

PRETREATMENT EFFECTS ON THE RATES OF ALDRIN METABOLISM IN PEA PLANTS (*PISUM SATIVUM*)

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Abstract

A study has been made of the rate of aldrin metabolism by a number of plant species. In roots of pea seedlings grown in medium containing aldrin, the disappearance of aldrin was greater than the production of dieldrin, including that which could be accounted for by transport to the tops. By contrast, peas without aldrin pretreatment metabolized aldrin mainly to dieldrin by epoxidation.

Measurements of enzymic activity with pea root extracts confirmed the inductive effect of aldrin pretreatment on the rate of aldrin metabolism.

The aldrin-metabolizing activity showed similar characteristics to mammalian systems in that pretreatment with substances such as phenobarbital and aniline affected activity.

Compared with other plant species grown in the presence of aldrin, peas maintained low levels of aldrin and dieldrin, although rates of aldrin uptake were similar, without showing the adverse effects on normal development which occurred in some plant species.

I. INTRODUCTION

There are many reports of metabolic transformations of pesticides in plants (Casida and Lykken 1969). *N*-Dealkylation of substituted urea herbicides (Frear *et al.* 1969) and epoxidation of aldrin and isodrin insecticides (Yu *et al.* 1971) have been reported, these authors demonstrating that the microsomes were the intracellular site of such metabolism. The present study confirms these reports of the site of activity and provides additional evidence that plant microsomes exhibit many of the characteristics of mixed-function oxidases found in animal microsomes.

It is commonly assumed that aldrin is metabolized exclusively to dieldrin in plants and that dieldrin is not further metabolized. Recently Yu *et al.* (1971) showed that aldrindiol was also a metabolite of aldrin. In addition, Weisgerber *et al.* (1970) demonstrated large conversions of organochloride insecticides including aldrin to water-soluble metabolites by a number of plant species after a 4-week period. However, there are few such studies of aldrin metabolism in plants and none known to the authors designed to detect possible inductive effects by pesticides.

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II. MATERIALS AND METHODS

(a) Reagents and Chemicals

Analytical grade aldrin was supplied by the Shell Chemical Co. Chromatographic standards of aldrin and dieldrin were purchased from the Polyscience Corporation. The purity of analytical aldrin was confirmed by the absence of extraneous peaks in gas chromatograms of standards and boiled tissue controls. Phenobarbital was purchased from May and Baker Ltd.; glucose 6-phosphate, glucose-6-phosphate dehydrogenase, and NADP from the Sigma Chemical Co., and bovine serum albumin from the Nutritional Biochemical Co. The hexane and isopropanol used were redistilled analytical reagents and all other chemicals and solvents were analytical reagent grade.

(b) Plant Materials

Sources and varieties of seed were as follows: wheat (*Triticum aestivum*) cv. Yalta T67, Sydney University Experimental Farm at Castle Hill, N.S.W.; maize (*Zea mays*) cv. GH128 hybrid and subterranean clover (*Trifolium subterraneum*) cv. Clare, H. G. Elrington Pty. Ltd., Kempsey, N.S.W.; peas (*Pisum sativum*) cv. Progress No. 9, F. Cooper Ltd., Wellington, N. Z.; barley (*Hordeum vulgare*), grown at Narrabri, N.S.W.; lupins (*Lupinus luteus*) cv. Weiko 3, Wesfarmers, Perth, W. A.; pumpkin (*Cucurbita pepo*) cv. Yates Queensland Blue; broad beans (*Vicia faba*) cv. Early Long Pod and climbing beans (*Phaseolus vulgare*) cv. Stringless Blue Lake, Arthur Yates Pty. Ltd.

