe translocation occurs in advance of this process. In this respect it is worth

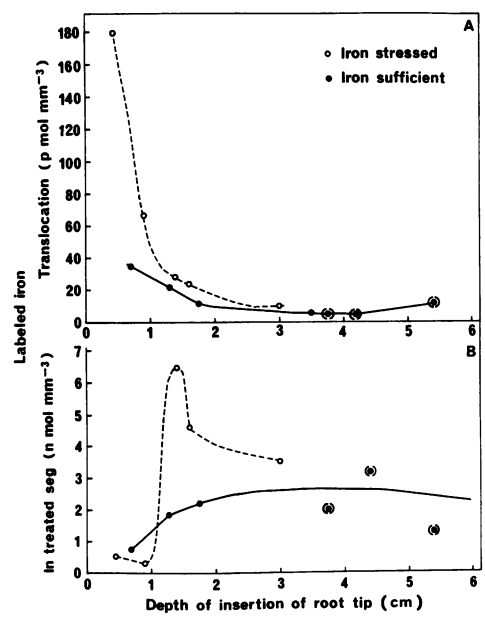

FIG. 4. Amount of labeled Fe translocated in 24 hr (A) or and bound to treated segment (B) by portions of primary lateral roots of barley. Plants had been pretreated in solutions with or without 10 μM Fe chelate and were treated with 10 μM FeEDDHA + 2 μCi of ^{59}Fe ml^{-1} during the experiment.

