TABLE 1
PERCENT OF THE TOTAL ACTIVITY IN THE FILTRATE FROM A PHOSPHOMOLYBDATE PRECIPITATION OF AN ALIQUOT OF AN IRRADIATED UNIT OF KH_2PO_4

Portion of sample examined	Method of counting	Relative amounts of sample	Activity in cts/sec	Percent of total activity
Total	Solid	1 ×	41.3	
Filtrate	Solid	1 ×	1.81	4.4
Total	Liquid	4 ×	32.3	
Filtrate	Liquid	4 ×	1.42	4.2
Total	Liquid	8 ×	64.7	
Filtrate	Liquid	8 ×	2.72	4.4

and a fourth aliquot were made to the same volume for the total activity determination. The radioactivity of the solutions was determined by means of a solution counter. An additional aliquot was prepared for solid counting with an end window counter. Instead of making the final solutions to volume, the phosphorus was reprecipitated as magnesium ammonium phosphate. The results are presented in Table 1. An average of 4.3% of the activity was left in the filtrate when phosphorus was precipitated as the phosphomolybdate.

TABLE 2
PERCENT RADIOACTIVE CONTAMINATION BY LOWER VALENCE FORMS OF PHOSPHORUS IN SHIPMENTS OF ORTHOPHOSPHATES

Material	Date of shipment	Percent contamination
Rock phosphate	10/18/48	12.1
KH_2PO_4	4/19/49	4.3
KH_2PO_4	5/10/49	0.0
KH_2PO_4	6/25/49	0.0

A check was made to determine whether the activity in the filtrate was due to incomplete precipitation or the presence of a possible lower state of oxidation of phosphorus. Samples of the stock solution were oxidized in one case by means of bromine water and in a second case by means of $KMnO_4$. The phosphorus was then precipitated as the phosphomolybdate, and the radioactivity in the filtrate was measured. No detectable amount of activity was found in the filtrate. This suggested that some lower oxidation state of phosphorus was present in a radioactive form. A further check on the nature of the contaminant was made. From another aliquot of the $KH_2P^{32}O_4$ phosphorus was precipitated in alkaline solution as magnesium ammonium phosphate. Phosphites are not soluble under these conditions. Pyro and meta phosphates are soluble, although small amounts may conceivably be adsorbed. The fact that no activity was found in the filtrate suggests the contaminant is a phosphite. Hull (*1*) working with P^{32} separated by cyclotron bombardment found $H_3P^{32}O_3$ as a contaminant in an $H_3P^{32}O_4$ solution. These results were repeated using H_3PO_3 as carrier and the same percent of contamination was found.

Three separate samples of $KH_2P^{32}O_4$ from Oak Ridge and one sample rock phosphate irradiated in a neutron pile have been examined for this contaminant, using H_3PO_3 as carrier. The results are presented in Table 2.

The reason for the difference in extent of contamination is not known. The half-life of the contaminant has been determined and found to equal the theoretical 14.3 days for P^{32}. In all probability the contaminant is principally a salt of phosphorus acid. Wilson (*4*) has shown that phosphorus in this form will not exchange for orthophosphate phosphorus. Libby (*2*) has shown that bombardment of P^{+5} with neutrons from a Ra-Be source surrounded by water results in a 50% conversion to P^{+3}. Phosphite salts are quite stable. The lower oxidation states of phosphorus are relatively easily oxidized to the phosphite in the presence of air and moisture.

The implications in the practical use of neutron-irradiated phosphates are twofold. First, any possible use of radiophosphorus, either in tracer work or therapeutically, should take into account the possible presence of this phosphite contaminant of probably high specific activity. Second, a possible method for the preparation of carrier-free phosphorus suggests itself. It would be valid only if the lower oxidation state of phosphorus were caused by the ejection of the phosphorus atom due to some specific nuclear reaction. This would probably be the reaction with sufficient recoil energy for ejection. It would necessitate a slow rate of reoxidation of the P^{+3}.

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Recovery of Growth Regulator from Plants Treated with 2,4-Dichlorophenoxyacetic Acid

Robert L. Weintraub, James W. Brown, and John N. Yeatman[1]

Camp Detrick, Frederick, Maryland

Although plant growth regulators are being extensively employed in a variety of practical applications, relatively little is known of the physiological mechanisms involved and virtually nothing as to the fate of such substances after absorption by the plant. Many of the developmental responses of plants following local application of exogenous growth-regulating chemicals serve to demonstrate that there is transport of a stimulus within the organism. In a few instances evidence has been obtained that the translocated substance is the applied compound itself, or some closely related derivative thereof, rather than an endogenous hormone the formation or movement of which has been influenced by the treatment (*2*, *3*, *5*). Experiments with a radioactively labeled growth regulator (2-iodo^{131}-3-nitrobenzoic acid) have indicated that the *radioactivity* becomes concentrated in meristematic

[1] The authors are indebted to Don C. Wood for performing the indoleacetic acid color tests and to Jean C. Nickerson, Katharine N. Taylor, and Edwin L. Wilson for assistance with the bioassays.

regions, and it has been stated that the intact molecule can be isolated from such tissue (*3*).

The present note reports recovery of *physiologically* active material from sites to which it must have been transported internally following application of 2,4-dichlorophenoxyacetic acid (2,4-D).