(c) Growth of Plants

All seed was washed in distilled water and surface-sterilized by soaking in 0.5% aqueous sodium hypochlorite for 10 min. After several washings in distilled water the seed was planted in treated or untreated perlite and watered with the following nutrient solution at pH 6.0: MnSO_4 (0.004 mM), K_2SO_4 (0.080 mM), MgSO_4 (0.060 mM), CaCl_2 (0.040 mM), KH_2PO_4 (0.040 mM), NaNO_3 (0.070 mM), Sequestrene 138 (Fe-chelate) (7 mg/l), H_3BO_3 (0.700 μM), Na_2MoO_4 (0.160 μM), ZnSO_4 (0.060 μM), CuSO_4 (0.025 μM) and CoCl_2 (0.015 μM).

Plants were transferred from untreated to treated perlite by hand with gentle shaking to remove adhering perlite followed by careful replanting. Before analysis or preparation of cell-free extracts, plants were removed, shaken, and washed in running tap water followed by distilled water and partially dried with paper towels.

(d) Treatment of Perlite

Aldrin was ground with a small amount of perlite in a mortar and mixed thoroughly with additional perlite to give a final concentration equivalent to 2.2 kg/ha and spread in trays to a depth of 10 cm. Equimolar amounts of phenobarbital, aniline, or dieldrin were distributed in perlite by the same procedure.

(e) Whole-plant Analysis

Triplicate samples of plant material containing 1–3 g of roots were homogenized in 6 ml of isopropanol–hexane (1:1 v/v) in a Sorvall Omni-mixer for 5 min with cooling in an ice-bath. Distilled water (6 ml) was added and the mixture shaken and centrifuged for 2 min at 5000 g in an M.S.E. bench centrifuge. The upper organic phase was decanted and washed twice by shaking with an equal volume of 2% (w/v) aqueous sodium sulphate solution before analysis.

(f) Enzyme Preparation

Plant root extracts were prepared and assayed according to the method of Yu *et al.* (1971). Roots were homogenized with 0.1M sodium phosphate buffer, pH 6.5 (1 g root in 1.5 ml), squeezed through muslin, and the homogenate centrifuged at 22,000 g for 25 min. The pellet was discarded and the supernatant, termed the 22,000 g supernatant, was centrifuged at 105,000 g for 2 hr in a Beckman L2-65 ultracentrifuge. The final supernatant is referred to as the soluble fraction. The pellet was resuspended in buffer to make a final concentration equivalent to 1 g of plant tissue per 1.5 ml. All operations were carried out at 0–5°C. Protein concentrations of each preparation were determined by the method of Lowry *et al.* (1951) using bovine serum albumin as a standard.

(g) Enzyme Assays

To 1 ml of tissue extract was added 1.8 μ moles of NADP, 18 μ moles of glucose 6-phosphate, 1 unit of glucose-6-phosphate dehydrogenase, and 0.1M sodium phosphate buffer pH 6.5 to a final volume of 6 ml. Aldrin (5 μ g) in methylcellosolve (10 μ l) was added and the mixture was incubated at 30°C with continuous shaking. Reaction was stopped by extraction with 6 ml isopropanol-hexane (1:1 v/v).

(h) Analysis

The hexane extracts were analysed in a Perkin-Elmer F11 gas chromatograph equipped with a Ni-63 electron-capture detector. A 1.5-m glass column packed with 3% QF-1 on Gas Chrom Q (100-120 mesh) was used. The column was maintained at 190°C, the injection port at 200°C, detector at 200°C, with a nitrogen carrier gas flow of 15 ml/min.

Standard solutions of aldrin and dieldrin in hexane were prepared fresh on the day of analysis. Retention times were 1.6 min for aldrin and 3.5 min for dieldrin. Samples and numerous standards were injected in a random order to compensate for possible changes in the sensitivity of the detector during the period of measurement. Quantities of aldrin and dieldrin were computed using peak area by comparison with standard curves which were linear in the range 0-1.0 ng injected in 5 μ l of hexane. Variation in replicated analyses was consistently less than 2% of the mean in this range.