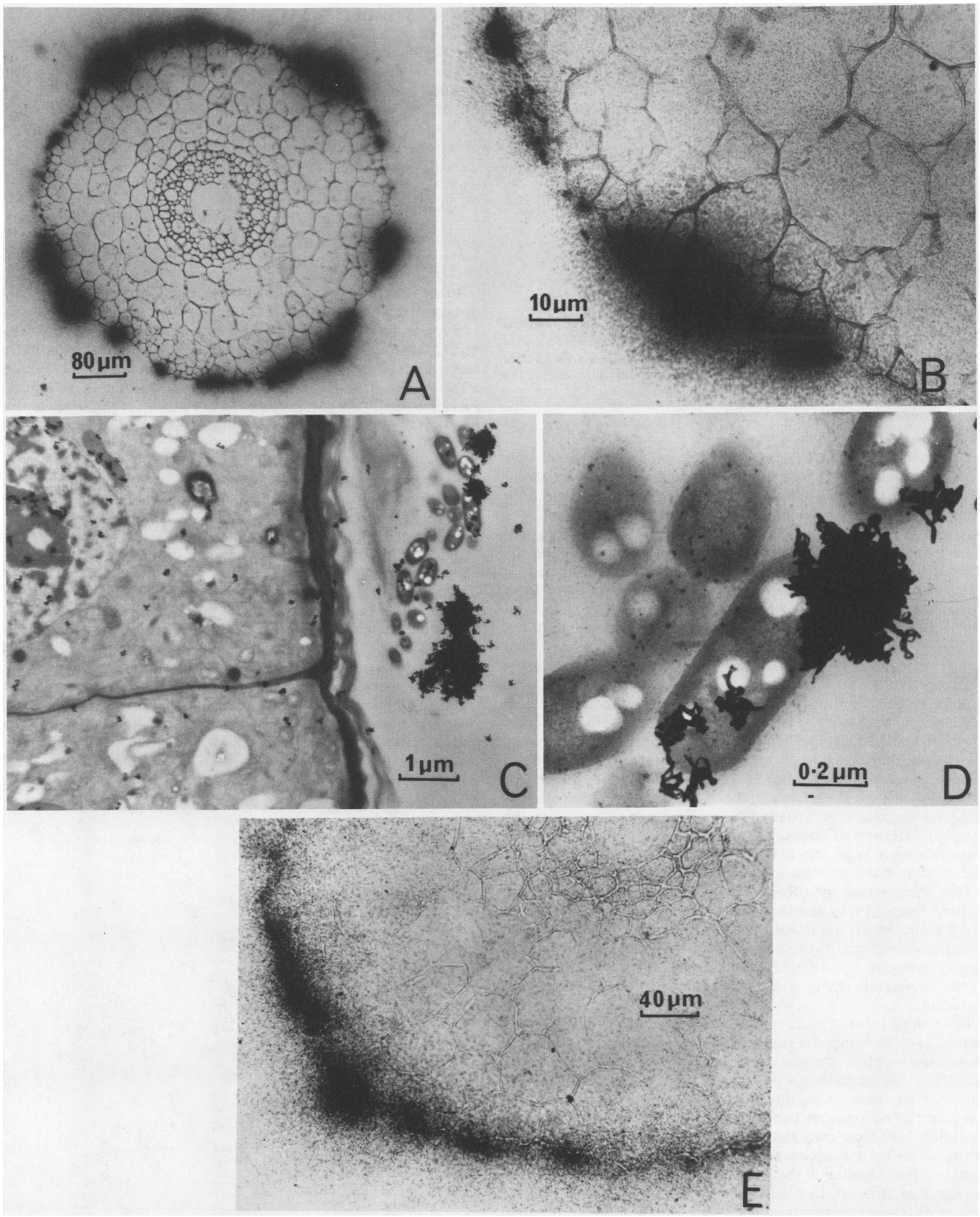

FIG. 5. Distribution of labeled Fe in root tissues of barley. A: section of unfixed frozen root approximately 1.5 cm from tip of a seminal axis labeled for 1 hr with 10 µM FeEDDHA + 3.27 µCi of ^{55}Fe ml^{-1}. Section thickness, 10 µM; emulsion, AR10; exposure, 49 days. B: high power view of (A) showing radioactive accumulation on the outer surface of epidermal cells. C: electron microscope autoradiograph of part of our epidermal cell and bacteria at the root periphery. Section of fixed material approximately 2 mm from tip of a root treated with 10 µM $Fe(NO_3)_3$ + 7 µCi of ^{55}Fe ml^{-1}. Section thickness, 200 nm. D: high power view of bacteria in (C) showing clumped distribution of radioactivity associated with outer surface of a colony. E: section of unfixed frozen root approximately 1 cm from tip of a sterile seminal axis labeled for 1 hr with 10 µM FeEDDHA + 4.8 µCi of ^{59}Fe ml^{-1}. Section thickness, 10 µM; emulsion, AR10; exposure, 26 days.

Table I. A comparison of the radioactivity of ^{59}Fe in various cell types by autoradiographs of transverse sections of barley root grown in sterile and non-sterile conditions.

Roots treated for 1 hr at 20 C with 10μM FeEDDHA plus 4.8 μCi ^{59}Fe ml^{-1}, pH 4.0
Frozen sections (10μM thick) exposed to AR 10 film at -25 C for 26 days.

Cell type	Density of silver grains (arbitrary units ± S.E)			
	Non-sterile		Sterile	
	Root 1	Root 2	Root 1	Root 2
Epidermis + periphery	6.9 ± 0.21	6.7 ± 0.28	7.15 ± 0.40	4.0 ± 0.23
Mid-Cortex	0.51 ± 0.09	0.75 ± 0.04	1.01 ± 0.08	0.63 ± 0.05
Endodermis	0.52 ± 0.03	0.85 ± 0.06	0.62 ± 0.06	0.31 ± 0.03
Xylem parenchyma	0.57 ± 0.03	0.74 ± 0.05	0.62 ± 0.06	0.37 ± 0.03

