animals show HSA still remaining from preinjection (700 ± 50 μg/ml), the inhibition of uptake of labeled HSA can probably be accounted for by isotope dilution. There is no change in coelomic fluid *p*H resulting from preinjection.

This capacity to selectively inhibit uptake was further studied in vitro. In these studies, cells from normal animals were used in the concentration of 5×10^5 per milliliter. Figure 2 shows that HSA (320 μg/ml) added to HSA-C^{14} (320 μg/ml) causes a reduction in the uptake of the HSA-C^{14}. Figure 3 shows that the uptake of BSA-C^{14} (80 μg/ml) is decreased by added BSA (80 μg/ml) but not by added HSA (80 μg/ml). The results of these experiments show that coelomocytes of *S. purpuratus* can discriminate between two closely related proteins.

A model predicting such discrimination has been put forward by Boyden (*8*), and while the results presented here fulfill predictions of his model, they indicate nothing about the nature of the actual uptake mechanism. The constellation of characteristics shown by these experiments is what one might find if one eliminated the proliferating effector cell lines (lymphocytes and plasma cells) from the vertebrate immune response, but retained the effector cell functions in a cell population which exhibits no lasting increase in number of cells or number of receptor molecules per cell as a result of contact with antigen.

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Calcium Supply to Plant Roots

Abstract. *Direct observations of the calcium-45 distribution around the roots of plants growing in soil showed a pattern in direct contrast to that predicted from calculations based on the calcium concentration of the soil solution, transpiration rates, and plant calcium content. Whereas calcium-45 accumulation at the root surface was predicted, depletion was observed. It is suggested that preferential water movement in larger pores may decrease the expected solution calcium accumulation at the root surface. However, autoradiographs may give no indication of soil solution concentration depletion or accumulation because of the relatively high level on the colloid surface. The nature and extent of the depletion indicates how profoundly the plant root alters its immediate environment in the soil.*

The two processes moving ions from the bulk of the soil to the root are mass flow and diffusion (*1*). These processes are complemented by the effect of plant-root extension. The contribution of mass flow, where ions are passively carried toward the root, has been calculated from the product of the amount of water moving to the root and the concentration of the soil solution. Barber, Walker, and Vasey (*2*) suggested that, on many of the soils they studied, calcium would accumulate around the root as a result of movement of calcium ions by mass flow in the soil solution. This conclusion was based on a range of 1 to 380 parts per million (ppm) calcium in soil solutions extracted from 135 soils of the North Central United States and a figure of 0.3 percent calcium in the corn plant. Al-Abbas and Barber (*3*) found a similar result for soybeans. Their calculations suggested that four times as much calcium reached the root as was absorbed by the plant.

In our experiment, wheat plants, with a calcium level at the end of the experiment (14 days) of 0.30 percent, absorbed an average of 256 μg of calcium per plant, whereas mass flow could have transported 2522 μg of calcium in the soil solution (with a level of 178 ppm in the soil solution, and while the plants transpired at a rate of 170 ml/g). It would appear that these results support the hypothesis of Barber *et al.* in indicating the presence of accumulated calcium at the root surface. But no such accumulation was observed in any of the autoradiographs taken (Fig. 1a). A similar experiment with a lower, but still adequate level of calcium in the soil solution (151 ppm) gave an even more marked depletion zone (see Fig. 1b).

The technique used in this study was similar to that described earlier (*4*). Wheat seeds were sown in soil in a narrow container so that the root grew against a thin mylar film. After the movement of ^{45}Ca in the soil, x-ray film was placed against the mylar overnight. Three wheat seedlings were

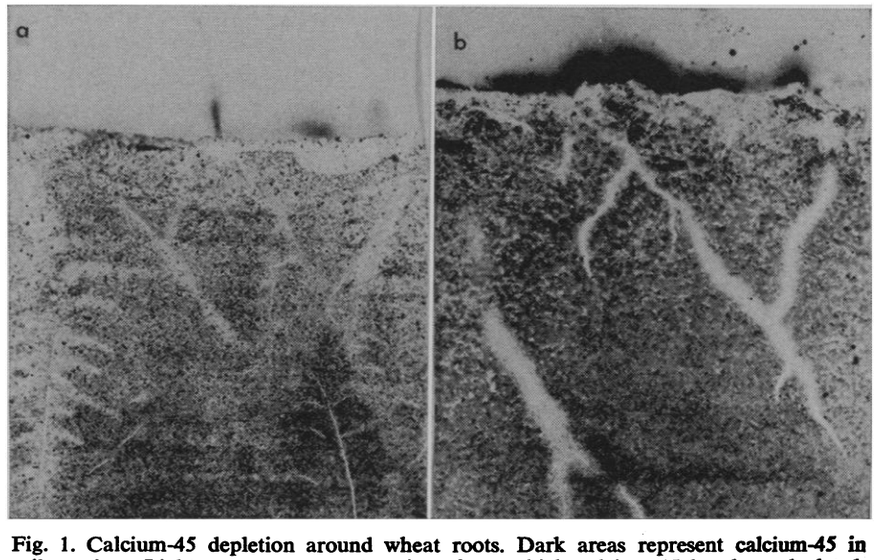

Fig. 1. Calcium-45 depletion around wheat roots. Dark areas represent calcium-45 in soil or plant. Lighter areas represent regions from which calcium-45 has been depleted. (a) In experiment 1, the calcium-ion concentration was 178 ppm in soil solution on day 12. (b) In experiment 2, the calcium-ion concentration was 151 ppm in soil solution on day 8.

