or albinism *per se* could not be ascertained from the present observations.

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[1] Nilan, R. A., *Conference on Radioactive Isotopes in Agriculture*, 151 (U.S. Atomic Energy Commission, 1956).

Metabolism of Carboxyl-^{14}C-labelled 2,4-Dichlorophenoxyacetic Acid by Bean Stems: Heterogeneity of the Ethanol-soluble, Ether-insoluble Products

There is a considerable disagreement in the literature concerning the nature and number of radioactive metabolites which can be isolated from bean plants following exposure to 2,4-dichlorophenoxyacetic acid (2,4-D) labelled with carbon-14 (refs. 1–6). Because of this situation it was decided to re-investigate this problem in connexion with our work on the induction of callus growth in bean stem sections by 2,4-D (ref. 7).

The details of the preparation and isolation of the radioactive, water-soluble products from bean stems which were treated with carboxyl-^{14}C-2,4-D will be reported elsewhere. Briefly, bean stems were vacuum infiltrated with 2 per cent sucrose and 10^{-4} *M* carboxyl-^{14}C-2,4-D (2 mc./m*M*), surface sterilized by brief immersion in 0·5 per cent sodium hypochlorite solution, rinsed in sterile distilled water, and incubated in nutrient medium for 2 days. They were lyophilized, pulverized, and continuously extracted with wet acetonitrile. After removal of the solvent by distillation, ether-soluble material was extracted at *p*H 1·5, and sedimenting insoluble matter discarded. The aqueous layer was chromatographed on a 'Dowex-1' formate column using a gradient of increasing formic acid concentration. The radioactive peak was freed from formic acid and further purified by passage through a water-saturated silicic acid column using water-saturated 35 per cent *n*-butanol in chloroform as the eluting agent. The product was homogeneous by paper chromatography in the solvent of Jaworski *et al.*[4] and contained no detectable ninhydrin-positive contaminants.

When this material was absorbed on to a small amount of charcoal ('Nuchar') in a sintered glass funnel, the elution pattern shown in Table 1 was obtained. Separate re-application of each of these four fractions to fresh charcoal resulted in their elution at the same relative positions. When another aliquot of the purified material was applied to a charcoal column which differed from that described by Stambaugh and Wilson[8] only in that the column was doubled in length while column diameter and volume of eluting solvent were halved, the distribution of radioactivity shown in Fig. 1 was obtained. This pattern was very reproducible and suggests the presence of some 10 different components in this mixture. In practice, it was found that not all these materials were equally stable, and if great care was not taken during the chromatography on 'Dowex-1', there was considerable hydrolysis, resulting in the release of some radioactivity in ether-soluble form accompanied by the appearance of at least 10 distinct ninhydrin-positive materials in the water-phase. The remaining water-soluble radioactivity was eluted from charcoal only with 1 per cent ammonium in 25 per cent ethanol.

Table 1. Elution of Purified Radioactive, Water-soluble and Ether-insoluble Bean Extract from 'Nuchar'

A total of 3,000 c.p.m. were applied to 0·5 gm. charcoal. Three 10-ml. fractions were collected with each solvent

Solvent	Total c.p.m. eluted
Water	144
25 per cent ethanol (aqueous)	763
1 per cent NH_4OH in 25 per cent aqueous ethanol	1,496
50 per cent aqueous acetonitrile	306
Total recovered	2,709

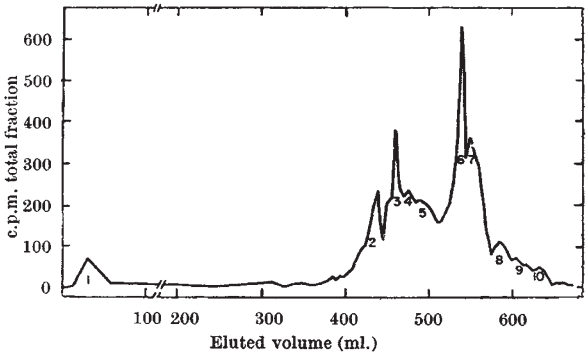

Fig. 1. The elution of radioactivity from 12-in. charcoal columns. 5 ml. fractions were cut between 350 and 700 ml. eluted volume

Holley[2] suggested that the ether-soluble hydrolysis product of the water-soluble bean fraction might be 6-OH-2,4-D, even though the compound was unknown at that time. Since then, 6-OH-2,4-D has been prepared by Cavill and Ford[9]. Using a small sample of this material, generously supplied by Dr. D. L. Ford, we found that it travels on paper indistinguishably from 2,4-D in the solvents previously used. However, when methylethylketone–conc. ammonium–water (100 : 5 : 3·2 v/v/v) was used the two compounds were readily separated. In this solvent the ether-soluble radioactive hydrolysis product from the bean extract was clearly separated from both these known substances (R_F: 2,4-D = 0·35; 6-OH-2,4-D = 0·61; bean extract hydrolysate = 0·0). Counter-current distribution of the ether-soluble material at *p*H 5·1 between phosphate buffer and diethylether resulted in the separation of four radioactive components. These results suggest that the previously reported main product of 2,4-D metabolism in beans[1–4] is indeed a complex mixture of amino-acid amides of several different and as yet unknown acid metabolites of 2,4-D. Further work towards the identification of these acids is in progress.

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[1] Butts, J. S., and Fang, S. C., in *Conf. Radioisotopes in Agriculture*, 209 (U.S. Government Printing Office, Washington, D.C., 1956).
[2] Holley, R. W., *Arch. Biochem. Biophys.*, **35**, 171 (1952).
[3] Jaworski, E. G., and Butts, J. S., *Arch. Biochem. Biophys.*, **38**, 207 (1952).
[4] Jaworski, E. G., Fang, S. C., and Freed, V. H., *Plant Physiol.*, **30**, 272 (1955).
[5] Weintraub, R. L., *Proc. North Central Weed Control Conf.*, 6 (1953).
[6] Weintraub, R. L., Yeatman, J. N., Lockhart, J. A., Reinhart, J. H., and Fields, M., *Arch. Biochem. Biophys.*, **40**, 277 (1952).
[7] Bach, M. K., and Fellig, J., in *Proc. Fourth Intern. Conf. on Plant Growth Regulation*, edit. by Klein, R. M., Boyce Thompson Inst., N.Y., 1959 (State Univ. of Iowa Press, Ames, Iowa) (in the press).
[8] Stambaugh, R. L., and Wilson, D. W., *J. Chromatography*, **3**, 221 (1960).
[9] Cavill, G. W. K., and Ford, D. L., *J. Chem. Soc.*, 565 (1954).