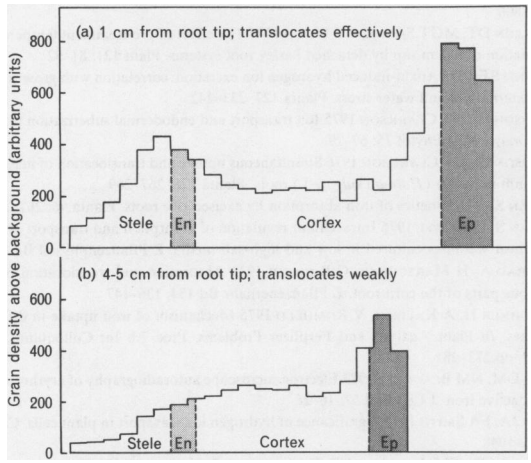

FIG. 6. Distribution of labeled Fe in transverse sections cut from seminal axes of barley. Roots were treated for 24 hr with 10 μM $Fe(NO_3)_3$ + 7 μCi of ^{56}Fe ml^{-1}. Section thickness, 10 μM; emulsion, Ilford G5; exposure, 4 days. En: endodermis; Ep: epidermal layer + root surface. Mean values from grain density measurements on eight sections at each location from three replicate roots; measurements by Leitz microscope photometer.

recalling that in *Z. mays*, Fe translocation to the shoot persisted in old regions of the root axis known to have endodermal suberization (14). The present work and studies of pea plants (3) show that the transport of Fe into the xylem depends strongly on coupled respiration. This suggests that metabolic factors may be responsible for translocation of iron from the younger zones. Autoradiographs show that iron supplied in the 1- to 2-cm zone moves radially through the cortex and into the xylem. The much greater mobility in this zone might be explained by the release of one, or a combination of several factors into the free space from the cortical cells. In soybean and tomato it has been suggested that "reductants" may be preferentially released in this zone (1, 4, 5). We have tried, without success, to observe sites of ferricyanide reduction using the technique of Ambler *et al* (1). The release of protons might also aid penetration of the free space by ionic forms of Fe; current ideas on proton extrusion during cell expansion (13) and in the maintenance of the electrical potential difference across the plasmalemma (21) are compatible with this suggestion. The work of Marschner *et al.* (19) showed, however, that roots of −Fe corn and barley plants did not release sufficient quantities of protons to produce any change in the pH of the culture solution. If the present results are to be explained in terms of the release of acid it seems likely that the amounts are small and their effects localized in the free space or at the root surface. In this respect we would point out that the rapid penetration of other trivalent metal cations, *e.g.* Sc^{3+} (9, 10) and La^{3+} (25, and unpublished observations), has also been noted in cells of the root apex and expansion zone. These ions are not reducible in the same way as Fe, but share the common characteristic of very low solubility in solutions greater than pH 4.5, especially in the presence of phosphate ions.

A third explanation of the results might be that some organic ligand is released in the translocating zone which complexes iron and increases its mobility in the root tissues.

More detailed studies of the physicochemical conditions at the root surface are highly desirable in view of the possibilities just mentioned. The present results indicate that comparative observation between translocating and weakly (non-) translocating zones of the barley root axis might be of great value in deciding on the salient features regulating iron translocation. If the same factor, or combination of factors, promotes Fe translocation in *Z. mays*, then a further comparison with barley should help to identify its nature, because it clearly operates over a much greater part of the root length (18).

The large accumulations of Fe which occurred in the more mature zones of barley roots, like those in pea (2), are extracellular and appeared to be precipitates. This process was assisted by, but was not dependent on microbial contamination (Fig. 5, A and E) and occurred when both ionic and chelated ferric iron were supplied to the root. Preliminary observations indicate that tracer Fe is desorbed reluctantly from the roots, but further data, particularly of an autoradiographic nature, are clearly needed about the location of Fe remaining in the root after desorption. Fe at the root periphery accumulated in pea roots at low temperature and in the presence of uncouplers (3) and in barley where translocation to shoots was 93% inhibited by CCCP. When Fe was presented to roots accompanied by the reducing agent ascorbic acid (Table III) the accumulation of tracer by the root was greatly diminished. This may explain how credible kinetic data have been reported (16) on the initial rates of Fe uptake by excised roots of rice, since the roots in those experiments were supplied with ferrous sulfate at pH 5.5. In general, however, the present work sounds a note of caution about the interpretation of studies on excised root systems in terms of the iron *nutrition* of plants. Quite apart from the extracellular accumulation of Fe, which may or may not be a factor, it seems likely that in barley, at least, only a limited proportion of the root surface supplies iron to the xylem and thence to the shoots. This conclusion is supported by the rapid approach to isotopic equilibrium of the tracer iron in the xylem sap from excised roots (Fig. 1), suggesting that previously accumulated Fe at the root periphery or in the cortical cells contributes little Fe to the xylem. The situation would appear to be the same in intact wheat seedlings (17) where very little of the ferrous iron accumulated in a 4-hr pulse label was translocated to the shoots