grown in fertilized Wongan loamy sand (*p*H 5.0 in 1:5 0.01*M* $CaCl_2$, clay content 13 percent and a water content at field capacity of 9 percent) for approximately 2 weeks. Calcium-45 (3 μc per gram of soil) was mixed thoroughly through the soil before germination. The plants were grown under controlled conditions of temperature, light, and humidity; containers were made up to weight with water once, then twice daily. Autoradiographs were obtained every 2nd day, and the calcium content on acid-digested plant material grown as a control under identical conditions was determined by atomic absorption spectrometry (*5*). The soil solution was extracted by centrifugation (*6*).

Beginning on the 5th and definitely obvious by the 7th day, slightly lighter areas on either side of the root developed on the autoradiographs (Figs. 1a). These areas continued to widen until the 14th day, at the end of the experiment. The well-delineated boundary of the depletion zone which has been reported for molybdenum (*7*), phosphorus (*8*), and zinc (*4*) was not apparent, which suggests that the concentration gradient between the soil and the root was appreciably affected by mass flow.

The observation of a depleted zone around the root seems anomalous in view of the calculated possible mass flow contribution of more than ten times the plant absorption, which should cause calcium to accumulate at the root surface. Further conflicting evidence came from the fact that transpiration rates had little effect on calcium absorption in a duplicate experiment: plants transpiring at high rates had calcium contents (270 μg per plant) similar to those transpiring at one-third of the high rates (245 μg per plant), while maintaining similar growth.

The first anomaly may be related to the fact that the depletion zone around the root shown on the autoradiographs is a measure of the total ^{45}Ca. The absence of activity near the root indicates that ^{45}Ca has been reduced to a low level and, for this to happen, considerable quantities of exchangeable calcium must have been removed from the colloids within the depletion zone. When such quantities of ^{45}Ca are displaced from the soil surfaces in the vicinity of the root, the calcium concentration in the soil solution should initially increase, but will subsequently be modified by plant uptake and back diffusion. The quantity of calcium appearing in the plant is considerably less than a conservative estimate of that removed from the depletion zone, so that calcium must be moved away from the root by back diffusion or the downward movement of water following irrigation. However, ^{45}Ca in solution may be at a greater concentration near the root than in the bulk soil solution and still not be detected in the autoradiographs because the total ^{45}Ca concentration in the region has been appreciably decreased.

The small effect of transpiration may be associated with the fact that the accumulation of calcium at the root surfaces cannot be calculated simply by assuming that the soil solution concentration is constant in the larger pores carrying water to the root. If the soil pores were regarded as a simple capillary system, the ions in the soil solution would be moved toward the plant root, and when more water was added to the plant system, ions would not be available to desorb calcium from charged sites on the colloid to replenish the soil solution. Thus, particularly in the larger pores, water with a much lower ^{45}Ca content may be carried to the root. Increasing transpiration therefore may not appreciably increase the movement of ^{45}Ca to the root.

Irrespective of the detailed method of estimating the mass flow contribution, the fact remains that water flowing to the plant root will be carrying some calcium into the surface region, and yet the overall effect is that mobilized exchangeable calcium has moved away from the root. The situation at the boundary of the depletion zone is very complex, and the processes of mass flow and back diffusion may each occur concomitantly, but their relative effects will vary in relation to the pore size. It would be expected that the coarser pores with a low ratio of surface to volume would be mainly involved in mass flow and finer pores with considerably larger ratios of surface to volume may be the principal path for back diffusion.

Despite the problems in calculating the mass flow contribution to calcium movement, the most significant feature of the autoradiographs presented is that a large amount of the exchangeable calcium in the region adjacent to the root has been mobilized in 5 days, and it appears likely that hydrogen ions or possibly other substances from the root are implicated. This observation indicates how profoundly the plant root alters its immediate environment and suggests that much of the work seeking to analyze movement of ions to root surfaces needs reappraisal.

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Nucleic Acid Molecules: New Microdiffusion Technique for Visualization

Abstract. *A microdiffusion technique, developed for visualization of nucleic acid molecules in the electron microscope, requires less than 0.01 microgram of nucleic acid. Although originally developed for free nucleic acids, the method can be applied to virion suspensions for direct visualization of their genomes; less than 10^{10} virions per milliliter are required. Results agree well with those yielded by the diffusion technique of Lang, Kleinschmidt, and Zahn.*

The length and molecular weight of intact viral nucleic acid molecules have been successfully measured by the technique of Kleinschmidt and Zahn (*1*), in which macromolecules are adsorbed and fixed in a structureless protein monolayer before transfer to the grid of an electron microscope for visualization. In the original procedure (*1*), DNA molecules were spread on water; subsequently (*2*) the molecules were allowed to attach to the monofilm by diffusion-