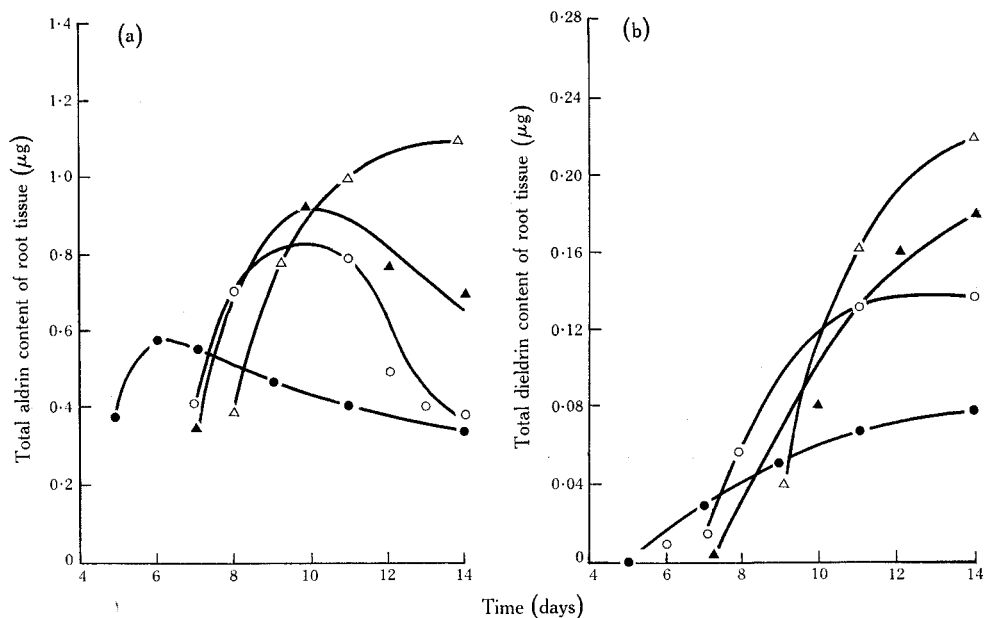


Fig. 1.—Changes in the aldrin (a) and dieldrin (b) contents of pea roots from seedlings transferred to aldrin 0 (●), 3 (○), 5 (▲), and 7 (Δ) days after planting. Analysis was performed as described in Section II(e).

III. RESULTS

An experiment was performed to determine the total levels of aldrin and dieldrin in pea roots with time of exposure to aldrin. One set of pea plants was grown for 14 days in the presence of aldrin while other plants, grown initially without aldrin, were transferred to perlite containing aldrin at 3, 5, and 7 days after planting. Triplicate samples of 1-3 g of plant root were analysed at intervals. Figure 1(a)

shows that the total aldrin content of root tissue reached a maximum 6–7 days after the plant came into contact with aldrin. For exposure periods longer than 7 days the aldrin level decreased. This decrease was far greater than the rate of appearance of dieldrin shown in Figure 1(b), indicating that reduction of the aldrin level in pea roots occurs by a process additional to metabolism to dieldrin.

A second experiment was designed to overcome the possibility of variation resulting from experimental technique and the stage of growth of the plant. All tissue extracts were prepared on the 14th day from roots of pea seedlings transferred to aldrin in perlite at 0, 3, 5, 7, 10, and 13 days after planting, and analysed for aldrin and dieldrin. Peas transferred on the fifth day were thus in contact with aldrin for 9 days before analysis. Figure 2 shows aldrin and dieldrin content recorded