Table II. The effect of root length on amount and concentration of labeled iron in xylem sap exuded from excised roots of iron-stressed barley plants

Roots treated for 24 hr at 20 C with 10μM FeEDDHA plus 0.2 μCi ^{59}Fe ml^{-1}, pH 5.5. Values quoted ± standard error of the mean.

Root length	Exudate volume	Labeled iron	Concentration of labeled iron
cm	μl	p mol	μM
1.5	4.7 ± 0.6	14.0 ± 2.6	3.0 ± 0.26
4.5	8.9 ± 1.5	42.3 ± 6.2	4.7 ± 0.90
7.5	10.6 ± 1.9	38.9 ± 7.9	3.6 ± 0.90

Table III. Effect of CCCP and L-ascorbic acid on binding and translocation of labeled iron by roots of iron-stressed barley plants.

Roots treated for 18 hr at 20 C with 10μM Fe $(NO_3)_3$ + 0.2 μCi ^{59}Fe ml^{-1}, pH 4.0. Values quoted ± standard error of mean.

Treatment	Labeled iron uptake (% control)	
	Shoot	Root
	n mol g^{-1} root (fresh weight)	
Control	177 ± 61	2844 ± 510
+3μM CCCP	12 ± 3.9 (7%)	3197 ± 598 (112%)
+100μM Ascorbic acid	147 ± 41 (84%)	363 ± 120 (13%)
+3μM CCCP + 100μM Ascorbic acid	10 ± 1.5 (6%)	754 ± 77 (27%)

during the subsequent 24-hr chase even though the root tracer concentration was 30 to 60 times higher than that of the shoot throughout.

In barley, in common with many other plants, Fe deficiency enhances iron translocation to the shoot. The results in this paper show that this is due to increased activity within the normal pattern of absorption rather than to bringing into operation zones of the root axis which are normally inactive in absorption.

In earlier studies, measurements were made of the absorption and translocation of a number of ions by various parts of the barley root system. From this work two contrasting patterns of behavior were observed with respect to distance from the root tip (root age) which were probably related to the mobility of ions in the symplast and to anatomical development. Phosphate (11), potassium (23), and ammonium (8) ions were translocated to the shoot from all locations tested in roots of 3- to 4-week-old plants. These ions probably enter the symplast near the root periphery and cross, even thickened endodermal cells, via plasmodesmata (22). By contrast, Ca and Mg appear to cross the cortex by an extracellular (apoplast) pathway, being absorbed directly at the endodermis. The suberization of the endodermis drastically reduces translocation of these ions to the shoot (15, 22); this observation implies that Ca^{2+} and Mg^{2+} move sparingly in the symplast. The translocation of Fe differs from both of these patterns of behavior, and is probably determined by metabolic factors unrelated to root anatomy.

Acknowledgments—We wish to record our thanks to R. K. Jones and B. Y. Lalit for help in initiating this project, to C. Basham for adept technical assistance, to D. Parry for preparing the electron micrograph autoradiographs, to the Chemistry and Electronic services of the Letcombe Laboratory for chemical and radio assays, to H. Marschner and J. C. Brown for their helpful discussion of points raised in this work, and to M. G. T. Shone for his help in preparing the manuscript.

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