is unlikely. Such traces, if present, would exhibit tryptic activity also upon incubation with unprocessed x-ray film. Control experiments showed that this was not the case.

In an attempt to prevent the agglutination by protamine, red cells were suspended in 0.5 to 2.0 percent saline dilutions of bovine albumin. Differences were observed between the dilutions necessary to protect normal cells, cells sensitized with anti-D antibody, or coated with autoantibodies (patients' cells). Similar results were noted with saline suspensions of cells exposed to varying concentrations of protamine sulfate. The critical values varied with cells from different individuals. However, normal cells were always the most resistant, and Rh-sensitized cells were the most susceptible to agglutination (Table 1).

Table 1. Effect of mixtures in promoting (+) or inhibiting (−) the agglutination of test cells. Red blood cell suspensions, 2 percent. Volume of reactants, 0.1 ml each. Titer of direct Coombs' test on patients' cells and sensitized cells, 1: 256. Cell suspensions incubated with the mixtures for 10 min at 37°C and subsequently centrifuged for 1 min at 1000 rev/min.

Agglutinating medium	Normal cells	Patients' cells (auto-antibodies)	Sensitized cells (isonati-bodies)	Trypsinized cells
Protamine 1% Saline	++++	++++	++++	−
Protamine 0.08% Saline	−	+	++++	−
Protamine 1% Albumin 2%	−	−	++++	−
Protamine 1% Albumin 0.5%	−	++	++++	−
Protamine 1% Trypsin 1%	−	−	++++	−
As above, pre-incubated 10 min	−	−	++++	−
Trypsin 1% Saline	−	−	++++	−
Saline alone (Control No. 1)	−	−	−	−
2% albumin alone (Control No. 2)	−	−	−	−

When trypsin and protamine were added simultaneously to the cell suspensions, only cells coated with anti-D antibody agglutinated. Incubation of protamine and trypsin for 10 min prior to their addition to these cell suspensions had the same effect. Since trypsin digests protamine, it became apparent that the enzyme itself must function as the agglutinating agent. Tests performed with trypsin alone confirmed its selective ability to agglutinate cells coated with anti-D antibodies (Table 1).

This effect is independent of the concentration of trypsin from 0.05 to 1.0 percent but requires incubation and seems to diminish with decreasing direct Coombs' titers.

These observations substantiate the reported differences between auto- and isoimmune antibodies (*1*). The trypsin effect also suggests a connection between two seemingly unrelated phenomena: (i) trypsinized cells are more susceptible than normal cells to agglutination by gamma globulin (*8*); (ii) the isoantibodies in hemolytic anemia of the newborn are gamma globulins, whereas the autoantibodies in acquired hemolytic anemia, are not (*9*).

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4. 5.0 ml of a 2-percent O/CDE cell suspension in saline incubated for 30 min with 0.015 ml of anti-D (albumin) typing serum gave a direct Coombs' test titer of 1:256.
5. 1-percent saline solution of "Difco 1:250" trypsin plus 1/10 its volume phosphate buffer *p*H 7.1; filtered before use. Trypsinization of cells was achieved by incubating a normal cell suspension with 1/10 volume enzyme mixture for 10 min.
6. Cell suspensions made up in a 0.02*M* NaCl-phosphate buffer, *p*H 6.0.

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On the Sources of Soil Phosphorus Absorbed by Mycorrhizal Pines

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Previous studies have indicated limited absorption of phosphorus by nonmycorrhizal pines in certain soils (*1, 2*). Either successful mycorrhizal inoculation or heavy applications of inorganic phosphates were effective in overcoming acute phosphorus deficiency (*2*). The mode of action of the mycorrhizal association is unknown. Among hypotheses are the possibility that activity of the extensive mycelium ramifying from the root tips might release phosphorus from the soil organic matter in the immediate vicinity of unsuberized root tips (*1, 3*) or the phosphorus might be transmitted to the root through fungal hyphae (*4*). Release into the soil solution remote from the root tips is unlikely, since benefit to pine was not shared by a companion grass (*2*).

Distinction between soil inorganic and organic phosphorus forms is possible. When inorganic P^{32} is added to the soil, it rapidly undergoes dilution and isotopic exchange with the inorganic soil phosphorus in accessible sites (*5*). Phosphorus in organic forms, being held by covalent bonds, remains unlabeled except as P^{32} is gradually incorporated by microbial synthesis (*6*). Accordingly, in a soil so treated, a plant able to utilize organic forms would be characterized by a

phosphorus content of lower specific activity than one absorbing entirely from inorganic sources.