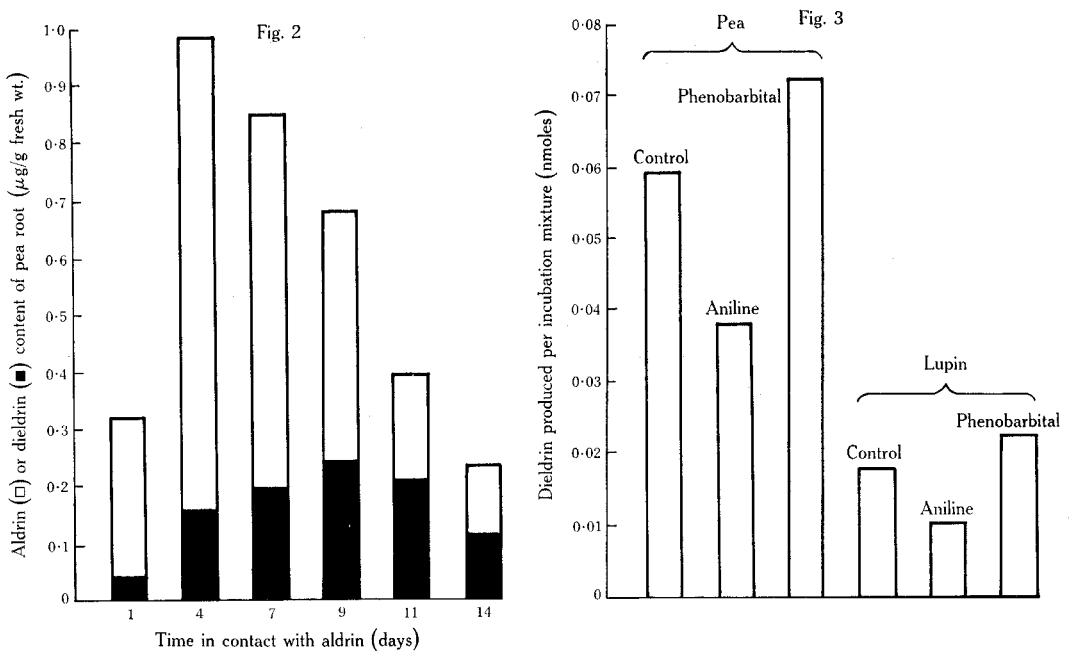


Fig. 2.—Aldrin and dieldrin in pea root 22,000 g supernatant from plants grown in contact with aldrin for varying periods of time. Homogenates were prepared as described in Section II(f) and analysed according to methods given in Section II(g).

Fig. 3.—Dieldrin produced from 5 μg of aldrin in 2 hr by 22,000 g supernatant of roots from plants grown normally or in the presence of aniline or phenobarbital. Assays were performed as described in Section II(g).

against time in contact with aldrin. With increasing time of exposure to aldrin the aldrin level rapidly reached a peak and then fell. Dieldrin increased in concentration more slowly and fell after 9 days.

Crude enzyme preparations from both pea and lupin roots converted aldrin to dieldrin, the reaction being stimulated by an NADPH-generating system and by Mg^{2+} and eliminated by boiling. Most of the activity was found in the microsomal fraction (105,000 g pellet). These results confirm those of Yu *et al.* (1971) with beans

and peas and indicated that a mixed-function oxidase system was operating. To determine whether plant mixed-function oxidases are subject to the non-specific types of induction characteristic of animal systems, peas and lupins were grown in the presence of aniline and phenobarbital. Root extracts from plants grown in the presence of phenobarbital converted aldrin to dieldrin more rapidly than did controls (Fig. 3). For plants grown in the presence of aniline the rate of dieldrin production was reduced. The mechanism of these effects upon the enzyme reaction was not examined and it is possible that aniline competes with aldrin for metabolism. However, the similarity with the animal system is striking.

Figure 4(a) shows the enzymic production of dieldrin in 4 hr by root tissue extracts of control peas and peas grown in the presence of aldrin. There was no difference in the net rate of total dieldrin formation. In Figure 4(b) changes in aldrin