As an illustration of the results obtained, a single experiment may be described. A 0.0025-ml drop of 95% ethanol containing 5 μg of 2,4-D and 25 μg of Tween-20 was placed near the base of each of the two primary leaves of 141 eight-day-old greenhouse-cultured seedlings of *Phaseolus vulgaris* var. Black Valentine. This dose is sufficient to stop growth of the terminal bud but would permit subsequent vigorous development of axillary shoots. The plants were left in the greenhouse 24 hr, and then the primary leaves were severed at the base of the petioles and the remainder of the aboveground portion of the shoots was dried overnight in a forced draft oven at 56° C. The tissue was ground in a Wiley micromill to pass 40 mesh, and duplicate 2-g aliquots were refluxed for three successive 6-hr periods in fresh 50-ml portions of 80% ethanol. The six extracts were prepared individually for assay by evaporating off the alcohol, acidifying the aqueous residue with tartaric acid, extracting with several portions of diethyl ether, adding absolute ethanol to the combined ether extracts, and evaporating off the ether. The ethanol solutions were brought to the desired volumes by adding sufficient ethanol, water, and Tween-20 to give final concentrations of 95% ethanol and 1% Tween-20. Activity was measured by the leaf repression bioassay of Brown and Weintraub (*1*) and expressed in terms of an amount of 2,4-D which would produce an equal response. The recoverable fraction of the applied activity depends presumably upon a variety of factors, including the size of the applied dose, the interval between treatment and harvest, the environmental conditions during this period, and the extraction technique.

The 2,4-D microgram equivalents found in the duplicate samples were: first extraction—108,122; second extraction—6,8; third extraction—3,2; total of three extractions—117,132. Similarly prepared extracts from untreated plants exhibited no activity by the leaf-repression test. The total amount of 2,4-D applied to the plants was $5 \times 2 \times 141 = 1410$ μg. The dry weight of the deleaved shoots was 9.95 g. Hence an average of 124 2,4-D microgram equivalents was obtained from stem tissue of plants which had received $2/9.95 \times 1410 = 283$ μg of 2,4-D; the recovery was thus 44% of that applied.

As it seems unlikely that all the 2,4-D placed on the leaves actually entered and was exported from them, the recovered activity probably represents a major proportion of the translocated growth regulator. The experiments of Mitchell *et al.* (*3*), Wood *et al.* (*5*), and Rice (*4*), which are, however, in many respects not directly comparable with the fore-going, indicated maximum exports from leaf to stem of roughly 50% to 70% of the applied dose.

The leaf-repression response is not specific for 2,4-dichlorophenoxyacetic acid but is produced by a wide variety of compounds exhibiting so-called formative activity. Hence the results described do not constitute evidence that the active constituent in the extracts is identical with that applied to the plant. Other possibilities are that the extracted compound is (1) a derivative of 2,4-D or (2) an endogenous growth regulator of which the concentration in the extracted tissue has been greatly increased by application of 2,4-D to other parts of the plant.

With regard to the first possibility, solvent partition experiments indicate that the extracted growth regulator is an acid, or easily dissociable salt thereof, and not a neutral or basic compound. It can be extracted by ether completely from an aqueous solution of pH 3 but practically not at all from one of pH 7.6. From ether solution it is extracted completely by 1% aqueous sodium bicarbonate. Furthermore, the potency of the extracts cannot be due to such derivatives as 2-(2′,4′-dichlorophenoxy)-ethanol, 2,4-dichloroanisole, 2,4-dichlorophenetole, 2,4-dichlorophenol, 2-chlorophenoxyacetic acid, and phenoxyactic acid, as the activities of these compounds in the leaf-repression bioassay are less than 0.5% of that of 2,4-D. 4-Chlorophenoxyacetic acid exhibits activity of the same order of magnitude as that of 2,4-D and has not been eliminated as a possibility, although it seems unlikely that this compound would be produced from 2,4-D *in vivo*.

The following results indicate that the activity is not due to auxin *a*, auxin *b*, indole-3-acetic acid, or indole-3-acetaldehyde, the only naturally occurring growth regulators which have so far been identified. The extracted growth regulator shows no diminution of activity after standing in 13*N* sulfuric acid or after boiling in 1*N* NaOH. Auxin *a* is alkali-labile, indole-3-acetic acid is acid-labile, and auxin *b* is sensitive to both acid and alkali. The activity of indole-3-acetic acid in the leaf-repression bioassay is less than 1% of that of 2,4-D, so that the indole-3-acetic acid content of the shoots would have to be of the order of 10,000 μg/g to yield the assay results found. This quantity would be easily detectable with the Salkowski acid-ferric chloride reagent, which was found to produce an unambiguous color with as little as 2 μg of indole-3-acetic acid, but no color with highly active extracts from 2,4-D-treated plants. Indole-3-acetaldehyde would seem to be excluded also by the solvent partition result.

In summary, after application of 2,4-dichlorophenoxyacetic acid to the primary leaf of a bean seedling, a considerable fraction of the applied growth-regulatory activity can be extracted from other parts of the plant. The available evidence is consistent with the view that the recovered activity is due to 2,4-dichlorophenoxyacetic acid itself or to some dissociable salt thereof.

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