This possibility was tested, employing two prairie soils naturally lacking mycorrhizal fungi: A Carrington silt loam from Wisconsin contained 5.3 percent organic matter and 6 and 36 lb inorganic P per acre, respectively, as determined by Peech's (*7*) and Bray's (*8*) methods. Comparable values for an O'Neill sandy loam from Iowa were 2.8 percent, and 1 and 22 lb. Phosphorus as $KH_2P^{32}O_4$ (*9*) was added at rates of 10 and 100 lb P per acre. For uniform distribution, solutions of radiophosphorous were sprayed upon the air-dried soil as it tumbled in a rotating drum; 1000 g of the Carrington soil or 930 g of the O'Neill soil were placed in 1-qt pots.

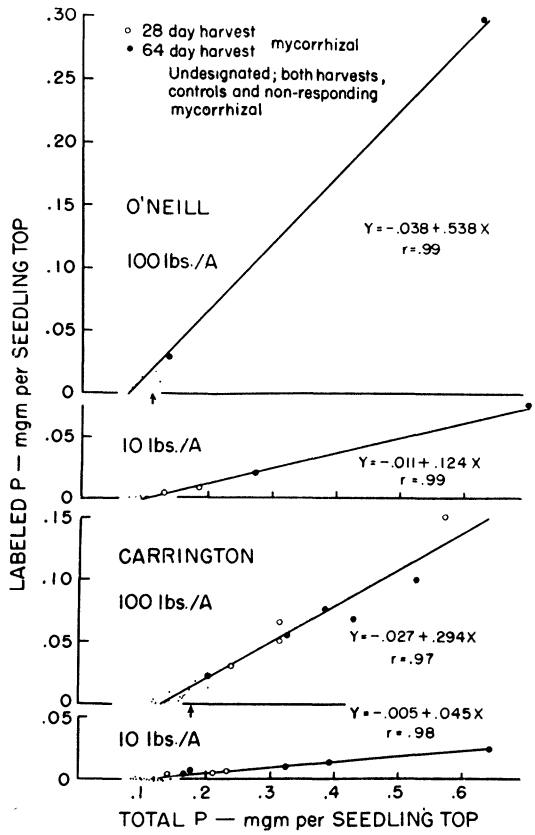

Fig. 1. Uptake of labeled phosphorus as related to increased total phosphorus in the tops of Monterey pine seedlings. Arrows mark average content at time of transfer.

Two seedlings that had been grown for 7 wk in 0.1 Hoagland's solution lacking phosphorus were transplanted to each container. Those transferred to the Carrington and O'Neill soils had mean dry weights of 0.23 and 0.16 g, respectively, and an average phosphorus content of 0.086 percent, an amount slightly less than the initial seed content. Segments of mycorrhizal pine root were used as inoculum, and the controls received similar segments autoclaved. A week later Italian rye grass was sown into designated treatments with and without pines. The grass and one-half of the pine treatments were harvested after 28 days, and the remainder of the pine after 64 days. Only the tops were analyzed for total phosphorus (*10*) and radiophosphorus (*11*).

Table 1. Percentage of total phosphorus in the tops of grass and mycorrhizal pines derived from labeled inorganic phosphorus added to soil.

Soil and rate of addition (lb/acre)	Rye grass (%)	Monterey pine	
		Regression coeff. Fig. 1 (%)	Mean of individual estimates* (%)
Carrington, 10	5.5 ± .1	4.5	5.6 ± .6
Carrington, 100	40.1 ± .2	29.4	35.0 ± 2.9
O'Neill, 10	14.7 ± .5	12.4	12.5 ± 1.0
O'Neill, 100	64.8 ± .6	53.8	63.0 ± 7.

* Including only those containing, in the 100 lb/acre series, more than 0.020 mg labeled P, or, in the 10 lb/acre series, more than 0.002 mg.

Slow and irregular response of the inoculated seedlings, apparently resulting from the initial deficiency, destroyed the original statistical design. All seedlings evidencing appreciable increases in dry weight or phosphorus content were mycorrhizal, however, whereas none of the nonmycorrhizal controls responded. Inoculated seedlings with few or recently formed mycorrhizae at harvest likewise showed little or no response. Although the tops of the nonmycorrhizal and nonresponding seedlings increased slightly in dry weight, they decreased in phosphorus content. This apparent loss was presumably due chiefly to transfer into the roots. Despite this reduction in total phosphorus, at the 100-lb rate 4.8 and 11.3 percent of the phosphorus in the tops from the Carrington and O'Neill soils, respectively, was derived from the added phosphate. These observations are in accord with the finding of Kramer and Wilbur (*12*) that nonmycorrhizal pine roots do accumulate phosphorus from solution but to a lesser extent than mycorrhizal roots.

As is shown by Fig. 1, the increased total phosphorus associated with mycorrhizal formation is linearly related to uptake from the inorganic addition. The relationship is affected by soil and rate of addition, but not by duration or extent of mycorrhizal activity as reflected in total phosphorus content. The constancy of the relationship is noteworthy only in that it corresponds precisely with the expected behavior of the common horticultural species.