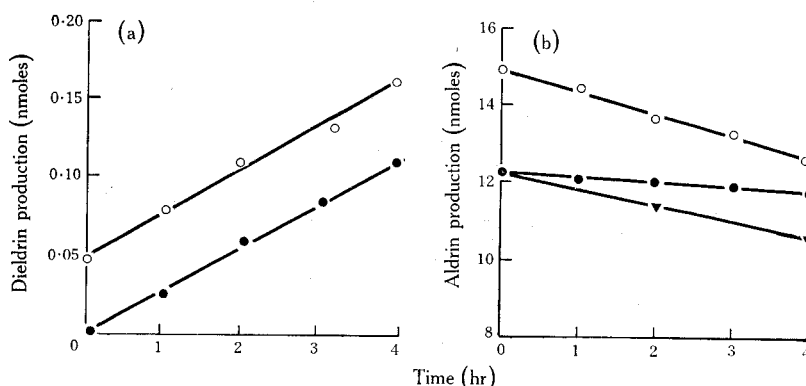


Fig. 4.—Enzymic production of dieldrin (a) and disappearance of aldrin (b) in pea root 22,000 *g* supernatant to which 5 μ g of aldrin was added, prepared from plants grown normally (●) or in the presence of aldrin (○) or dieldrin (▼).

concentration in the same incubation mixtures are shown. The decline of aldrin in control peas was only slightly greater than the dieldrin production shown in Figure 4(a). However, for peas grown in the presence of aldrin or dieldrin, removal of aldrin far surpassed dieldrin production by a factor of about 20.

By comparison with other plant species, peas grown in the presence of aldrin retained only small amounts of the pesticide at 3 weeks, as shown in Figure 5. Barley and clover are notable for the production of large amounts of dieldrin but pumpkin contained little dieldrin. The amount of aldrin plus dieldrin in the tops was relatively low. This experiment and others indicated that transport of pesticides to the tops of peas could not account for the removal from the roots. Clearly there are distinct species differences in the metabolism of aldrin by plants at this stage of growth.

Tests with 3-week-old plants showed that after exposure for only 3 days to aldrin, the total uptake of aldrin was similar in all species. The large differences in concentration of aldrin and dieldrin between species (Fig. 5) appeared only after longer exposures and were presumably the result of differential rates of metabolism or inductive effects.

IV. DISCUSSION

In this study rapid techniques of extraction which minimized solvent evaporation were used. This allowed the measurement of the absolute amounts of pesticide residues in intact plants. As indicated in Section III, peas exposed to aldrin for a period showed no greater rates of dieldrin formation than controls. However, levels of aldrin decreased more rapidly. The ratio $[\text{dieldrin}]/([\text{aldrin}] + [\text{dieldrin}])$ has often been used to estimate dieldrin formation, but this would have been misleading, as this study demonstrates.

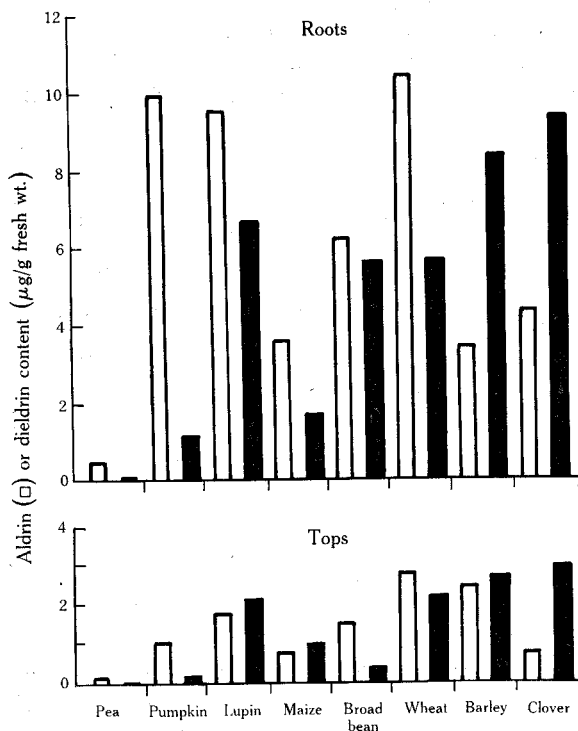


Fig. 5.—Aldrin and dieldrin content of both tops and roots of a number of seedlings grown in the presence of aldrin for 3 weeks [see Section II(e)].

