

the second electrophoresis, all components are uniformly 1 cm long, the width of the applied gel strip. The spots are, however, compressed as they enter the 8 percent gel by a factor which is the ratio of the respective migration rates in the two gels. The lactic dehydrogenase isozymes, for example, appear as rectangular spots 3 mm (5 percent direction) by 5 mm (8 percent direction).

SAMUEL RAYMOND
BARBRO AURELL

William Pepper Laboratory of
Clinical Medicine, University of
Pennsylvania, Philadelphia

References and Notes

1. M. D. Poulik and D. Smithies, *Biochem. J.* **68**, 636 (1958); O. Smithies and M. D. Poulik, *Nature* **177**, 1053 (1956).
2. P. E. Hermans, W. F. McGuckin, B. F. McKenzie, E. D. Baird, *Proc. Staff Meetings Mayo Clinic* **35**, 792 (1960).
3. G. C. Ashton, *Nature* **180**, 917 (1957).
4. S. Raymond and M. Nakamichi, *Anal. Biochem.* **3**, 23 (1962).
5. S. Raymond, *Clin. Chem.* **8**, 455 (1962).
6. A. L. Latner and A. W. Skillen, *Lancet* **1961-1**, 1287 (1961).
7. This work was supported by grants from the National Institutes of Health and the Institute for Cooperative Research of the University of Pennsylvania.

4 June 1962

Anion-Exchange Properties of Plant Root Surfaces

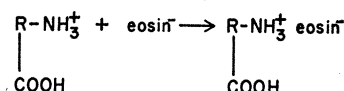
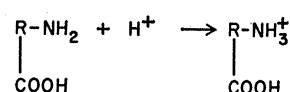
Abstract. Anion-exchange properties of plant root surfaces were demonstrated by using negatively charged anion dyes. The site of the positive charges may be amino groups which become positively charged in the presence of hydrogen ions but are neutralized under alkaline conditions.

Cation-exchange properties of root surfaces have been demonstrated by Williams and Coleman (1). Further experimentation has shown the (i) nature of the exchange sites, (ii) the cation exchange capacities of different plant species, and (iii) how differences in capacities can be used to explain differences in uptake of monovalent and divalent cations. Plant roots contain many —COOH groups whose negative charge can adsorb positive ions, such as methylene blue⁺, Ca⁺⁺, NH₄⁺, and H⁺. Plant cells also contain considerable quantities of protein (2) whose amino groups (NH₂) become positively charged under acidic conditions. These groups on root surfaces could be a potential source of positively charged spots for anion-exchange reactions. Plant roots placed in negative dyes, such as eosin or acid fuchsin, become highly colored, especially on the tips and on meristematic

tissue. If this adsorption is on positively charged spots, then anion exchange can take place on the root surface.

An experiment was performed in which eosin dye previously adsorbed on root surfaces was replaced by a series of sodium salts—NaF, NaCl, NaBr, NaI, NaNO₃, Na₂SO₄, Na₂HPO₄, NaHCO₃, Na₂CO₃, and NaOH. (Figure 1 shows the results.) A different quantity of dye is released by each anion, and the amount released increases with increasing salt concentration. A lyotropic series of replacement is observed in which the order of replacement of dye is: F⁻ < Cl⁻ < Br⁻ < I⁻ < NO₃⁻ < SO₄²⁻ < HPO₄²⁻, CO₃²⁻, HCO₃⁻, and OH⁻.

The replacing power of neutral salts increases slowly as the concentration of salt increases from 0.0001N to 0.1N. The shape of the curves resembles cation exchange curves for replacement of methylene blue by chloride salts (1). A peculiarity in the shape of the curve is observed with the alkaline salts Na₂CO₃, NaHCO₃, Na₂HPO₄, and NaOH. In 0.0001N solutions slightly more eosin was replaced by these salts than was replaced by neutral salts. However, in 0.001 to 0.1N solutions, a plateau of release was reached and further increases in concentration did not release more eosin. The data suggest that positive exchange spots are neutralized by basic salts, thus causing adsorbed eosin to be released. A possible adsorption site that would be affected in this manner is the NH₂ group of the protein molecule. The group could react as follows:



Addition of OH⁻ to eosin-saturated groups would bring about neutralization of the positively charged H-ion, thus forming water and neutral NH₂ groups and causing the release of eosin to the solution:

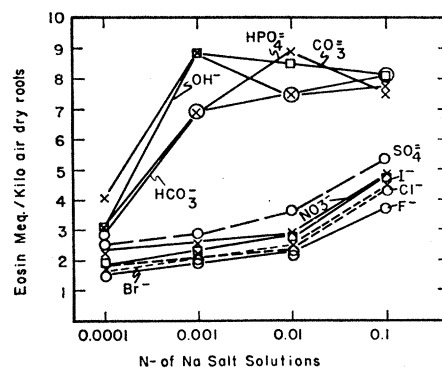
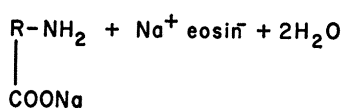
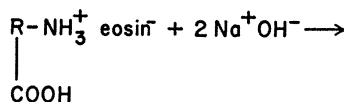


Fig. 1. Exchange of eosin from intact barley roots by salts as a function of concentration.

If this reaction takes place, adsorption of anion dyes should be affected by the pH of the medium. A number of NaCl solutions were prepared in which the pH was varied from 1 to 12 with HCl and NaOH. Three Algerian oat plants grown in Hoagland solutions for 2 weeks were washed in distilled water, placed in 0.1N HCl for 1 minute, rinsed five times in 200 ml of distilled water, and then placed in eosin at pH 7 for 30 seconds. The dyed roots were then rinsed five times in water and placed in 50 ml of 0.1N NaCl (adjusted to pH values from 2 to 12) for 30 seconds. The concentration of eosin in the solutions was determined with a colorimeter (Klett Summerson) with a blue filter (No. 42). (It was later observed that a No. 54 green filter, with eosin adjusted to pH 5.5, gave more reproducible readings.) The results plotted with x's are shown in Fig. 2. The data indicate that acid solutions were quite effective in keeping amino groups hydrogen-saturated, thus allowing eosin to remain tightly bound.

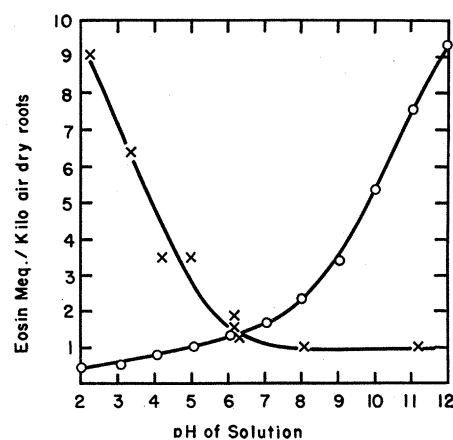


Fig. 2. The effect of the pH of solutions upon the amount of eosin removed from oat roots: (x) pH of saturating eosin solutions; (o) pH of displacing NaCl solutions.

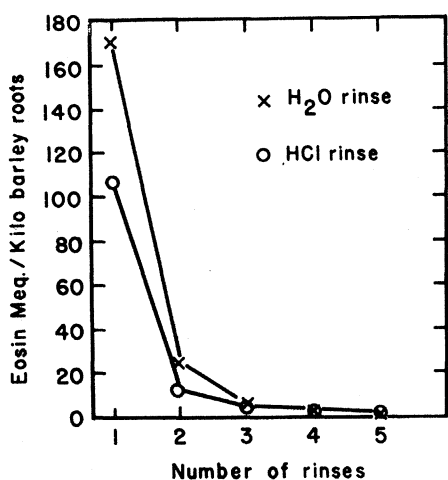


Fig. 3. Amount of eosin released from barley roots by rinsing with H₂O or 0.01N HCl.

The data plotted with o's in Fig. 2 were obtained by saturating oat roots with eosin at pH values ranging from 2 to 12 and releasing the dye from the roots with 50 ml of 0.1N NaOH. The resulting alkaline eosin solution was neutralized with 50 ml of 0.1N HCl, and the eosin concentration was determined on the colorimeter. These data substantiate the other data of Fig. 2.

The excess eosin solution which adhered to the root after immersion in the dye solutions was removed by five 10-second rinses in 200 ml of distilled water.

The amount of dye in each succeeding rinse appeared to be too high for mere dilution of excess saturating solution left clinging to the root surface. Calculation showed that released dye was in greater concentration than it should have been and, therefore, the rinse must have contained adsorbed dye released from the root. To minimize the loss of adsorbed dye, roots were washed in HCl at the pH of the saturating dye solution. A comparison of the amount of dye removed from root surfaces by HCl (at pH 2.0) and by H₂O is found in Fig. 3. In each instance the roots were agitated vigorously for 6 seconds in the wash solutions. An increase in pH of the wash solution results in a loss of exchange sites which is the difference in the sum of the milliequivalents of eosin removed from the roots in the first and second rinses. The use of HCl as a rinse has the advantage of maintaining the acidity at the pH used in saturating the roots with dye while the excess saturating solutions from the root surface are being removed. From Fig. 1 it is obvious that Cl⁻ itself has considerable replacing

power for removing adsorbed eosin from the root surface. However, of the anions examined, only F⁻ ion replaced less eosin than did Cl⁻.