One may also compare the ratio between added and native soil phosphorus in the totals absorbed by the mycorrhizal pines and by rye grass in comparable cultures. Allowance must be made for the initial phosphorus content of the pine, either (i) through use of the regression coefficients of Fig. 1, or (ii) by deducting the mean content of nonresponding plants at harvest from the individual values for responding seedlings. Table 1 presents the two estimates for the pine

seedlings compared with the values for the grass at the end of the 28-day period.

Both pine seedling estimates are weighted by data from the 64-day harvest, in contrast to the 28-day harvest of the grass. Microbial synthesis and possibly chemical exchange as well would have continued through the intervening period, gradually reducing the specific activity of phosphorus available from inorganic forms. The slopes of the regressions are further diminished by the lowered phosphorus content of nonresponding plants. Allowance for these factors indicates fair agreement between the respective values for pine and grass.

Thus it appears that in each of the four combinations of soil and fertilizer, Italian rye grass and mycorrhizal pine seedlings utilized the added inorganic phosphorus and native sources to a very similar degree. It may be concluded that the mycorrhizal roots possessed no exceptional facility for utilizing phosphorus from the soil organic matter.

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Separation of *dl-cis* from *dl-trans* Labeled and Unlabeled Chrysanthemumic Acids on Paper

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Carbon-14 labeled *dl-cis,trans*-allethrin has been synthesized (*1*), and its physiological and insecticidal behavior in houseflies and cockroaches is now being investigated (*2*). Obviously, the value of this investigation would be greatly increased by the comparison of the eight isomeric labeled allethrins with one another.

The unlabeled compounds have been prepared from the isomeric chrysanthemumic acids and allethelones following their resolution by standard chemical techniques (*3*). Because only 2 g of the *dl-cis,trans*-2 C^{14} chrysanthemumic acid was available to us, other methods of separation were required, and a method applicable to microgram quantities was very desirable. The first step toward such a procedure has been accomplished by the successful separation on paper of the *dl-cis* from the *dl-trans* chrysanthemumic acid.

The solvent was prepared by shaking 50 parts of isopropyl acetate with 25 parts of 10-percent aqueous ammonium hydroxide. The mixture was then allowed to stand in the chromatographic chamber overnight for separation and for saturation of the atmosphere. Saturation was facilitated by hanging wide paper strips in both layers of the solvent mixture. Whatman No. 1 paper in 1-in. strips was drawn once through 1-percent aqueous ammonium chloride and air-dried in the hood. Five to 20 μg of both labeled and unlabeled *dl-cis,trans*-chrysanthemumic acids and also of authentic samples (*4*) of *dl-cis* and *dl-trans* acids (mp 115° to 116° C and mp 51° to 54°C, respectively), were applied to separate strips of paper. The strips were irrigated for approximately 4 hr by ascension of the organic layer of the solvent mixture. The strips were then air-dried, and the zones of ammonium salts were located with the potassium permanganate and benzidine sprays applied as described by Winteringham (*5*).

The developed chromatograms of the *dl-cis,trans*-chrysanthemumic acids had dense zones of Rf 0.37 and 0.60 (*6*) and, by comparison with the developed chromatograms of the authentic samples, these zones were found to be *dl-trans* and *dl-cis* acids, respectively. On standing, the polarity of the solvent increased, owing apparently to slight hydrolysis of the isopropyl acetate. As a consequence these Rf values gradually shifted during a 2-wk period to 0.51 and 0.72, respectively. This change in no way interfered with the use of the procedure, particularly when authentic samples were run simultaneously.

In addition to the zones of *dl-cis* and *dl-trans* acids, zones of Rf 0.0–0.01, 0.20, and 0.98 have been observed. The zones of Rf 0.0–0.01 and 0.98 have been common to all chromatograms that contained *dl-trans* acid. The zone of Rf 0.20, although visible only in chromatograms obtained from 20-μg samples of *dl-cis,-trans* acid, was detected radiometrically in the labeled product. The zone of Rf 0.98 also was obtained on all chromatograms of the *dl-cis* acid, but it occurred to a lesser extent in the authentic samples than in the *dl-cis,trans* acids. Although the zone of Rf 0.98 was detectable on strips run as blanks and was probably due partly to traces of impurities in the solvent, there is no doubt that additional material traveled to this zone when the acids were chromatographed. The presence of materials having these Rf values has also been demonstrated in a commercial sample of *dl-cis,trans*-chrysanthemumic acid.

To learn more about the impurities, unsprayed chromatograms from 20-μg samples of labeled acid were sectioned according to sprayed duplicates and