Assay of tissue homogenates of pea roots incubated with air and an NADPH-generating system confirmed the results with intact plants. Yu *et al.* (1971) reported that in bean homogenates aldrin disappearance was accounted for by production of dieldrin (80%), aldrindiol (10%) (Fig. 6), and water-soluble metabolites (2%). No attempt has yet been made to recover aldrindiol or other metabolites in this study but it is probable that the metabolism of aldrin is through the conversion to such substances rather than to dieldrin. Yu *et al.* (1971) found no conversion of dieldrin to aldrindiol, although this is the mechanism in animal systems, and argued that the systems producing dieldrin and aldrindiol were separate even though they had identical requirements, including pH optima. It is not clear from the present work whether an induced rate of dieldrin metabolism, paralleling aldrin disappearance, occurs in pea roots. However, dieldrin also induced aldrin metabolism [Fig. 4(b)]. Although some movement of aldrin and dieldrin to the tops of all plants tested occurred, this was clearly insufficient to account for the large decrease in the aldrin level in the roots of pea plants pretreated with aldrin. Tests showed that rates of

initial uptake of aldrin by all plants were similar; however, under the experimental conditions described peas were uniquely active in reducing aldrin levels.

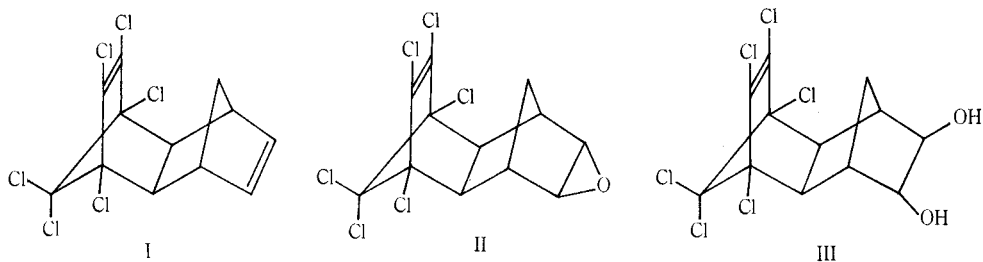


Fig. 6.—Structures of aldrin (I), dieldrin (II), and aldrindiol (III).

The results of Yu *et al.* (1971) demonstrated similarity between the aldrin-metabolizing systems in plants and animals. Our work confirms this similarity and extends it by demonstrating the inductive effects of aldrin and other substances known to induce drug metabolism in animals. Pretreatment with phenobarbital, a substance which has been used as a systemic fungicide (Summers 1968), caused an increased rate of epoxidation in pea and lupin roots. Aniline, an inducer of oxidations of a group of drugs distinct from those induced by phenobarbital, produced a lowered epoxidation rate; phenol, aldrin, and dieldrin did not affect this rate. Of other pesticides tested, monuron increased epoxidation and simazine caused a decrease.

There are possibly important implications of these results in agricultural practice. Long-lasting pesticides in soil could affect the usefulness of newer pesticides by inducing enzyme systems in plants for their metabolism. In addition, effects usually associated with hormone imbalance were noticed in several aldrin-pretreated plants. As an extreme case, beans died after a week's exposure and in broad bean there was reduced growth with leaf curling. Peas and other plants were not obviously affected. The apparent ability of the pea to detoxify aldrin may account for its greater resistance. High levels of organochlorine pesticides have been reported to cause adverse effects upon the growth of cacao seedlings (Enrique-Smith 1968). At this stage it is not possible to define these effects but plant microsomal preparations similar to those studied here have been shown to be concerned in gibberellin synthesis (Murphy and West 1969) and thus direct competition for oxidase activity by aldrin is possibly one explanation.

We intend to isolate and identify aldrin metabolites produced by pretreated plants and examine in detail the enzymic mechanisms concerned with oxidative metabolism of aldrin and other pesticides.

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