Lundegårdh (3) found that anions had a marked effect on root potentials. He concluded that "potential measurements favor the assumption that anions of neutral salts are adsorbed to the surface layer of the protoplasm by exchange. Hence the surface layer also possesses positive valencies, which in the absence of anions of acids are saturated only by OH⁻ (and a small amount of HCO₃⁻) ions." My results are in agreement with Lundegårdh's conclusion of positive adsorption sites. However, the data in Figs. 1 and 2 are more suggestive of the type of site which amino groups would produce and these groups, rather than being saturated with OH⁻, appear to be neutral in alkaline solutions.

D. EMERTON WILLIAMS

Department of Soils and
Plant Nutrition,
University of California, Berkeley

References and Notes

1. D. E. Williams and N. T. Coleman, *Plant Soil* **2**, 243 (1950).
2. D. K. Dougall and K. Shimabayashi, *Plant Physiol.* **35**, 396 (1960); T. A. Lamport and D. H. Northcote, *Nature* **188**, 665 (1960).
3. H. Lundegårdh, *Kgl. Skogs-Lantbruksakad. Tidskr.* **8**, 234 (1940).

27 July 1962

Particle-Size Analysis of Valders Drift in Eastern Wisconsin

Abstract. Analysis of the fraction of Late Wisconsin deposits less than 2 mm in diameter has shown a wide range in textural composition of glacial tills. Massive lake sediments were high in clay content and contained less sand than the till of the same textural class. Glaciofluvial and eolian deposits were loamy to sandy and sandy, respectively.

Valderan drift of Late Wisconsin age (1) constitutes the parent material from which soils in a large area of eastern Wisconsin (see inset, Fig. 1) are formed. This drift was derived in part from pre-existing lake sediments, and as a result has a characteristic reddish-brown hue (2, 3). Some of these deposits have been shown previously to be of finer texture than associated Cary (Middle Wisconsin) drift (2, 3). The primary objective of our study has been to characterize in more detail the particle-size-distribution of Valderan deposits in this region.

Samples of drift were collected

throughout the region at depths ranging from 2 to 15 feet (4). Most of them were obtained from C horizons—a layer of relatively unweathered drift below the soil solum believed to be similar to that from which at least part of the overlying soil was formed. When lithologic discontinuities were found below the solum, the significant contrasting layers were sampled. All samples were classified as to genetic origin in the field on the basis of their lithology and the physiographic characteristics of the land forms on which they were found. Care was taken not to sample disturbed or contaminated material.

Bulk samples were air-dried, and gravel was removed with a sieve (No. 10 U.S. Standard). The particle-size distribution of the fraction less than 2 mm in diameter was determined according to the method outlined by Day (5); a hydrometer was used to determine the percentage of silt and of clay. Carbonates were not removed before analysis. After the results had been plotted on cumulative curves, percentages of sand (2.0 to 0.05 mm), silt (0.05 to 0.002 mm), and clay (< 0.002-mm diameter) were obtained and plotted on a textural triangle (U.S.D.A.) (6) (Fig. 1).

Results of these analyses showed that 169 of 288 samples contained more than 27 percent clay. These were included in four fine or moderately fine textured classes as follows: (i) 67 clay loams, (ii) 60 clays, (iii) 25 silty clays, and (iv) 17 silty clay loams. The high clay content of soils formed from these sediments and the characteristic red color, have resulted in the local designation, "red clays." In a similar manner, the area in which these sediments occur is commonly called the "eastern red clay region."

Of the 119 samples containing less than 27 percent clay, 97 were medium to moderately coarse textured and were included in the following textural classes: (i) 48 sandy loams, (ii) 43 loams, and (iii) 6 silt loams. One sample of sandy clay loam texture containing less than 26 percent clay was also included in this group. The remaining 21 samples were coarse textured and included 7 loamy sands and 14 sands.

An interesting characteristic of most of the samples was their silt content. Out of the 288 samples, 209 contained between 30 and 50 percent silt. Of these 209 samples, 185 consisted of glacial till, mainly of loam texture or finer; 23 were glaciolacustrine sediments; and 1 was a sandy loam glaciofluvial deposit.